Pilot Project - New Knowledge for an integrated management of human activities in the sea

Second Deliverable

Outcomes of the assessments for multi-disciplinary and multi-state programmes

(01 / 04 / 2014 – 31 / 05 / 2015)

Final version

IRIS-SES

INTEGRATED REGIONAL MONITORING IMPLEMENTATION STRATEGY IN THE SOUTH EUROPEAN SEAS

www.iris-ses.eu

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Grant Agreement:
07.0335 / 659540 / SUB / C

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Guidelines on spatial and temporal extent of monitoring water column and seabed habitats indicators based on their scales of natural variation. May 2015. **IRIS-SES project**.

Edited by: Alberto Basset, Francesco Cozzoli, Maurizio Pinna, Monia Renzi, Giannoudi Louisa, Streftraris Nikos, Pagou Kalliopi

European Commission
Funded by the European Commission – DG Environment

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Summary

This Second Deliverable of the project IRIS-SES is structured in three different parts:

Part I – Guideline on spatial and temporal extent of monitoring water column and seabed habitats indicators based on their scales of natural variation and relative Annexes;

Part II – Gap analysis and relative Annexes;

Part III – E-learning activity.

In Part I, the issues related to spatial and temporal extent of natural variability are generally discussed. In particular, the focus is on the variability of the water column and the seabed habitats indicators (Descriptors D1, D5, D6) in the Mediterranean and the Black Sea. The main document deals with the variability issues under general/theoretical perspectives, however having some practical examples reported extensively.

In Part II, there is the description of the gap-analysis on the existing institutional data on marine ecosystem monitoring. This analysis takes into consideration the existing gaps along the spatial and temporal scales between available data and the MSFD requirements and discusses the potential gaps related to the natural variability of the descriptors and the parameters that are included in the MSFD.

In Part III, part of the e-learning activity developed by the IRIS-SES project using the Lifewatch platform, is described briefly.

This deliverable can be accessed also through the projects website: www.iris-ses.eu

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PART 1

Guideline on spatial and temporal extent of monitoring water column and seabed habitats indicators based on their scales of natural variation and relative Annexes

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07.0335/ 659540 / SUB / C

INTRODUCTION

Variability is an inherent characteristic of the natural processes. We could consider natural variability any change that is not induced by human intervention. Variability affects both, the environmental factors and the biological components of the systems (changes in abundance, species composition, structure, etc.) and manifest on space (heterogeneity) and on time (dynamics). Variability is promoted by changes in physical and chemical conditions and by biological activity. Additionally, different human actions could also affect the natural variability. Ecosystems and more particular the organisms must cope with this variability in order to stabilize their own system and make it as predictable as possible. This involves developing adaptive mechanisms to extreme conditions, to adjust its life cycles to the frequency of the changes.

Living beings (including humans) require certain capability to anticipate the changes and incorporate them through natural selection, into their genomic background. These adaptations can be physiological, or behavioural (migrations and rhythms) or structural (species composition and abundance) and could take place at different time scales and organization levels. It implies adaptation at individual level (physiological mechanisms), populations (genetic structure), species (evolution) and ecosystem (ecological succession).

To address variability in monitoring programs, it is necessary and of interest to detect changes in ecosystems produced by human impacts or to evaluate the consequences of management and restoration actions, as well as to separate the effects of natural variability from the anthropogenic changes.

To the best of actual knowledge, a key point is represented by the need to align the scale of the assessment with the ecosystem temporal and spatial natural variability, also, prioritizing areas where pressures and impacts are important. In spite of that, actual knowledge on spatial and temporal variability of selected variables and indicators for the eleven Descriptors of the MSFD are not complete and the principal gap is represented by the lack of clear and rationale criteria on the basis of which to decide choosing opportune scales of observation to cope natural fluctuations in marine ecosystems.

This document, in generally discusses the issues related to spatial and temporal extent of natural variability. In particular, it focuses on the variability that water column and seabed habitats indicators for Descriptors D1, D5, D6, both in the Mediterranean and the Black Seas. Attached to this report are Annex I and Annex II, which are the Glossary and the Abbreviation list, respectively. The main document is dealing with the variability issues under general/theoretical perspectives, however some practical examples are extensively reported in Annex III.

Rational on the selection of D1, D5, and D6 Descriptors

The selection of these descriptors (D1, D5 and D6) was mainly based on the existing literature and the fact that there is a better knowledge and data collection on the spatial and temporal extent of their natural variability, with a focus in the water column and the seabed habitat indicators, in the Mediterranean and Black Sea.

1. KEY ELEMENTS ON THE MSFD FOR THE MONITORING OF MARINE ENVIRONMENTAL STATUS

1.1. Introduction to MSFD

The Marine Strategy Framework Directive (MSFD, 2008/56/EC) of the European Parliament and of the Council (17th June 2008) established a framework for community action in the field of marine environmental policy. This policy was first realized on the 15th of July 2008. It established that EU Member States have to define Good Environmental Status, GES (Article 9), to set environmental target (Article 10), to develop operative monitoring programmes (Article 11) and to assess every six years the environmental status of their marine water (Article 8; Article 17(2)). GES defines the environmental status of the marine waters, as these should provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions, as well as sustainable, thus safeguarding the potential for uses and activities by current and future generations (Article 3(5)). Defined GES has to be achieved by the year 2020. On an operative point of view, monitoring programmes have to be sized and set by 2016 following a six-year cycle for a new evaluation (Claussen et al., 2011).

The MSFD follows a 'holistic approach', taking into account the structure, function and ecological processes of the ecosystem, imposing to define GES using the set of eleven Descriptors that are numbered from D1 to D11 and that cover very distinct fields, from Biodiversity (D1) to Energy

and noise (D11), and that together, summarize the way in which the whole system function.

In this context, the choice of indicator aggregation rules is essential, as the final outcome of the assessment may be very sensitive to this (Prins et al., 2012).

The Commission Decision 2010/477/EU (1st September, 2010) on criteria and methodological standards of good environmental status of the marine waters, decided that of the eleven Descriptors defined by the MSFD (2008/56/EC), 56 indicators could be used for their evaluation and these are obtained by the EU Commission through the analysis of recent scientific literature (Cochrane et al., 2010; Olenin et al., 2010; Piet et al., 2010; Rogers et al., 2010; Ferreira et al., 2010; Rice et al., 2010; Law et al., 2010; Swartenbroux et al., 2010; Galgani et al., 2010; Tasker et al., 2010).

Article 3(5) of the MSFD (2008/56/EC) requires that GES could be determined at the level of the marine region or sub-region as referred to in Article 4, however the geographical scale that could be used for the assessment, is not well defined by the Directive. In the first cycle of the implementation the geographic scales and frequencies applied by Member States (MSs) for the assessments varied between descriptors and there were large differences among MSs.

The Regional Seas Conventions (RSCs) have developed approaches to define assessment areas for specific aims (e.g. Ecosystem Approach for some biodiversity aspects, fisheries, eutrophication, contaminants), while the main aspects such as chemistry and biology are monitored in the Black Sea according to the Final ¨*Diagnostic Report on the state of the Black Sea environment*”.

The Ecosystem Approach (EcAp) goes beyond examining single issues, species, or ecosystem functions in isolation. Instead, it recognizes ecological systems for what they are: rich mixes of elements that interact constantly with each other. This understanding is particularly important for coasts and seas, where the nature of water keeps systems and functions connected (COP, 2002).

Natural variability is strongly related to physiographic, climatic and geographic conditions. Variability is a common trait of marine ecosystems worldwide and it could affect habitat type, spatial and temporal distribution and abundance of the species living in a specific habitat. The concept of habitat includes aspects related to the quality, the occurrence, the distribution and the abundance of the species and communities that are living within (Cochrane et al., 2010). It is recognized that natural variability of intrinsic environmental conditions does not necessary means something negative but that stimulates biodiversity.

The conservation of biodiversity represents the principal target to be achieved to guarantee ecosystem functioning, resource conservation, and future exploitation of them, including services (i.e. food supply, recovery, recycling, purification processes, renewal of resources etc.) that nature provides for humans (Costanza et al., 1997).

As an examble of the importance of the key resources given to us by the marine ecosystems for the human-life itself, consider that the atmospheric oxygen is principally due to photosynthesizing primary producers as phytoplankton and phytobenthos (i.e. *Posidonia oceanica* meadows). Phytoplankton photosynthesis fixes up to 50 Gt of carbon per year, contributing nearly half of the global primary production, on average 140 g C m^{-2} yr^{-1} and half of the total amount of O_2 produced by all plant life (Falkowski et al., 1998).

Relationships among marine ecosystems and humans are as old as the humanity itself. Marine waters represent the principal exploited resource for feeding, transports, oil-pumps, energy production, commerce and leisure, contributing in this way to even the cultural development of our existence. The negative side of this relationship is the strong exploitation of the marine ecosystem, which is represented by severe human pressure that it is mainly concentrated in the coastal areas and that produces important impacts on the near-shore and off-shore zones. It was estimated that, at the turn of the last century, about 136 million people, which live in the Mediterranean coastal areas, directly and indirectly impact on the marine ecosystem (Clark, 1997). Even if deep-sea ecosystems represent the largest biome of the global biosphere, the importance of the near-shore and off-shore zone to the global biodiversity of marine ecosystems is a relatively recent discovery (Danovaro et al., 2010) contrasting with the current use of some European Countries to discharge dredged sediments in off-shore disposal sites. To achieve the full economic potential of oceans and seas, protection and restoration actions would be performed mainly for reducing eutrophication, water and sediment pollution and biodiversity losses. Marine ecosystems represent the principal route via which pollutants are transferred from the abiotic compartments towards the organisms that accumulate along the trophic web. As the direct spillage of crude petroleum and refined petroleum products, especially relevant for the Mediterranean basin due to the high boat traffic and the increasing number of occasional accidents and marine oil-spill occurrence. As a characteristic example it can be referred that in 1979 the levels of total hydrocarbons of 16 μgL^{-1} were reported in the water (Neff, 1979), associated to 148 ngL^{-1} of the total PAHs and in the Mediterranean Sea these concentrations are expected to grow with increasing

human exploitation of the resources. Hydrocarbon pollution is not a problem confined in harbours (Renzi et al., 2009) and coastal marine areas (Lipiatou et al., 1997; Baumard, et al., 1998a; Baumard, et al., 1998b; Rogers, 2002), but also impact offshore habitats (Danovaro et al., 2010) and marine protected areas (Renzi et al., 2010).

The European Union by recognizing the importance of marine ecosystems and the severe threats to its integrity, has launched the MSFD as a key part of the Integrated European Maritime Policy.

The principal aims of the MSFD are:

I) To protect and to preserve the marine environment, preventing its deterioration or, where possible, restoring ecosystems that are adversely affected by human activities;

II) To prevent and to reduce inputs from pollutants, ensuring that the impacts on or the risks to marine biodiversity, marine ecosystems, human health and legitimate use of the marine resources, could be considered insignificant (Article 1(2)).

The viewpoint of the MSFD imposes on the Member States, an ecosystem-based approach to the management of human activities; in fact, the collective pressure due to human activities should be controlled to achieve GES (Article 1(3)). Some overlaps between MSFD and Water Framework Directive (WFD) exist and are explicitly recognized by the MSFD itself, which makes it clear that in coastal waters the latter Directive only intends to tackle aspects, which are not covered by the WFD (e.g. litter, noise, biodiversity). Furthermore, the MSFD requires a coordinated approach to the European MSs towards coherent implementations and the development of common actions to prevent pollution and to protect the marine resources.

The MSFD requires MSs to develop strategies for their marine waters (within European Marine Regions and sub-regions), detailing the state of the environment (e.g. the current condition of habitats and species and pressures impacting upon them), defining GES and establishing environmental targets and indicators, monitoring programmes and measures designed to achieve or maintain a good status .

To achieve these goals, monitoring programmes had to be established by European MS within July 2014, with the aim to evaluate if GES is achieved and to evaluate environmental status evolution during that time. Furthermore, monitoring programmes have to be adequate, coherent, coordinated,

and integrated across Regions and Sub-regions, as well as having specified the size of existing pressures and impacts, including trans-boundary features and impacts. Moreover, monitoring programmes have to be consistent, coherent, compatible and complementary with requirements imposed by other EU legislations and by the RSCs. In fact, previous European legislation as WFD (WFD, 2000/60/EC), Habitats Directive (HD, 1992), Birds Directive (BD, 2009) and international agreements, such as the Regional Seas Conventions (RSCs), are partially superimposed in terms of geographical competences, descriptors and frequencies of monitoring to the MSFD.

Summing up, the MSFD imposes: *i)* the determination of GES; *ii)* the development of harmonized monitoring programs on the basis of methods that ensure consistency and allow comparison between marine sub-regions to the extent of which GES is being achieved by the Member States (Art. 9.3); *iii)* the development of national and international based strategies to reduce human pressures; *iv)* the restoration of critical sites.

To achieve these goals "*the structure, functions and processes of the constituent marine ecosystems, together with the associated physiographic, geographic, geological and climatic factors, allow those ecosystems to function fully and to maintain their resilience to human-induced environmental change. Marine species and habitats are protected; human-induced decline of biodiversity is prevented and diverse biological component function in balance. Hydro-morphological, physical and chemical properties of the ecosystems, including those properties, which result from human activities in the area concerned, support the ecosystems as described above. Anthropogenic inputs of substances and energy, including noise, into the marine environment do not cause pollution effects*" (Art. 3.5.).

Pressures able to affect diversity within species, between species and of ecosystems, reduce biodiversity *sensus* CBD (1992) and these different levels of complexity in terms of ecological organization have to be considered during monitoring and biodiversity assessments (Cochrane et al., 2010). The relevance of biodiversity to the marine environments is widely defined by Cochrane and colleagues (2010). According to this study and citations therein, biodiversity could be intended as follows:

i) "Within species" variation is expressed by the occurrence of discrete sub-species and populations and by genetic diversity. Such intra-specific variability is important, for example, in the survival of a species when facing a new or multiple natural and

anthropogenic pressures, and also for evolutionary change. At the intra-specific level, ecological and phenotypic traits (e.g. geographical range and size distribution within a population) and genetic traits (e.g. genetic structure and diversity) are important features of the overall state of a species

ii) *"Between species" variation is expressed by the wide range of marine animal and plant species in many taxonomic classes. Maintenance of species diversity is a major goal for international biodiversity policies, in view of the accelerating rate of extinction of species in some ecosystems and the increasing numbers of species being listed for protection (e.g. by IUCN).*

iii) *"Of ecosystems" variation within and between ecosystems represents levels of ecological organization above the species level, and provides both wide regional variation and represents aspects that are vital to the overall functioning of ecosystems. Although the term Ecosystems can be applied at many different scales, they are often considered to be very large marine systems (termed Large Marine Ecosystems), similar in scale to the regions and sub-regions provided in the Directive. These large systems (ndr. are divided) into marine landscape and habitat/community types, representing two different ways of characterizing the marine environment at organizational scales between large marine ecosystems and species. Habitats and their associated communities of species – on either the seabed (benthic species living on the seabed, attached to it as epibiota or living in the sediment as infauna) or in the water column (plankton). Habitats are defined on the basis of their physical, hydrological and chemical characteristics (e.g. substrate, temperature, salinity, water movement, nutrient and oxygen levels). Communities of species are associated with particular types of habitat. This combination of abiotic and biotic elements is technically termed a biotope. Landscapes – topographically-defined features, generally large in scale, (e.g. estuaries, fjords, seamounts, deep-sea canyons) which comprise combinations of particular (seabed) habitats/communities and also often certain mobile species (e.g. anadromous fish in estuaries, bentho-pelagic fish on seamounts), due to the physical and hydrological characteristics of the feature. Ecosystems - The habitat/community level (seabed and plankton) and species level (for large/highly mobile species) are expected to be the main units of assessment for Descriptor 1. However, for an*

ecosystem-orientated assessment, as required by the Directive, species and habitats/communities should not be considered in isolation from each other, but as part of the wider ecosystem. This can in part be addressed by considering broader aspects of habitat diversity and their spatial pattern and the overall composition and community structure of pelagic/mobile species.

In spite of the need to quickly achieve the development of monitoring programmes, a general lack of knowledge affects the opportunity to reach the proposed goals. In fact, actual gaps concerning natural spatial and temporal variability of elements listed in Annex III and Annex V of the MSFD affect the chance to define well sized monitoring programmes in term of an optimum balance between the sampling efforts and the significance of obtaining results.

Descriptors considered by MSFD (MSFD Appendix 1) with their associated meaning of GES are detailed in Table 1.

Table 1. Descriptors used in MSFD and their associated meaning of GES

Descriptor	Descriptor Acronym	Definition of GES
Biological diversity	Descriptor 1 D1	The quality and the occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.
Non-indigenous species	Descriptor 2 D2	Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems
Commercial fish shellfish	Descriptor 3 D3	Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of healthy stock
Food webs	Descriptor 4 D4	All the elements of marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity
Eutrophication	Descriptor 5 D5	Human-induced eutrophication is minimized, especially adverse effects thereof, such as losses of biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters
Seabed habitats integrity	Descriptor 6 D6	Seabed habitat integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected
Hydrographical	Descriptor 7	Permanent alteration of hydrographical conditions does

changes	D7	not adversely affect marine ecosystems
Contaminants	Descriptor 8 D8	Concentration of contaminants are at levels not giving rise to pollution effects
Contaminants in seafood	Descriptor 9 D9	Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards
Marine litter	Descriptor 10 D10	Properties and quantities of marine litter do not cause harm to the coastal and marine environment
Energy and Noise	Descriptor 11 D11	Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment

In accordance to Article 9, it is imperative for the Member States to determine the characteristics of GES and therefore define the precise specifications of this main MSFD objective. By the 15th of July 2012, the Member States had to provide information on the initial assessment (Article 8), on the determination of GES (Article 9) and on the establishment of environmental targets and associated indicators (Article 10). The European Environment Agency press release on the 14th of February 2014 "*Europe's seas: A valuable asset that must be used sustainably*" concludes that "*The current way we use the sea risks irreversibly degrading many of the ecosystems*", "*Species surveys have found that 'good environmental status' can be applied to less than a fifth of species and a similar proportion of habitats*". To witness real improvements in our marine environment, the briefing recommends a two-fold approach:

I) MSs need to implement the MSFD in a more consistent and coherent manner that allows progress towards good environmental status to be monitored across regions.

II) Reducing environmental pressures will require us to shift our economies and our values to a more sustainable way of living, including producing and consuming, in order to comply with the vision of 'living well within the limits of our planet' contained in the 7th Environmental Action Programme which sets out Europe's environmental policy priorities.

The key tool for the achievement of the MSFD goals is the Programme of Measures (PoMs) (Article 13) which must be established by 2015 following two preparatory steps: the initial assessments, GES and targets, and secondly the preparation of the monitoring programmes (Article 11). All these elements form part of the marine strategies (Article 5). As a result, MSFD implementation is a step-by-step process in which each one builds upon the previous one.

The correct definition of both GES and monitoring efforts for each Descriptor and indicator needs to reach a well-defined knowledge on the actual assessment of each "object" that has to be monitored including ranges of natural variability in terms of spatial and temporal natural

fluctuations. Unluckily natural fluctuations are not yet well defined in the marine environments and to achieve MSFD goals could be strictly affected by the lack of knowledge.

1.2. Variables and indicators considered for the D1, D5 and D6 Descriptors

A synthetic view on indicators described in this Guideline for Descriptors D1, D5, and D6 is reported in Table 2. The existent link among the MSFD Descriptors (2008/56/EC) and indicators listed in the Decision 2010/477/EU4 (EC, 2010) is also highlighted. Additionally, the variables that need to be measured to obtain the parameters necessary for a given indicator to be applied, are reported in the same table. In some cases, a single variable can be considered as an indicator, for example, the total abundance of a given species. On the other hand, the mean age of a given population, or the age range of such population could be considered an indicator, but the age itself is listed as a variable.

In this Guideline some of the variables and indicators listed in Table 2(see asterisk) are extensively discussed in terms of actual knowledge on their natural variability and some examples are given (see Annex III of this Document) on spatial and temporal scales and in terms of optimization of their monitoring frequencies. This Table is organized starting from Descriptors and then linking habitats, taxonomic groups, indicators listed in Decision 2010/477/EU4 (EC, 2010), indicators considered by other Projects (e.g. PERSEUS), variables to be determined to assess indicators or used as indicators themselves.

Concerning Descriptor D1, Annex III of the Directive, lists the main groups of the marine species that should be considered. The biological components of the MSFD are: microbes, phytoplankton, zooplankton, angiosperms, algal macrophytes, invertebrates, fishes, mammals (cetaceans and seals), reptiles (turtles) and seabirds.

Some important aspects have to be here underlined:

i) Areal extent of migratory species should be considered beyond the jurisdictional limits of the Directive (as defined by the Art. 3.1.);

ii) Microbes, jellyfishes, pelagic cephalopods and the range of marine habitat types that occur within the jurisdiction area of the Directive are, also considered to fall within the scope of the MSFD and they are grouped under the Descriptor of Biological Diversity (D1). In spite of that, homogeneous and structured data are not actually available concerning these taxa. These aspects are actually neglected by most national monitoring

programmes and data on microbes, pelagic cephalopods as well as data concerning other key taxa of the marine ecosystems including Cnidaria (i.e. jellyfish) and Ctenophora, are available from research programmes often based on private segnalations and other sources.

An example is given by the CIESM JellyWatch Program, a research program developed on the basis of temporary funds (http://www.ciesm.org/marine/programs/jellywatch.htm). Several projects in the Mediterranean have developed a Jellyfish watch and/or measures including: PERSEUS, Projecte Medusa, ACRI, MED-JellyRisk, Spot the Jellyfish and Cote d'Azur.

iii) Some of the listed indicators are included because of their increased importance arising from their evaluation on better understanding ecological dynamics of the marine ecosystems. As example, the importance of taxonomic variation (or distinctness) has recently attracted attention in the marine environment (e.g. Clarke and Warwick, 2001) where the value of maintaining variety at higher taxonomic levels (e.g. at phylum or class levels) is advocated.

Table 2. List of considered indicators and variables for the D1, D5 and D6 Descriptors

Descriptor	Habitat	Group	Indicator Annex I Directive 2008/56/EC	Variable or Indicator
D1, D4, D6	Water column & Terrestial habitats	Reptiles	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.2.1 Population abundance** 1.3.1 Population demographic characteristics **1.3.2 Population genetic structure**	Abundance (*) Structure (adults vs juvenile) Sex ratio Fecundity rates Survival/ mortality rates (*) Number and distribution of nests (*)
		Mammals	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.2.1 Population abundance** 1.3.1 Population demographic characteristics 1.3.2 Population genetic structure	Abundance (*) Structure (adults vs juvenile) Sex ratio Fecundity rates Survival/ mortality rates
		Birds	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.2.1 Population abundance** 1.3.1 Population demographic characteristics 1.3.2 Population genetic structure	Abundance (*) Structure (adults vs juvenile) Sex ratio (*) Body size (*) Fecundity rates Survival/ mortality rates
	Water column habitats	Fish	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.2.1 Population abundance and biomass** **1.3.1 Population demographic characteristics** 1.3.2 Population genetic structure	Abundance (*) Structure (adults vs juvenile) Sex ratio (*) Body size (*) Fecundity rates Survival/ mortality rates Species richness of fish Age (1) Length
	Seabed habitats	Phytobenthos	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.1.3 Area covered by the species** **1.2.1 Population abundance and**	Abundance (*) Number of species (*) Total biomass (*) Biomass of seagrass

			biomass **1.3.1 Population demographic characteristics** 1.3.2 Population genetic structure	Species richness (*) Abundance of seagrass (*) Depth distribution of seagrass Areal extent of marine angiosperms(*) Substrate type (*) Areal extent of *P. oceanica* meadow (*) Presence/Absence of *P. oceanica* meadow (*) Survival rate of *P. oceanica* Presence of sensitive and/or tolerant species Surf. area/biomass ratio of macroalgae species Abund. of shade-adapt, slow grow. calc. sp. (*)
		Zoobenthos	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.1.3 Area covered by the species** **1.2.1 Population abundance and biomass** **1.3.1 Population demographic characteristics** 1.3.2 Population genetic structure	Abundance (*) Number of species (*) Total biomass Relative biomass Species richness BENTIX (*) Depth distribution of communities Diversity Indices Shannon Index Abundance ratio above specified length Biomass ratio above specified length Presence of sensitive and/or tolerant species AMBI-AZTI M-AMBI
	Water column habitats	Phytoplankto n	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.2.1 Population abundance and biomass** **1.3.1 Population demographic characteristics** 1.3.2 Population genetic structure	Abundance (2) Number of species (3) Relative biomass Species richness Evenness (Sheldon) of phyt. Biomass ratio of diatoms/dinoflagellate (spring) Abundance of dinoflagellates (C-strategy) Genetic diversity
		Zooplankton	**1.1.1 Distributional range** **1.1.2 Distributional pattern** **1.2.1 Population abundance and biomass** **1.3.1 Population demographic characteristics** 1.3.2 Population genetic structure	Abundance (4) Number of species Relative biomass Biomass of mesozooplankton Biomass ratio Copepods/mesozooplankton
	Common for all habitats and groups for the D1, D4, D6 Descriptors		**1.4.1 Distributional range** **1.4.2 Distributional pattern** 1.5.1 Habitat area 1.5.2 Habitat volume 1.6.1 Condition of the typical communities 1.6.2 Relative abundance and/or biomass 1.6.3 Physical, hydrol., chemical conditions	EEI-Ecological evaluation index (*) Areal extent of maerl-type biogenic sediments Evenness of selected biological component PREI IBI
	Sea-floor integrity		**6.1.1 Type, abundance, biomass and areal extent of relevant biogenic substrate** 6.1.2 Extent of the seabed significantly affected by human activities for the different substrate types 6.2.1. Presence of particularly sensitive and/or tolerant species **6.2.2 Multi-metric indexes assessing benthic community condition and functionality, such as species diversity and richness, proportion of opportunistic to sensitive species** 6.2.3 Proportion of biomass or number of individuals in the macrobenthos above some specified length/size 6.2.4 Parameters describing the characteristics (shape, slope and intercept) of the size spectrum of the benthic community	Species level taxonomy (*) Areal coverage (*) Presence/Absence (*) Species diversity, richness and ratios (*) Length Body-size (*)

D5	Water column Habitats	None	**5.1.1 Nutrients concentration** 5.1.2 Nutrient ratios **5.2.1 Chlorophyll concentration** **5.2.2 Water transparency** **5.2.3 Abundance of opportunistic macroalgae** **5.2.4 Species shift in floristic composition** **5.3.1 Abundance of peren. seaweeds and seagrasses** **5.3.2 Dissolved oxygen**	Chlorophyll-a (*) Dissolved Oxygen (*) Orthophosphates (PO_4) (*) Transparency (*) Nitrites (NO_2) (*) Nitrates (NO_3) (*) Ammonium (NH_4) (*) Silicates (SiO_4) (*) Total Nitrogen (TN) (*) Total phosphorous (TP) (*) Total Suspended Solids (*) Turbidity (*) Temperature (*) Salinity (*) pH (*)

NOTES:
Note 1: Age extimations are more frequently performed by the length or weight data. Otholites determinations are scarce and referred to scientific researches.
Note 2: very few data on picoplankton and in the open sea, not all seasons covered (winter), inappropriate frequency of sampling
Note 3: Although genetic methods boost species identification the uncertainty in species number estimate is very high due to lack of adequate spatio-temporal resolution of sampling and difficulty of species identification under conventional microscope
Note 4: few data on microzooplankton and in the open sea, not all seasons covered (winter), inappropriate frequency of sampling.

1.3. State of the art of marine monitoring in the light of natural variability

1.3.1 Matching current marine monitoring and MFSD needs

The optimization of monitoring strategies in terms of spatial and temporal sampling efforts represents a critical point for the implementation of the MFSD. One critical aspect is represented by the definition of the natural variability of the indicators used to define GES for each Descriptor. Stakeholders, operators and scientists should focus efforts to link spatial and temporal samplings to the natural variability of monitored variables and indicators for improving monitoring strategies in the marine ecosystems, (Figure 1).

The key role of stakeholders is to fund new monitoring and research activities aimed at fulfilling knowledge gaps, while scientists analyze results, improve monitoring tools and strategies and propose to stakeholders and operators new and better-sized management tools.

Optimization should arise from a joint effort of the three elements: stakeholders (administrators) who should define precisely the goal of a given monitoring system, and then scientists together with operators should design a better sampling scheme, taking into account the natural variability, (temporal and spatial), and the adequate sampling methodologies. Pervasive and difficult cross-scale and cross-level interactions in managing the environment are evidenced by a recent research (Cash et al., 2006). The complexity of these interactions and the fact that both scholarship and management have only recently begun to address this, represent the principal limit to develop a constructive dialog between issues involved during the managerial process. In particular, dynamics of cross-scale and cross-level interactions are affected by the interplay between institutions at multiple levels and scales (Cash et al., 2006).

In spite of difficulties, the collective interest towards the definition of natural variability is growing.

To give an example, a recent study identified the following 13 different categories of stakeholders that have an explicit interest on seabirds biodiversity (and, indirectly, on seabirds variability since it is one of its attributes): National governments; regional and local governments; National Park or Protected Area managers; BirdLife partners; other NGOs; research institutes, groups and individuals; industrial fisheries; local (artisanal) fishermen; tourist businesses (tourism companies, hoteliers, fish restaurants, tourists); wind energy community; island inhabitants; and oil companies (Derhé et al., 2012).

The correct definition of the natural variability of different ecosystem components represents the starting point for a well-sized monitoring program able to integrate stakeholders' needs and give responses on ecosystem quality detecting human pressures and ecosystem health evolution. Principal aspects involving stakeholders, operators, scientists in detecting and quantifying natural variability and using the gained knowledge (e.g., in designing monitoring programmes as well as planning mitigation and recovery strategies) are synthesized in Figure 2**.**

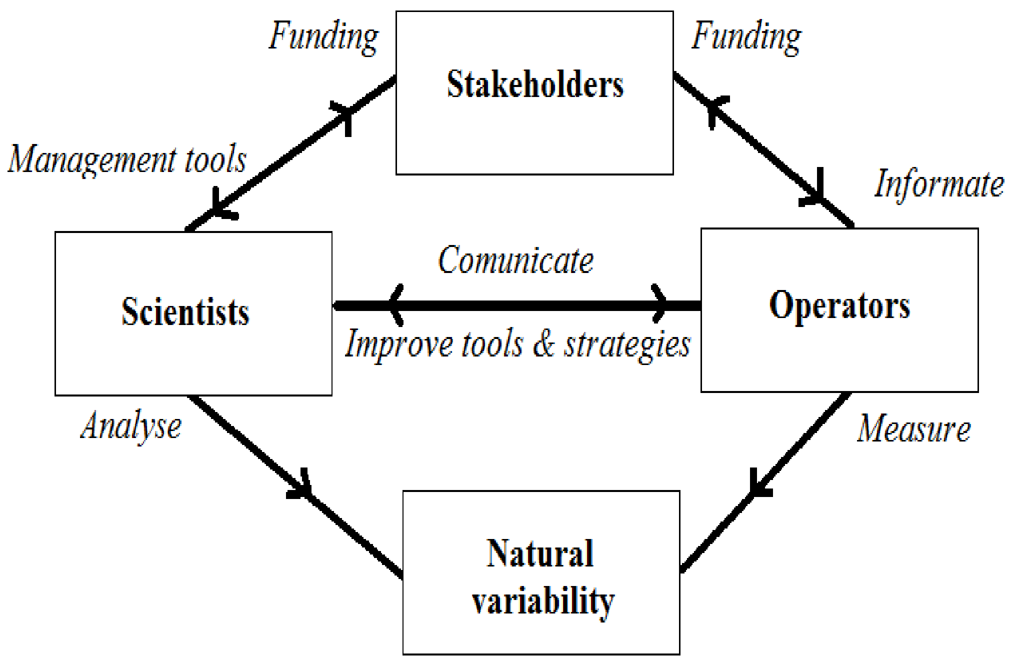

Figure 1. Actors and interactions involved in monitoring activities, general view.

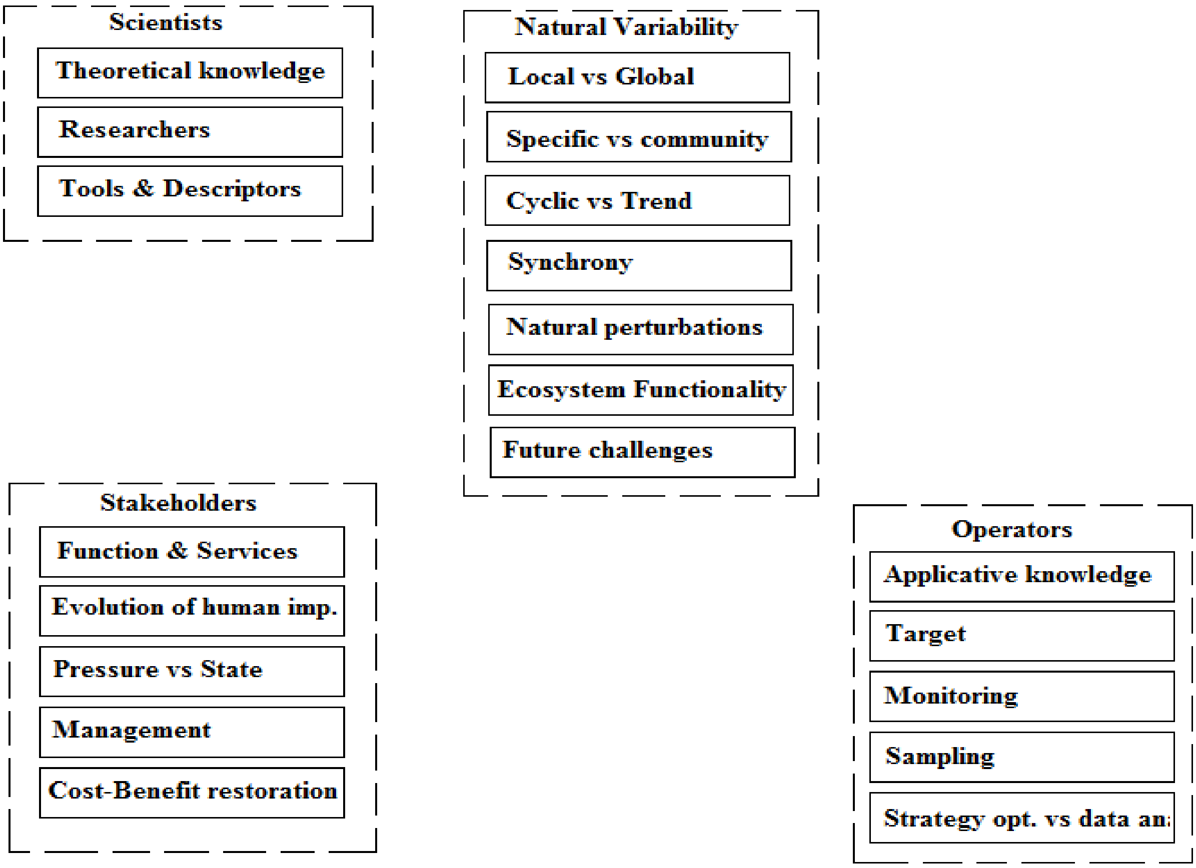

Figure 2. Actors and interactions involved in monitoring activities, detailed view.

1.3.2. Challenges to MFSD: filling in the gaps

Actually, numerous and different monitoring programmes are performed in the Mediterranean and the Black Sea. Available data on marine monitoring are collected by different sources that are: national monitoring programmes, long term research schemes funded by national governments, long term research schemes funded by the European Community, local monitoring and research developed with specific purposes and funded by public or private sectors, etc. Many of the biological data reported in the Mediterranean and European Union Countries of the Black Sea are collected from national monitoring programs, developed in the implementation of Water Framework Directive, Habitat Directive and Common Fisheries Policy (CFP). The Habitat Directive applies to all the marine areas where the target habitats and species occur, whereas the WFD apply to coastal (<1 NM, from the shoreline). Existing monitoring programmes partially only answer to the MFSD needs and some important gaps exist between those and MFSD targets. An overview of the gaps that arise by comparing the actual marine monitoring with the MFSD needs, is summarized here.

The analysis from the inventory of the IRIS-SES project (Grant Agreement 07.0335/659540/SUB/C) on the elements monitored by the involved to the project Mediterranean and Black Sea countries, highlighted some gaps (in spatial and temporal coverage) for some of the eleven Descriptors of the MSFD (http://iris-ses.eu/final-metadatabase-for-iris-ses/). On a general basis, to comply with the MSFD needs, the monitoring network has to be extended in off-shore waters, taking also into account the indicators of the open sea. In fact, the principal data acquisition source is the monitoring programmes developed for the WFD and those focus on limited ecosystem components.

The largest part of the existing monitoring programmes have data on: Biological Diversity (D1), Non-indigenous species (D2), Population of commercial fish & shellfish (D3), Eutrophication (D5), Seabed habitats integrity (D6) and Hydrographical changes (D7). Among these, eutrophication (D5) is the best and widely monitored descriptor. Concerning D7, a distinction for the objectives of the monitoring and the spatial scales of the data availability is needed, despite the fact that as a descriptor is basic for providing the framework to interpret the results from the rest of the monitoring systems. There are not many hydrographical monitoring systems that directly detect or estimate "permanent changes in hydrodynamic conditions", as it is the alterations in the water flow dynamics caused by the anthropogenic impacts (channels, large ports etc), which was the original aim of this descriptor. However, the "global" monitoring (on a large spatial scale) of the hydrographical and hydrodynamic conditions, not answering directly to any descriptor, but as a necessary background for interpreting the results of the different monitoring systems in relation to each one of them, provides massive data acquisition, taken from satellite imagery, moorings, buoys, CTD casts carried out in most of the research surveys, or even by using gliders, radars, and tidal registers.

Concerning Food webs (D4), Contaminants (D8) and Contaminants in seafood (D9) the "theoretical" sampling scheme is not well implemented in all the countries, or at the same level, and that creates an additional gap. In fact, even if the monitoring systems under MEDPOL program, functioning several decades ago in most Mediterranean countries, should be sufficient to fulfil most of the MSFD requirements, even the number of analyzed samples or sampling sites is not a sufficient indicator, to measure the adequacy of the monitoring intensity, as it is not comparable among different descriptors. Marine litter (D10) and Energy and Noise (D11) have a general lack of data that should be highlighted for all the countries considered in the IRIS-SES project. As reported also by the PERSEUS Deliverable Nr. 5.2 "Identified *gaps on MSFD*

assessment elements" (2013) it is considered necessary to establish appropriate monitoring programs and further review and harmonize the indicators and methodologies applied for the assessment of the environmental status among the MSs.

Moored and free-floating buoys can measure a large variety of physical, chemical and biological variables such as salinity, temperature, turbidity, dissolved oxygen, trace metals, pCO_2 and other water constituents, and the data are transmitted in real-time to land-based observatories (Zampoukas et al., 2012). All of this, is dependant on the number of instruments that the buoys could accomodate. Ships of opportunity, merchant fleet such as ferries, and satellite remote sensing analysis could be useful means for data acquisition. Figure 3 and Figure 4 summarize the data availability in the Mediterranean Sea and Black Sea, respectively.

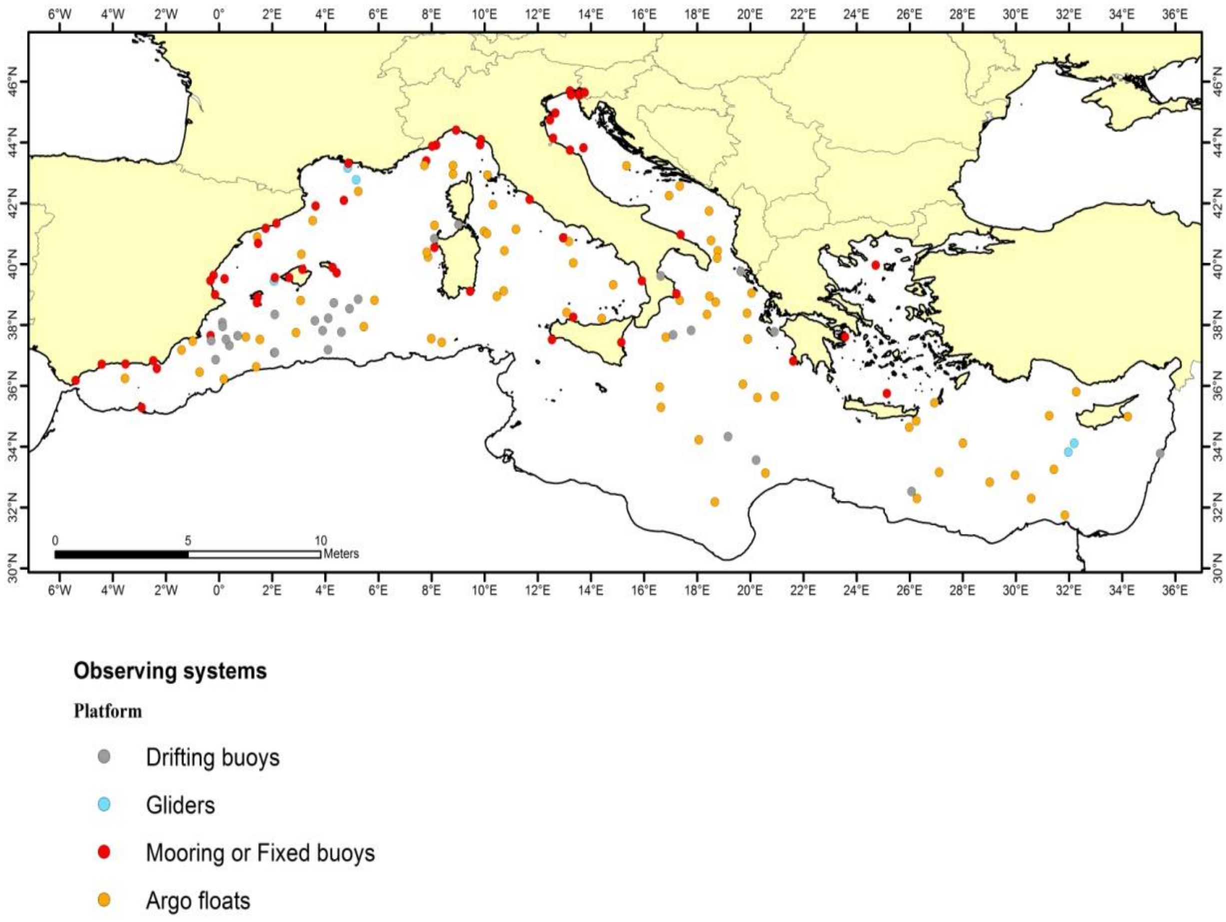

Figure 3. Actual total coverage in the Mediterranean Sea of the different observing systems platforms (highlighted by different colors).

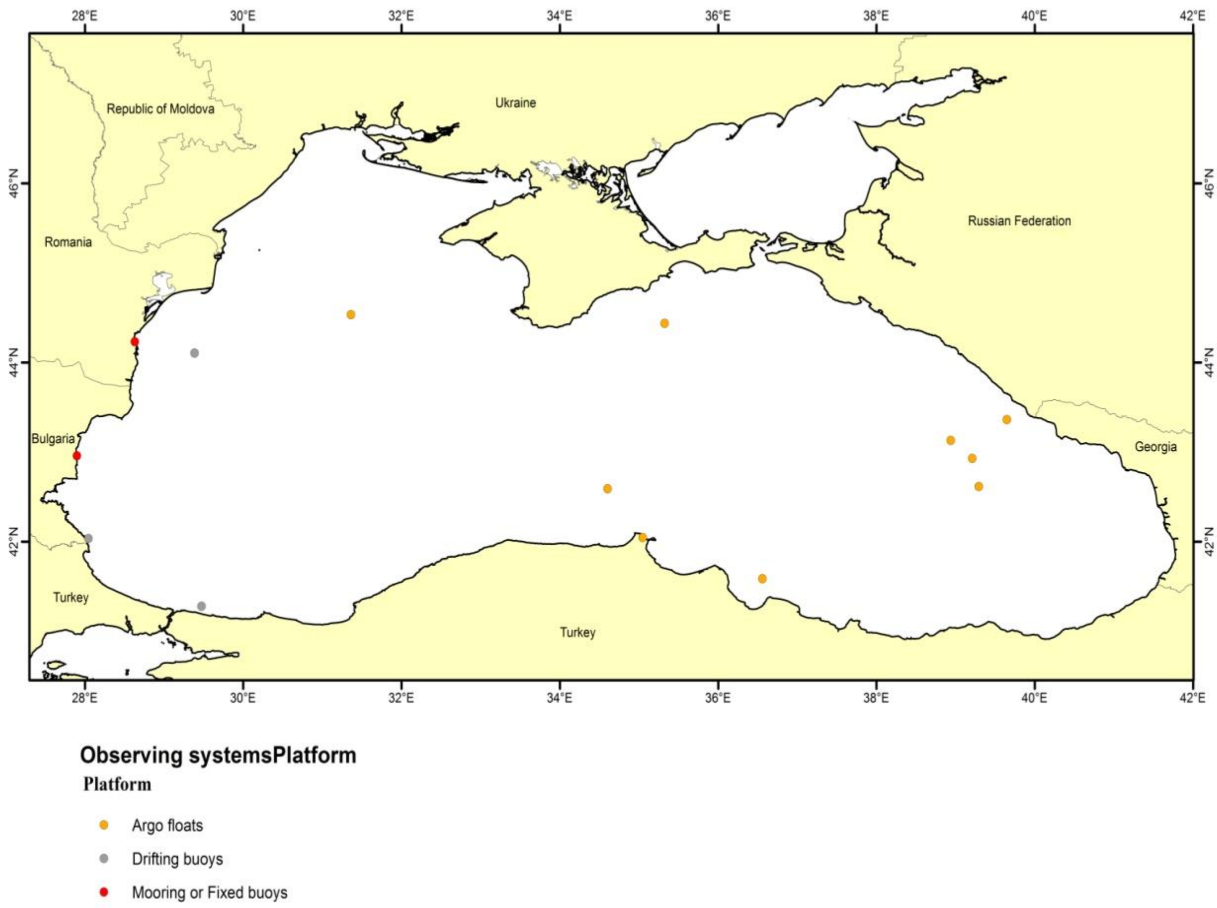

Figure 4. Actual total coverage in the Black Sea of different observing systems platforms.

The analysis on the spatial coverage of the operation of the autonomous mobile instruments (drifters, floats and gliders) showed that the southern areas of the Mediterranean Sea and the whole of the Black Sea are under-sampled and denser observations and implementation strategies are required. This gap can be partially filled by involving scientists from North African countries in the new observatory initiations. For the Black Sea, more integrated observation programs are needed among its surrounding countries and an active collaboration with other European initiatives (Poulain et al., 2013).

Currently, the remote sensing outcomes in the Mediterranean and Black Sea are limited to core variables (e.g. Sea Surface Temperature, Sea Surface Height, Chlorophyll, etc). The development of the remote sensing datasets more suitable to evaluate the ecosystem attributes relevant for the MSFD Descriptors (e.g. productivity, biological diversity, turbidity, etc) is required, and it is part of the activity planned in the EU project PERSEUS on WP4.

2. NATURAL VARIABILITY: DRIVERS, SPATIAL AND TEMPORAL SCALES & PATTERNS

A theoretical analysis of drivers, spatial and temporal scales and patterns of variability is reported in this Chapter while a wider description including some practical examples concerning the natural variability of Descriptors D1, D5 and D6 is reported in the Annex III of this Document.

Landres and colleagues (1999) defined natural variability as the ecological conditions and the spatial and temporal variation of those conditions, that are relatively unaffected by humans, within a period of time and a geographical area that is appropriate to an expressed goal. Natural variability involves the linkages of habitats, species, communities, and ecological processes at multiple spatial and temporal scales (Noss, 1991). It is an inherent characteristic of natural processes as it affects both the environmental factors and biological components of the ecosystems. The correct determination of natural variability could allow distinguishing effects produced by natural fluctuations from those induced by human-due pressures on data collected during monitoring programmes. One of the major aims of characterizing natural variability is to understand how driving processes vary from one site to another, how these processes influenced ecological systems in the past and how these processes might influence ecological systems today and in the future. Natural variability concept offers both a challenge and an opportunity for ecologists to become meaningfully involved with managers in defining ecologically appropriate goals and practices for a specific area of study or a specific region.

Natural variability is a multidimensional concept (Purvis and Hector, 2000) and encompasses many scales of variation in biological organization (from genes to ecosystems). Following Gibson and colleagues (2000) we define "scale" as the spatial, temporal, quantitative, or analytical dimensions that are used in order to measure and study any phenomenon. One can describe the natural variability in terms of scale of magnitude (low, high), frequency (short, long, regular, irregular) and spatial patterns (small scale, large scale). Ecological processes are scale-specific in their effects, and create heterogeneous landscapes with scale-specific structure and pattern (Turner et al., 2001). On one hand, larger scale dynamics set the boundary for smaller scale dynamics. On the other hand, the final, large scale outcome of environmental evolution is the cumulative results of smaller scale processes. The common features of the marine species distribution in all environments is represented by patchiness, that is present in all spatial scales starting from the distribution of individuals of a population in their habitat to the mosaics of faunal benthic communities (Petersen, 1918; Thorson, 1951; Peres and Picard, 1964) or to the faunal provinces

and zones at a biogeographical level (Briggs, 1995). The definition of scales at which structural changes (i.e. species abundances) occur in a certain population could allow determining the ecological process affecting the patterns of distribution. This knowledge is therefore essential for developing and testing hypotheses about processes and essential for designing sampling strategies for environmental impact assessments, in which the changes produced by human activities need to be differentiated from the sources of natural variability (Pérez-Ruzafa et al., 2007). The scale at which spatial patterns of variation are observed affects our level of perception on their variability. Due to this , the methodological aspects related to sampling strategies in terms of spatial and temporal efforts (Anderson et al., 2005) and also in terms of number of sampling replicates (Underwood, 1997) are key aspects for marine monitorings. In fact the absence of a correct sampling strategy could affect the possibility to segregate, on a statistical basis, natural variability of the ecosystem from human pressures (Benedetti-Cecchi, 2004). The choice of the opportune spatial and temporal scale during monitoring is the "*conditio sine qua non*" the occurrence of pseudo-replicates (Hulbert, 1984; Underwood, 1997) could be prevented.

Interactions may occur within or across scales, leading to substantial complexity in dynamics. Functional redundancy across scales provides reinforcement of function, increasing resilience. Cross-scale processes can propagate, amplify or buffer site-level actions (Cash, 2006). Ignoring cross-scale dynamics within spatial and temporal dimensions is common and leads to a range of management problems (Holling, 1986; 1995; Clark, 1987).

Community and species responses to a single external driver can be modulated across scale levels by subsidiary factors (Thrush, 2005). Choosing the appropriate geographic extent is particularly critical where management issues extend beyond the scale of the planning area, as is commonly the case (Ibanez et al., 2013). A regional scope is often needed to inform planning efforts that address local ecological and social issues. Many bioregional assessments and conservation strategies deal with this, providing context for local analysis and planning. Working along environmental gradients is the most feasible approach for natural variability investigation. Still, it presents challenges. First, such approaches can require mid- to long-term datasets collected over intensive and extensive temporal and spatial extents (Bolker, 2009).

Second, integration of responses across different studies is most effective if ontogenetic stages and spatial and temporal scales are similar. Finally, disentangling the relative contributions of multiple covariates, including climate, that jointly influence individual performance, is complex (Bolker, 2009). In many cases, these challenges can be overcome by multi-investigator collaborations

intended to ensure uniformity of field methods and/or by the use of alternative analytical approaches. Data collected along environmental gradients can be analyzed by means of hierarchical or multilevel models that link scales (individual organisms, sites, landscapes, and regions) and make inferences about species performance at each scale and as a function of the many biotic and abiotic factors expected to affect these processes (Clark, 2005).

Separating attributes at their population, community and ecosystem levels in the marine environment is important because there are conservation implications at each level of the hierarchy (Zacharias and Roff, 2000). Variations in biological diversity (D1), seabed habitats (D6) and eutrophication (D5) are strictly linked between them in the marine ecosystems. As example, organic matter in sediments is an important source of food for benthic fauna, but an overabundance can cause reductions in species richness, abundance, and biomass due to oxygen depletion and buildup of toxic byproducts (ammonia and sulphide) associated with the breakdown of these materials. Moreover, often other chemical stressors co-varying with sediment particle-size are associated to the increase of the organic content in sediment (Shine, 2005). Different environmental studies emphasize different aspects of biodiversity at different scales, e.g., eutrophication and habitat homogenization affecting gamma-diversity (Velland et al., 2007) vs. biodiversity-ecological functioning experiments focusing on alpha-diversity (Emmerson et al., 2001; Hooper et al., 2005). Cross-scale studies demonstrate that shoot studies only partly address the spatial structure of seagrass landscapes and further large-scale spatially explicit research, is required. Macrozoobenthic responses to sediment composition can be modulated from the spatial scale specific factors (Thrush, 2005) like the adopted management strategy. Focusing on biodiversity following an unimodal, large scale-basis response to increasing stress (e.g., Connell, 1978; Hacker and Gaines, 1997), lead to underestimate effects on beta-diversity. Hewitt (2010) showed that, even within a soft-sediment habitat apparently homogeneous at the 100 m scale, enough heterogeneity in species distributions can exist in such manner that the homogenization of these can still pose a threat to gamma diversity. Massive stress events should override patch dynamics by re-setting all patches to the same (early) successional state (Denslow, 1980), while at lower levels of stress; asynchronous dynamics of individual patches should produce heterogeneity (Connell, 1978; Huston, 1979). Relative shifts between different aspects of biodiversity at small spatial scales could be an early warning signal for large-scale biodiversity loss (Hewitt, 2010). Alteration of the relationship between the average species richness and its heterogeneity lead to the potential for regime shifts. The interplay observed between alpha-, beta- and gamma-diversity

has a major implication for resilience. Rare species have been implicated as providing insurance and functional resilience against change (Walker, 1992; Naeem and Li, 1997). Dornelas and colleagues (2006) showed that similar habitats adjacent to each other can have markedly different communities, thereby decreasing the scale at which we should consider heterogeneity to be important to biodiversity. Hewitt (2010) suggests including the response of gamma- and beta-diversity and rare species into theoretical models predicting resilience and regime shifts and to empirical studies trying to understand the role of rare species in different systems.

2.1. Drivers at different spatial and temporal scales

Organisms and ecosystems must cope with any variability in order to stabilize their own system and make it as predictable as possible. This involves developing adaptive mechanisms to extreme conditions, to adapt their life cycles to this frequency of changes.

Living beings (including humans) require certain capability to anticipate the changes and incorporate them to their genomic background through natural selection. These adaptations can be physiological or behavioural (migrations and rhythms).

To cope with variability it is necessary in monitoring programs, as usually we are interested in to detect changes in ecosystems produced by human impacts or to evaluate the consequences of management and restoration actions and hence it is essential to separate the effects of natural variability from the anthropogenic changes.

In marine ecosystems, the connectivity of marine environments causes and the threats, such as habitat loss, climate change, pollution and introduced species, operate on what Ricklefs (1987) terms "*processes beyond the normal scale of consideration*", cannot be mitigated by means of traditional marine conservation measures. The inability to progress beyond species or spaces approaches to marine conservation can be attributed partly to a lack of understanding of the mechanisms structuring marine biodiversity (Zacharias and Roff, 2000). Attributes at the population (e.g. migration) and ecosystem levels (e.g. water movement) tend to be easier to observe than community attributes such as competition and ecosystem ones (Zacharias and Roff, 2000). Processes such as productivity involve both biotic and abiotic components and could be not impacted by human activities (e.g. water motion, exception made for the indirect effects due to the global warming process) or impacted by human activity (e.g. biogeochemical cycles, events, productivity). To define the boundaries of natural variability of the observed descriptors is a fundamental step for evaluating monitoring outputs (i.e. if the observed conditions are within their natural state or if they have been altered by human impacts).

A crucial part for describing natural variability is selecting the time period and geographical extent used to characterize system dynamics. There is no single, widely applicable optimal period, and relevance is lost if a long time period is used, because conditions such as climate and species composition may have changed drastically.

Both biotic and abiotic drivers contribute in generating variability at different spatial scales. Any chemical or physical factor in the environment is considered as an abiotic driver. Biotic drivers, such as predation, disease, and competition for resources such as food, water, and mates, can also affect how a species is distributed. A biotic factor is any behavior of an organism that affects another organism, such as a predator consuming its prey. Due to limited resources, populations may be evenly distributed to minimize competition.

2.1.1. Abiotic drivers

As 'abiotic drivers' of natural variability we consider all those physical factors that generate heterogeneity in a habitat structure. The physical habitat variability set the species niche boundaries (potential niche) of which the realized niche is a subset defined by ecological integration (Hutchinson, 1959).

Sources of large-scale (temporal and spatial) variability are:

- Geographical barriers (including distance and fronts between currents): populations divided (isolated) by a physical barrier can follow independent evolutionary path, increasing variability.
- Climate, latitudinal gradient: climate is a main large scale driver of species turnover and affects many ecosystem functions
- Main water circulation patterns: they can determine the pathways of species spreading and nutrients large-scale distribution. Large-scale hydrodynamics can affect sedimentation processes, oxygen, nutrient availability, resources availability, species movements and spread, pollutants diffusion, salinity.

Sources of intermediate scale (temporal and spatial) variability are:

- Bottom topography and elevation in the benthic realm or eddies and fronts in offshore pelagic habitats: these factors determine habitat complexity at intermediate scales
- Exposure to local hydrodynamics, waves: it can affect hydrodynamic stress and nutrient distributions
- Marine eutrophication

Sources of small-scale (temporal and spatial) variability are:

- Bottom typology and structure: landscape patterns influence the ways organisms move on the landscape (Wiens and Milne, 1989). Topography and microclimate difference may create barriers to species dispersal, especially between water bodies. In isolated habitats, populations are more susceptible to environmental catastrophes and invasion of exotic species
- Hydrodynamic: water turbulence
- High, localized peaks of disturbance (chemical, eutrophication, physical, energy and noise): Paine and Levin (1981) demonstrated that natural regimes of disturbance and recovery, also, produce spatial and temporal variability
- Water properties: temperature, salinity, light exposure, nutrient levels, oxygen concentration, and carbon dioxide.

2.1.2. Biotic drivers

Ecological interactions define the natural variability within the physical niche boundary (Hutchinson, 1959). Ecological and evolutionary processes produce the pattern and connectivity of landscapes (Landres et al., 1999).

Sources of large-scale (temporal and spatial) variability are:

- Biogeography: Organisms and biological communities vary according to a highly regular fashion along geographic gradients of latitude, elevation, isolation and habitat area.
- Dispersion ability: species pathways of spreading.

Sources of intermediate scale (temporal and spatial) variability are:

- Population dynamics.
- Recruitment.

Sources of small-scale (temporal and spatial) variability are:

- Resource distribution: Distribution patterns can change seasonally, in response to the availability of resources.
- Community interactions: Levin (1976; 1978) showed that biotic predator-prey interactions, combined with spatial movement, could result in patchy spatial patterns of populations.
- Ecosystem engineering: by means of ecosystem engineering, changes in the spatial distribution of organisms (shift in areas, invasion, local extinction) can exacerbate or dampen ongoing physical trends (Crooks, 2002).

2.2. Body size component of natural variability: a general framework with applied implications

Organisms' characteristics vary predictably with their body size (e.g., Bartholomew, 1981; Peters, 1983; Calder 1984; Schmidt-Nielsen, 1984; Niklas, 1994; Gillooly et al., 2001; 2002; Sterner and Elser, 2002). Theoretical advances in ecology have shown explicitly how these characteristics can be quantified, related to each other, and explained in terms of basic principles of biology, chemistry, and physics. Many features of population dynamics and community organization are due to effects of body size on the performance of individual organisms. Strong, positive relationships exist between the spatial and temporal extent of ecosystem processes and patterns as well as the body size of the involved organisms (Table 3). For example, at the population level, the number of populations, the population density and the average body size of individuals are related through commonly decreasing functions. The extent of individual home range and, more in general, individual motility, is often a positive function of organisms' size (Haskell 2002). The scaling of rates of ecological interactions has important implications for coexistence and species diversity. The qualitative empirical patterns of biodiversity would suggest that many species are rare and only a few of them are common; many are small and few are large; moreover, small species are expected to be dense and large species sparse.

Table 3. Spatial elements and level of ecological organization are hierarchically structured. Different indicators can be used to monitor the ecological status at different levels of organization.

Spatial scale	Low	---------------------------------------→			High
Scaling natural variabilty with:	Hierarchical level	individual	Population	community	ecosystem
Biological elements:					
mammals			Abundance	Structure	
Birds			Abundance	Structure	
Reptiles			Seaturtles spawning populations		
fishes		Sex	Relative abundance	Species richness	
		Age	Relative biomass	Shannon index	
		Maturation age			
phytobenthos			Species biomass	Species level taxonomy	Substrate type
			Presence of *P. oceanica* meadow	Total biomass	
			Survival rate of *P. oceanica*	Abundance macroalgae (total cov)	
				Areal extent of marine angiosperms	
				Shannon-index macroalgae	
				Areal extent of *P. oceanica*	

							meadow			
							Abundance of seagrass			
							Biomass of seagrass			
							Depth distribution of seagrass			
zoobenthos					Relative abundance		Species level taxonomy			
					Relative biomass		Abundance of benthic inv			
					Depth distribution of *D. cornea; C. barbata, C. crinita, D. trunculus, M. phaseolina, P. crispa*		Shannon-index benthic inv			
					Biomass of *C. gallina; C. barbata; D. trunculus; M. leidyi; M. galloprovincialis; P. crispa; U. pusilla*		BENTIX			
					Body lenght distribution of *C. gallina, D. cornea, M. lineatus, M. galloprovincialis, U. pusilla; M. galloprovincialis*		Zoobenthos diversity indices			
							Abund. Ratio of cumulative proport. of size classes			
							Depth distribution of typical zoobenthic communities			
zooplankton					Species biomass		Species level taxonomy			
					Abundance ratio Copepods/mesozooplankto n		Total abundance			
							Abundance of mesozooplankton			
phytoplankto n					Species biomass		Species level taxonomy			
					Abundance of selected phytoplankton species and taxa groups		Total biomass			
					Abundance of selected dinoflagellates (C-strategy species)		Biomass of phytoplankton (spring and summer: coastal; shelf; open-sea)			
							Abundance ratio of diatoms/dinoflagellate			
							Eveness (Sheldon) of phytoplankton			
							Species diversity of plankton (Menhinick)			
integrative							Species richness		Areal extent of maerl-type biogenic sediments	
							Eveness of selected biological component			
							PREI			
							EEI-Ecological evaluation index			
							IBI			
							Turbidity			

Monitoring with benthic invertebrates is often based on different sensitivities of some taxa of macroinvertebrates (Norris and Georges, 1993) and is used extensively. Moreover, indicator species have a generally low sensitivity to weak disturbances, being unsuitable for detecting early signs of stress (e.g. Balloch et al., 1976; Murphy, 1978; Hellawell, 1986). In the last three decades, theoretical and experimental studies have focused on body-size–abundance distributions, biomass-

size spectra or dimensional structures as structural community features (Damuth, 1981; McMahon and Bonner, 1983; Peters, 1983; Lawton, 1990; Schmid et al., 2000). It has been found that body-size–abundance distributions respond to disturbance pressures through individual energetics, population dynamics, and interspecific interactions and species coexistence responses. Thus, they can provide tools for the evaluation of aquatic ecosystem health (Rasmussen, 1993). Indeed, body-size–abundance distributions are expected to be at a higher hierarchical level than taxonomic composition of communities. Therefore, these would be independent of the taxonomic composition and consequently, having fewer variables. Communities are expected to be organized hierarchically on a body size gradient. Therefore, the width of the size–abundance distributions is expected to decrease with increasing direct, or cascade, disturbance pressure. Moreover, body size is generally easy to measure and amenable to intercalibration procedures, it is comparable across taxa, guilds and sites, and, as a community feature, it is expected to vary on disturbance gradients, according to energetic and ecological constraints.

Predictions of the body size-energy constraints and the hypothesis on body-size–abundance distribution descriptors are based on the assumption that the body-size–abundance parameters are determined by interspecific more than intraspecific components. The ecological relevance of body-size–abundance distributions in macroinvertebrate benthic guilds is supported by two major evidences: (a) body-size–abundance distributions that are affected by the interspecific more than the intraspecific component, i.e. body-size–abundance distributions are more than a population-level description of a size structure of the dominant macroinvertebrate taxa, and (b) body-size–abundance distributions are relatively invariant when compared with the taxonomic composition of guilds and communities, i.e. body-size–abundance distributions seem to be at a higher hierarchical level than the taxonomic composition of communities, according to the body-size–energy constraints hypothesis (Basset et al., 2004). Some interesting features of the body size and the related descriptors are: (a) body-size–abundance distributions are consistently less variable than taxonomic composition; (b) the width of body-size–abundance distribution is mainly due to the interspecific component; (c) the descriptors of body-size–abundance distributions seem to respond on environmental gradients and generally covary with species density, richness and diversity, on which most of the monitoring programmes actually rely (Basset et al., 2004). The major intrinsic disadvantage of the body size related descriptors are the sample size required. A description of the size–abundance distribution requires large samples of individuals, and obtaining such data can be time consuming. In phytoplankton communities, literature data report sample

sizes as large as 300 individuals of the most abundant species being utilized to study body-size structure (Echevarria et al., 1990). Nevertheless, the potential advantages of body-size-related descriptors and the results already available indicate that they can represent a significant improvement to already established tools, particularly for the implementation of MFSD, which requires descriptors comparable across quality elements, across ecosystem types, regional areas and other spatial scales.

3. PRESSURES & PROCESSES

Different types of human activities could affect the natural variability of the considered descriptors for this document. An essay concerning the mining of the principal pressures affecting Descriptors D1, D4, D6 and D5 variability is following on the basis of existing literature.

The correct determination of natural variability could allow disentangling changes in the values of the indicators produced by natural fluctuations from variability induced by human-due pressures on data collected during monitoring programmes. However, this is a difficult task, since data collection could be affected by significant problems that could reduce representativeness of obtained results of monitoring and could consequently affect the assessment of natural variability. First of all the absence of literature data on natural variability (spatial and temporal) of the considered Descriptors represents an important gap that have to be quickly filled. Available data are fragmented, and usually it is difficult to compare among them as they are not acquired with the same or similar methodological approach. Concerning data acquisition programs, the information on the used methods, the quality assurance and the control procedures adopted to validate the dataset, are difficult to obtain and not always reported, making the data processing impossible or lacking completely.

Regarding the Descriptor D1, problems related to the integration process among indicators and variables used for the ecosystem quality evaluation are still completely unresolved. Marine habitat integrity could be evaluated on the basis of the species richness but, also, considering the spread of species across the higher taxa. Due to this, both species richness and taxonomic spread are important attributes of biodiversity that should be equally considered for environmental monitoring and conservation purposes (Warwick, 2005). A single indicator could not be sufficient to correctly describe the ecosystem health status. For example, the analysis of macrofauna could

not be sufficient to evidence signs of environmental stress (Karakassis et al., 2004) and large animals (such as demersal fish) with longer life spans are more likely to show response to subtle effects (Machias et al., 2004). Furthermore, in coarse sediments, such as those inhabited by *Posidonia oceanica*, benthic fauna could evidence high abundance; biomass and diversity although monitoring results regarding density of *Posidonia oceanica* meadows could show severe effects on the health of the seagrasses (Karakassis et al., 2005). Losses of seabed habitats such as *Halimeda tuna, Palmophyllum crassum, Zanardinia typu*s could be a combination of human induced stress, low light irradiance and recruitment reduction (Piazzi et al., 2012).

To obtain a good description of the natural spatial and temporal variability, that represents the very first step in the design of monitoring programs, it is necessary to develop a well-sized sampling strategy able to separate human-induced variability from the natural one. Furthermore, a well-sized sampling strategy based on the correct and exhaustive knowledge of natural variability of the considered descriptors could be effective to reduce sampling efforts (time consuming procedures) and consequently their costs, ensuring effectiveness of obtained results.

An essay of the spatial and temporal variability of the various indicators addressed in the Mediterranean region for the Descriptor D1– Biological diversity and the associated Descriptor D5 – Eutrophication and D6 - Seabed Habitats Integrity, is reported in the following chapter.

3.1. Descriptors D1, D6 – Biodiversity & Seafloor Integrity

Concerning **seabirds**, major threats are represented by habitat deterioration (Monteiro et al., 1996), oil spills, fisheries bycatch and over-fishing, that have an impact at all levels of marine food webs (Péron et al., 2012). Fisheries bycatch could be the main cause of low adult survival probabilities of Yelkouan Shearwaters breeding in Port Cros (García-Barcelona et al., 2010; Oppel et al., 2011) as well as the endangered Balearic Shearwater *Puffinus mauretanicus* (Oro et al., 2004). Also invasive, non-native predators (i.e. black rats *Rattus rattus* and domestic and feral cats *Felis catus*) are important threats (Baccetti et al., 2009), which are able to produce significant population declines (Sultana and Borg, 2006), and the impact of introduced predators are mostly severe when both adults and eggs are affected by predation (Cuthbert et al., 2001). Windfarms (Derhé et al., 2012), geological events (Fontaine et al., 2011), increasing levels of light pollution, and disturbance to colonies resulting from touristic and residential development are additional significant threats affecting the species in several countries (Rodríguez and Rodríguez, 2009; Rodríguez et al., 2011; Fontaine et al., 2011). IUCN (2012) reported in details a list of the principal

pressures both for **reptiles and marine mammals**. Concerning **turtles**, principal threats are the capture of adult turtles, human predation of eggs and the rapid beach development (Clarke et al., 2000), while concerning **marine mammals**, principal pressures are represented by bycatch, driftness, food resource depletion and pollution (IUCN, 2012).

Seagrass meadows are considered the most valuable component of shallow water environments (Hemminga, 1998), providing important nursery habitats for a number of fish species, with remarkable primary and secondary productivity rates (Tomasko and Lapointe, 1991). Seagrass meadows grow above the top layer of sediments and contribute to its aerobic oxidation by transporting to the rhizosphere a great deal of oxygen produced during photosynthesis. The respiration of the roots via lacunae oxygen is dispersed into the layers of sediments (Pedersen et al., 1998). The critical environmental factors limiting the growth of seagrass meadows are still poorly understood, since it remains unclear which biological interactions have a direct or indirect influence on such ecosystems health status. A recent study performed in 12 coastal systems indicates a progressive decline of about 65% of phanerogams and of 48% of other submerged aquatic vegetation taxa that occurred during the past 150–300 years (Lotze et al., 2006). The observed decrease is an incident of different factors such as chemical pollution, water eutrophication, physical impacts, modifications of the trophic structure, and impacts produced by urban settlements (Duarte, 2002; Orth et al., 2006; Short et al., 2006). Worldwide, significant management efforts are made for restoration purposes on the extent and the water quality of transitional waters aiming to recover productivity and habitat value of these important ecosystems (Lirman et al., 2008). Phyto-sociological dominance is a complex and not yet well-understood phenomenon, which depends on multifactor levels of interaction between abiotic and biological factors as the relative importance among each variable, is not fully explained. Furthermore, fluctuations on a yearly basis produce an ecological effect, of rapid changes in the population settlements and in phanerogams distribution (Orfanidis et al., 2008).

Seagrass loss results from direct human impacts, including mechanical damage (by dredging, fishing, and anchoring), eutrophication, aquaculture, siltation, effects of coastal constructions, and food web alterations; and indirect human impacts, including negative effects of climate change (erosion by rising sea level, increased storms, increased ultraviolet irradiance), as well as from natural causes, such as cyclones and floods (Duarte, 2002).

Certainly, as a result of **eutrophication**, a growth of opportunist epiphytes takes place, reducing dramatically the photosynthetic capacity of seagrass leaves, by covering them completely. Moreover, the availability of light itself is reduced as a consequence of increased phytoplankton biomass. Substantial quantities of nitrogen and phosphorus discharged from the effluents of the municipal wastewater treatment plants and of intensive aquaculture factories, especially within low renewal water environments, cause frequent macro/micro algal blooms, leading to a nearly complete screening of the sunlight needed by seagrass to survive. Under conditions of water eutrophication, seagrasses are also less competitive than the opportunistic macroalgae: while organic matter and an increase in sulphide sediment concentrations can result in the reduction of seagrass biomasses (Goodman and Dennison, 1995), as the opportunistic macroalgae grow up and displace them. Restoration work can improve sediment quality, and the removal of the eutrophication sources quickly leads to seagrass recovery (Ben Charrada, 1995; Plus et al., 2003). Thus the substratum quality is decisive for the seagrass settlement that itself contributes to modify the substratum.

Concerning **benthic invertebrates**, a recent research evidenced as nutrients and, in particular total organic carbon (TOC), can affect benthic population structure and in particular species richness (Hurlbert's $E(S_n)$). Results have shown that the risks of reduced species richness from the organic loading and other associated stressors in sediments, should be relatively low at TOC concentrations <10 mg/g, high at TOC concentrations >35 mg/g, and intermediate at concentrations in between (Shine, 2005). In pristine/natural marine ecosystems, the community diversity index (H') for benthic invertebrates (zoobenthos) is reported to be higher than 5 (Simboura and Zenetos, 2002).

Regarding fishes, the main pressure is the obvious effect from overfishing, not only on targeted populations but in the case of non selective gears, to the whole fish communities. However, the destruction of essential habitats such as spawning or nurseries ones, by physical disappearance due to human constructions or related activities (ports, dredging, beaches "regeneration"…) or pollution, represents an additional major threat to the Mediterranean fish populations. The impact of alien species, mainly Lessepsian migrants, is also a hazard for local biodiversity, as occurs in many other taxonomic groups.

The major pressures and principal processes that significantly affect the biological elements for each indicator considered by the Descriptor D1, D4, D6 are listed in Table 4.

Table 4. Principal Pressures & Processes related to biological elements (D1, D4, D6).

Indicator	Habitat	Major source of pressure	Processes
Number of Individuals	Biocenosis	Fishing Dredging Overfishing Pollution Eutrophication	Climate changes Hydrodynamic changes River inputs changes Submarine volcanic activity Violent sea storms Alloctone/Autochthone competition Invasive species Competition Migration/Adaptation processes
	Birds	Habitat loss Damages to nesting Pollution	Climate changes Competition/predation Changes in feeding resources
	Reptiles	Bycatch Nesting disruption Photopollution Litter and plastic bags Disturbance of nesting areas Tourism Habitat loss Sea level rising Ocean acidification Catching Coastal development Noise pollution Vessel collisions	Climate changes Hydrodynamic changes Feeding availability Predation Disease and pathogens
	Marine mammals	Energy Pollution Bycatch Driftness Catching Underwater noise pollution Vessel collisions Sonar Unregulated whale watchingactivities Invasive species Coastal development Prey depletion Agricoltural pesticides Antifouling paints Food resource depletion	Climate changes Hydrodynamic changes
Structure	Biocenosis	Habitat loss Pollution Physical damages Dredging Turbidity Eutrophication	Climate changes Hydrodynamic changes River inputs changes Submarine volcanic activity Violent sea storms Alloctone/Autochthone competition Invasive species Competition Migration/Adaptation processes
Number of nesting pairs	Birds	Habitat loss	Climate changes

		Damages to nesting Pollution	Competition/predation Availability of feeding resources
Reproductive success	Birds	Habitat loss Damages to nesting Pollution	Climate changes Competition/predation Availability of feeding resources
Age	Fish Pelagic and Benthic Fish	Overfishing Pollution Habitat loss	Protection of Nursery areas Climate changes Competition/predation Availability of feeding resources
Length	Fish Pelagic and Benthic Fish	Overfishing Pollution Habitat loss Eutrophication	Protection of Nursery areas Climate changes Competition/predation Availability of feeding resources
	Benthic decapods (macro- invertebrates)	Overfishing	Protection of nursery areas Climate change Connectivity
Relative Abundance	Fish Pelagic and Benthic Fish	Overfishing Pollution Habitat loss Eutrophication	Alloctone/Autochthone competition Invasive species
	Benthic macro- invertebrates	Overfishing Habitat loss	Protection of nursery areas Climate change Connectivity
	Phytoplankton	Eutrophication Temperature	Climate changes River inputs Sea storm
	Zoobenthos	Eutrophication Temperature Habitat loss Overfishing Pollution	Climate changes River inputs Invasive species Connectivity
	Zooplankton	Temperature Habitat loss	Climate changes Invasive species
Relative or Total Biomass	Fish Pelagic and Benthic Fish	Overfishing Pollution Habitat loss Eutrophication	Alloctone/Autochthone competition Invasive species
	Benthic decapods (macro- invertebrates)	Overfishing Habitat loss	Protection of nursery areas Climate change Connectivity
	Phytoplankton	Eutrophication Temperature	Climate changes River inputs Sea storm
	Zoobenthos	Eutrophication Temperature Habitat loss Overfishing Pollution	Climate changes River inputs Invasive species Connectivity
	Zooplankton	Temperature Habitat loss	Climate changes Food availability/predation
Sex	Fish Pelagic and Benthic Fish	Pollution Habitat loss Overfishing	
Species level taxonomy	Fish	Overfishing Pollution Habitat loss	Alloctone/Autochthone competition/predation Invasive species

			Climate changes Availability of feeding resources
	Phytobenthos	Turbidity Hydrodynamic changes Eutrophication Mechanic disruption	Alloctone/Autochthone competition Invasive species River inputs Climate changes
	Phytoplankton	Eutrophication Temperature	Climate changes River inputs Sea storm
	Zoobenthos	Eutrophication Temperature Habitat loss Overfishing Pollution	Alloctone/Autochthone competition Invasive species
	Zooplankton	Temperature Habitat loss	Climate changes Invasive species
Maturation Age	Pelagic and Benthic Fish	Overfishing Pollution Habitat loss	
	Benthic decapods (macro-invertebrates)	Overfishing	
Species biomass Substrate type Total Biomass Total Coverage	Phytobenthos	Turbidity Hydrodynamic changes Eutrophication Mechanic disruption	Alloctone/Autochthone competition Invasive species River inputs Climate changes

3.2. Descriptor D5 – Eutrophication

In this section, the knowledge on natural variability in terms of both spatial and temporal scales is detailed for each of the indicators considered for the Descriptor D5.

Variability of the environmental factors (nutrients, dissolved oxygen, transparency) and the related physical parameters (temperature, salinity, hydrological parameters, rivers' discharges, currents, waves and winds) and biological components of the systems (chlorophyll-*a*, changes in abundance, population structures, species composition – shift in species dominance, structure, etc.) represent a natural process that could significantly affect the data collected during monitoring of the marine ecosystems in relation to the indicators used for the Descriptor D5. Major sources of pressure and principal processes that could significantly affect the environmental factors for each indicator considered by the Descriptor D5, are listed in Table 5.

Table 5. Principal Pressures & Processes related to Environmental factors (D5).

Variable/Indicator	Major source of pressure	Processes
Dissolved Inorganic Phosphorous, orthophosphates	Rivers (freshwater input) Surface run-off (agriculture) Dumping of dredged material	Consumption and regeneration Sedimentation Release from sediments
Total Phosphorous, TP	WWTP	Regeneration
Oxidized Nitrogen concentrations, TNOx	Rivers Surface run-off (agriculture) Atmospheric deposition	Nitrogen fixation Denitrification and nitrification processes Volcanic emissions
Ammonium (NH_4)	WWTP Direct discharge Tourism	Regeneration Consumption Redox processes
Total Nitrogen (TN)	Rivers WWTP Aquaculture	Regeneration
Silicates (SiO_4)	Decreasing input from rivers	Consumption Regeneration Sedimentation
Dissolved oxygen	WWTP Industry (eg Desalination Temperature and Salinity)	Production and consumption Climate changes?
Transparency	Total Suspended Solids (TSS)	Cloudiness Light penetration
Colour	Nutrient enrichments Industry Pollution (i.e. oil spills)	Primary producers proliferations H_2S production (rare)
Turbidity	WWTP Industry Dredging or sediment resuspension	River inputs Sea storms Sedimentation
Temperature	WWTP Industry	Global Warming Processes Seasonal alternations

	Energy Production Processes	Underwater volcanic emissions
Salinity	Temperature alteration Desalination plants Rivers discharge	Global Warm Processes Season alternation Underwater volcanic emissions Global water circulation process Evaporation/condensation processes Freshwater inputs
pH	WWTP Industry Increased atmospheric CO_2 concentrations	Carbonate/Bicarbonates equilibrium Primary producers
Alcalinity	WWTP Industry	Carbonate/Bicarbonates equilibrium Primary producers

Note: For DO and Transparency two different components of pressure have been identifed: direct pressure – discharges (organic matter, total suspended solids, etc.) and indirect pressure due to nutrient enrichment and phytoplankton proliferation (meaning also total suspended solids), etc.

In Table 6, indicators considered for the Descriptor D5, are linked to indicators considered forDescriptors D1 (Biological Diversity) and D6 (Seabed habitat integrity). In this case also, major sources of pressure and principal processes that could significantly affect indicators variability are listed concerning biological elements.

Table 6. Biological elements to be considered for the Descriptor D5.

Parameters	Major source of pressure	Process
Chlorophyll	Nutrients enrichment and changes in N/P, Si/N, Si/P ratios	Photosynthesis
Abundance of opportunistic macroalgae	Direct discharges (TSS) and human activities – dredging Tourism	Natural selection
Species shifts in floristic composition (Diatoms/Flegellates, Benthic/Pelagic)	Changes in N/P, Si/N, Si/P ratios Temperature	Competition Natural selection
Harmful algal blooms	Nutrients enrichment and Temperature Human activities?	Reproductive strategy
Abundance of seagrasses and seaweeds	Direct discharges (TSS) and human activities – dredging Tourism	Competition Habitat changes

4. SCALING MONITORING TO NATURAL VARIABILITY

Coastal ecosystem dynamics are structured across different scale levels. On one hand, the realized habitat state represents a subset of the natural variability on large scale. On the other hand, large, long term trends are the cumulative result of smaller, faster changes happening at a finer scale. While some relevant factors of influence/stressors/pressures are scale-specific, others have cross-scale effect. Emergent scale-level properties contribute in adding complexity to the general frame.

Many evidence from the past few decades indicate that neither completely local-level management nor fully higher-level management works well by itself. The partitioning of the natural variability across and within scales determines a need to design and support management institutions at more than one level, with attention to interactions across scale from the local level up (Berkes, 2002). Environmental governance structures are also nested. Local/regional governance usually focuses on local interests and short-medium term objectives, while national or communitarian politics must integrate different needs and can operate on medium-long-term perspectives. Local institutions generally have a different way to collect and use knowledge with respect to centralized ones. Additionally, the former tend to use their own folk knowledge whereas centralized management agencies tend to use internationally accepted scientific practice and often assume away local knowledge and practice. There is a need for tools to enable common strategies for researchers to deal both with the people and the environment as an integrated system. A general feature that has to be faced to choose the opportune spatial and temporal scales is that all of them must be considered for interpreting results as well as the sampling efforts and data collection has to be able to reduce cost and optimize results. The parallelism between the hierarchical organization of jurisdictional units (city-district-region-state-UE)-institutional arrangements (regional, national, EU directives) and hierarchical organization of ecosystem processes can be a fruitful path for the implementation of an efficient cross-scale monitoring. In fact, the selection of the optimal spatial and temporal scale to perform monitoring has to fit with the need to optimize cost and benefits, minimizing sampling efforts that are time consuming and of economic loss. However, jurisdictional boundaries rarely coincide with ecosystem boundaries. Thus, cross-scale institutions are also needed. The fact that there is often a mismatch in scale between institutions and ecosystems is considered part of the reason for resource mismanagement (Folke et al., 1998). Cross-scale institutional linkages are needed to provide integration. Self-organization and adaptive capacity in monitoring plans (Berkes, 2002) should be considered. Due to the fact that the Marine borderline is usually in the order of several hundreds of kilometres and the temporal horizon for evaluation is every 6 years, the optimal spatial scale for evaluating the GES and for the definition of the basic reference scales for MSFD would be the meso-scale, having the temporal scales being annual. Obviously, regarding time scales, for monitoring design and a proper interpretation of result, smaller and larger time scales must be specially considered, mainly intra-annual seasonal variations and multi-decadal oscillations. In relation to the spatial aspects, the high habitat heterogeneity in Mediterranean Sea oblige us to adapt the sampling to the scale of the main target habitats, which is much smaller than that of the marine demarcations, going down at least to the

coarse-scale (1-10 km) and from there integrate results for a global evaluation at meso-large scale. Concerning Descriptor D5, spatial scales of natural variability for indicators are summarized in Table 7. For each indicator principal processes and drivers responsible of variability are listed. Both horizontal and vertical scales of variations were analyzed. In Table 8 temporal scales of natural variability for indicators related to the Descriptor D5 are summarized. For each indicators' the principal process responsible for variability, is listed. Both horizontal and vertical scales of variations were analyzed. Horizontal variability is defined concerning three different temporal variability levels: small (months), intermediate (seasons) and large scales (decades) while vertical variability is analyzed concerning shelf and offshore water. The effect of each process and driver is defined for different scales in three classes of intensity: Low (L), Medium (M), and High (H).

Table 7. Natural variability, spatial scales for D5 indicators.

Parameter	Processes	Driver	Spatial scale				
			Horizontal			Vertical	
			Small (water body, <10^1 km)	Intermediate scale (subregional, 10^1-10^2 km)	Large scale (Biogeographic region, 10^2-10^3 km)	Shelf	Off-shore
Nutrients	Consumption and regeneration	Biological activity intensity	H	H	L	H	M
	Sedimentation	Temperature, winds and currents regime (Stratified waters)	H	H	L	H	M
	Release from sediments	Oxygen deficiency Mixing phenomena	H	M	M	M	H
	Redox processes	Dissolved Oxygen content	H	M	L	H	M
Dissolved oxygen	Production and consumption	Light availability	M	H	L	H	H
	Climate changes	Temperature changes	H	H	H	H	H
Transparency	Cloudiness, Light penetration	Climate	H	M	M	H	H
	Turbidity	Sedimentary inputs	H	M	L	H	M
Color	Primary producers proliferations	Nutrients enrichment	H	H	M	H	M
	Suspended solids	Sedimentary inputs	H	H	M	H	M
Turbidity	River inputs Sedimentation	Sedimentary inputs	H	H	M	H	M
	Sea storms	Climate	H	H	H	H	H
Temperature	Global Warm Processes	Climate Sea/fresh water influence	H	H	H	H	M
	Season alternation	Seasonal weather	H	H	M	H	H
	Underwater volcanic emissions	Geology	H	L	L	H	H
Salinity	Global Warm Processes Season alternation Underwater volcanic emissions Global water circulation process Evaporation/condensation processes Freshwater inputs	Geomorphology Hydrodynamics Seasonal weather	H	H	M	H	H
pH	Carbonate/Bicarbonates equilibrium	Biological activity	M	H	H	H	H

	Primary producers	Climate changes					
Alkalinity	Carbonate/Bicarbonates equilibrium Primary producers	Biological activity Climate changes	M	H	H	H	H

Table 8. **Natural variability, temporal scales for D5 indicators.**

Parameter	Process	Driver	Temporal scale				
			Horizontal			Vertical	
			Small (Months, 10^{-2}-10^{-1} years)	Intermediate scale (Season, 10^{-1}-10^{0} years)	Large scale (Decades, 10^{1}-10^{2} years)	Shelf	Off-shore
Nutrients	Consumption and regeneration	Biological activity intensity	H	H	H	H	M
	Sedimentation	Temperature, winds and currents regime (Stratified waters)	L	L	H	H	M
	Release from sediments	Oxygen deficiency Mixing phenomena	L	M	H	M	H
	Redox processes	Dissolved Oxygen content	M	H	L	H	M
Dissolved oxygen	Production and consumption	Light availability	M	H	L	H	H
	Climate changes	Temperature changes	H	H	H	H	H
Transparency	Cloudiness, Light penetration	Climate	M	H	H	H	H
	Turbidity	Sedimentary inputs	H	L	L	H	M
Color	Primary producers proliferations	Nutrients enrichment	H	H	M	H	M
	Suspended solids	Sedimentary inputs	H	H	M	H	M
Turbidity	River inputs Sedimentation	Sedimentary inputs	H	H	M	H	M
	Sea storms	Climate	H	H	H	H	H
Temperature	Global Warming Processes	Climate Sea/fresh water influence	H	H	M	H	M
	Season alternation	Seasonal weather	H	H	M	H	H
	Underwater volcanic emissions	Geology	M	L	H	H	H
Salinity	Global Warming Processes Season alternation Underwater volcanic emissions Global water circulation process Evaporation/condensation processes Freshwater inputs	Geomorphology Hydrodynamics Seasonal weather	H	H	M	H	H
pH	Carbonate/Bicarbonates equilibrium Primary producers	Biological activity Climate changes	M	M	H	H	H
Alkalinity	Carbonate/Bicarbonates equilibrium Primary producers	Biological activity Climate changes	M	M	H	H	H

Concerning monitoring of the **benthic communities** at littoral areas, sampling in most cases concerning effect on species needs to integrate natural variability along with several factors such as habitat type, depth, season, light attenuation, temperature, and nutrient uploads. Many environmental and anthropogenic stressors are affecting Mediterranean coastal communities and ecosystems. Several studies have demonstrated high inter-annual variability associated with invasive species (Box et al. 2010a,b; Vazquez-Luis et al., 2014a) and coastal fishes associated to seagrass beds (Deudero et al., 2008). Not only changes at biodiversity level should be considered, but also shifts in functional aspects of the ecosystems. In this matter, examples of functional changes at food webs induced by invasive macroalgae have been demonstrated (Deudero et al., 2011; 2014). Key-sessile species are especially vulnerable to those human impacts, and therefore, monitoring should address pressures associated to species survival and conditioning spatial distribution (Vazquez-Luis et al., 2014b).

Concerning **water masses and the planktonic communities**, an attempt to scale a variety of ocean processes along with rough coverage domains of various oceanographic platforms in spatial and temporal scales of concern, was performed by Dickey and Bidigare (2005) as shown in Figure 5.

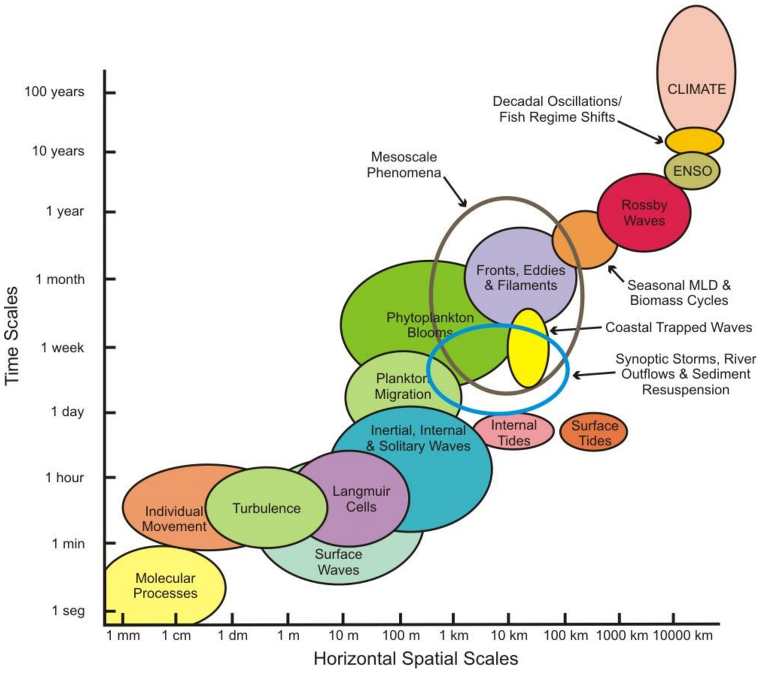

Figure 5. Time and horizontal space plot indicating a variety of ocean processes (top) along with rough coverage domains of various oceanographic platforms (bottom). The arrows on the figure are intended to draw attention to the cascade of energy and information.

5. INTEGRATION OF DESCRIPTORS

A technical guidance on integration across descriptors, indicators and variables including examples and direct experiences from other European projects such as PERSEUS FP7 is given in this Chapter.

Two different practical examples to highlight how different elements of biodiversity could be integrated is reported. The first example is referred to the construction of the biological evaluation maps, and the second one refers to a method of integrative assessment of biodiversity based on spatial scale rating approach.

5.1. Phytoplankton blooms assessment based on remotely sensed ocean color data

Phytoplankton blooms play a central role as ecological/environmental status assessment traits of high policy importance *sensus* those of the Water Framework Directive and Marine Strategy Framework Directive. One of the main challenges in their practical application however is the need of data with frequency corresponding to the natural spatial and temporal scales of phytoplankton variability.

The majority of *in situ* observations that are commonly used for ecological monitoring of the Black Sea, as an examble, are generally based on near-shore monitoring programmes or irregular oceanographic cruises that provide either non-synoptic, coarse resolution realizations of large scale processes or detailed, but time and site specific snapshots of local features. Sixteen years (1998-2013) of remotely sensed ocean color data were used to assess the interannual dynamics of spring and summer phytoplankton blooms (PBs) in two distinct regions (shelf < 200 m and open sea > 200 m) off the Bulgarian Black Sea waters (Slabakova et al., 2014), based on the constant threshold method (Kim et al., 2009) to estimate the intensity, spatial extend and frequency of major and minor blooms in the spring and in the summer. The satellite data reveal an overall decreasing trend of chlorophyll-a concentrations, in conformity to *in situ* data (Figure 6).

A pronounced general trend of decreasing values chlorophyll-a was observed in the coastal and shelf habitats. However a sustained annual maximum as well as seasonal variability of values around or above the thresholds was recorded in coastal and shelf areas, while at the open sea, in the majority of the measurements, concentrations were above the threshold (MISIS SoE Report, 2014).

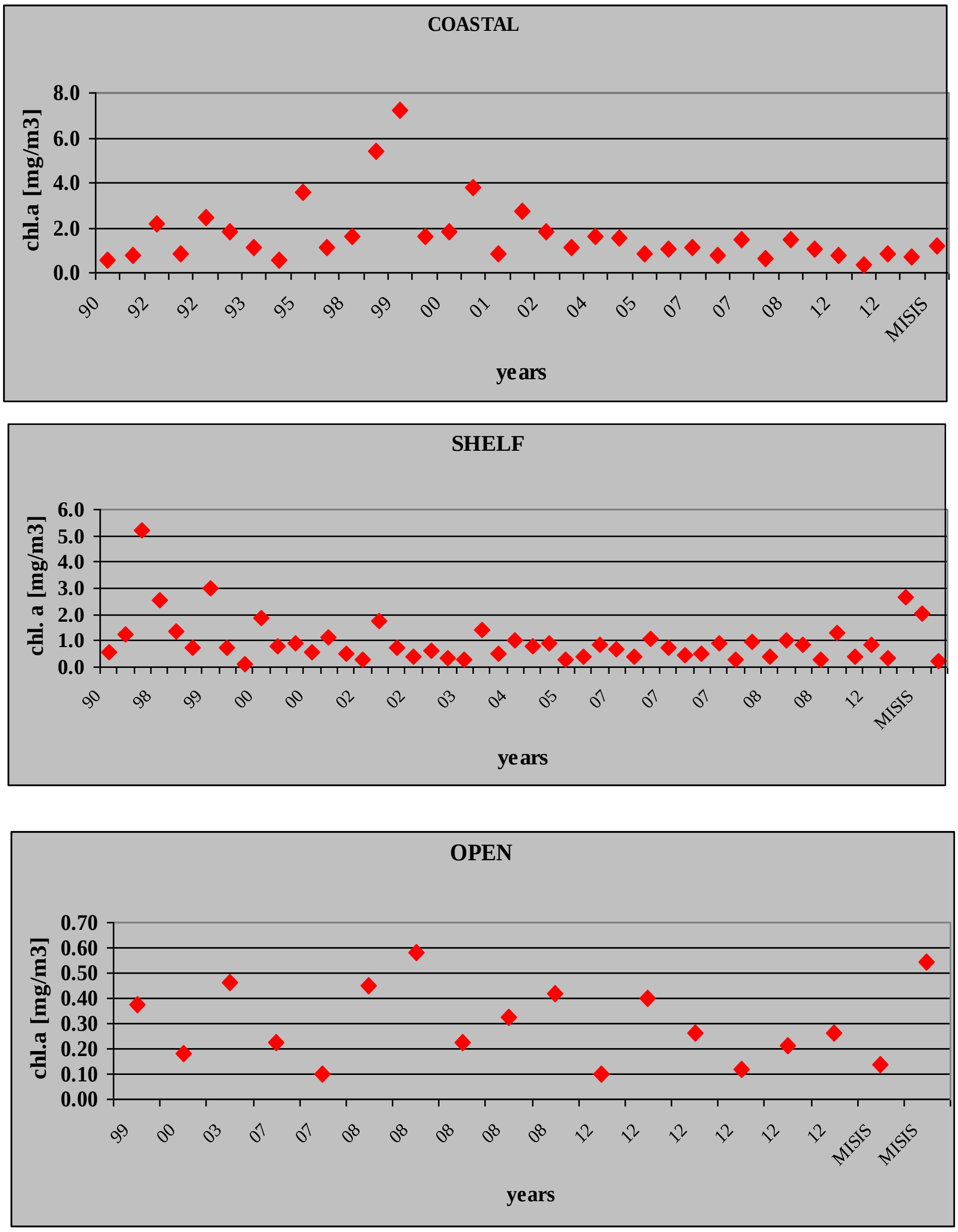

Figure 6. Long-term (1990-2012) variation of chlorophyll-a [mg/m^3] along the BG coastal, shelf and open sea habitats (Galata transect).

As expected, the *in situ* data sampled in different summer months and at different frequencies during the period 1990-2013 could not adequately capture the oscillations observed by the much higher frequency remote data (Figure 7 & Figure 8). The time evolution of PBs in the shelf was similar to that in the open sea with low intensity in summer and high in spring. The spring PBs in the two regions were featured as strong and long lived, with a steady increasing trend of major blooms magnitude, while the summer blooms were weak and widespread (Figure 9 & Figure 10).

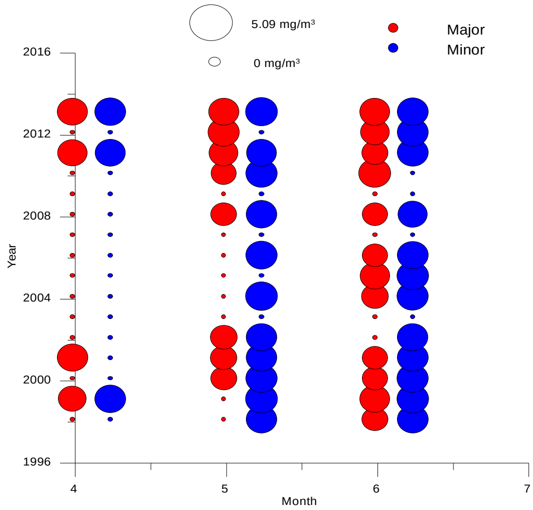

Figure 7. Monthly averaged spring PBs magnitude (mg/m3) in the shelf by years (Bulgarian Black Sea area, Slabakova et al., 2014).

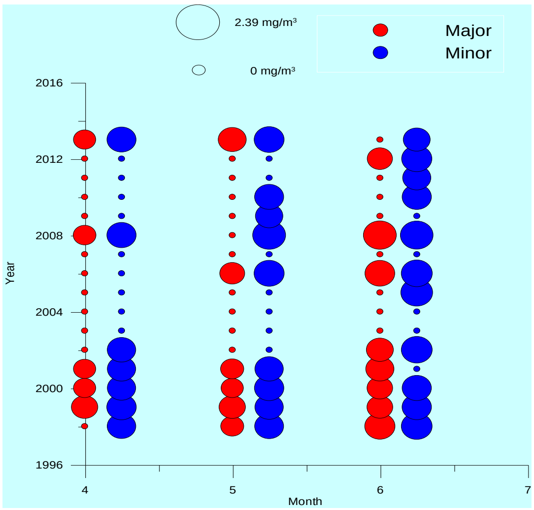

Figure 8. Monthly averaged spring PBs magnitude (mg/m3) at open sea by years (Bulgarian Black Sea area, Slabakova et al., 2014).

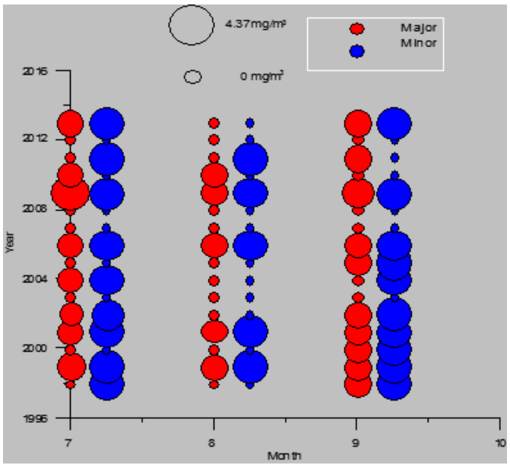

Figure 9. Monthly averaged summer PBs magnitude (mg/m3) in the shelf (A) and open sea (B) by years (Bulgarian Black Sea area, Slabakova et al., 2014)

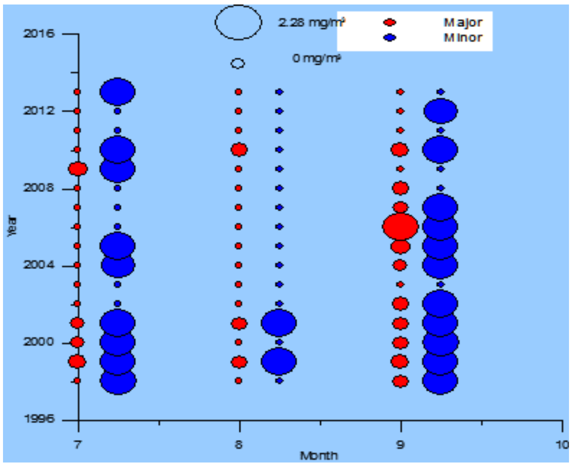

Figure 10. Monthly averaged summer PBS magnitude (mg/m3) in the shelf (A) and open sea (B) by years (Bulgarian Black Sea area, Slabakova et al., 2014)

5.2. Biological evaluation maps

Derous et al. (2007, referred in Prins et al., 2013) describe a method for constructing Biological evaluation maps that compile and summarize all available biological and ecological information

for a study area, and which allocate an overall biological value to subzones**.** This tool can be used to develop management strategies for sustainability and conservation of the marine environment, as well as baseline maps for future spatial planning at sea. However, integrated ecological information is needed. The marine biological evaluation is based on a literature review of existing evaluation criteria and the consensus reached by a discussion group of experts. Selected criteria include: a) first order criteria: aggregation, rarity and fitness consequences; b) modifying criteria: naturalness and proportional importance.

This methodology, apart from being a geographical scaling method, provides information for each of the components and their integrative evaluation, together with the reliability of the result, taking into account spatial and temporal data availability (Derous et al., 2007). Biodiversity evaluation maps aim to the compilation of all available biological and ecological information for a selected study area and allocate an integrated intrinsic biological value to the subzones (Derous et al., 2007). Thus it is also a method of indicators aggregation. Borja and colleagues (2011) acknowledge that this approach appears to have a conflict with the findings of the task group, which counseled against an integrated single assessment for this descriptor (Cochrane et al., 2010). However, they consider that the valuation approach can be a practical solution integrating large amounts of biodiversity information.

This is a scaling method of rating the results of each assessment unit according to the percentage or the length of the surface they cover in relation to the whole area. Then the results of each assessment unit are summed up in order to derivean assessment for the whole area.

5.3. Integrative assessment of biodiversity

Based on the Derous et al (2007) methodology Borja et al. (2011) developed a method of spatial scale rating presented for biodiversity (D1) in the Basque country, as reported in Figure 11.

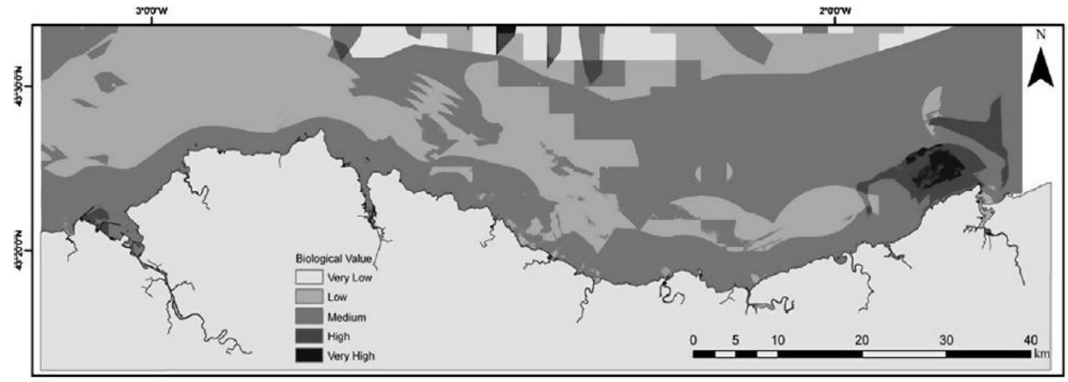

Fig. 2. Integrative biodiversity valuation of the Basque continental shelf.

Table 3
Integrating biodiversity valuation into a unique value, for the whole Basque continental shelf. Key: EQR: Ecological Quality Ratio.

	Very Low	Low	Medium	High + very high	Total
Equivalence (E)	1	2	3	4	
Percentage of the surface	6.9	36.0	53.3	3.8	100
Rate (per one) (R)	0.069	0.360	0.533	0.038	1.0
Total ($E \times R$)	0.069	0.719	1.599	0.150	**2.539**
EQR (Total −1/3)					**0.51**

Figure 11. Graph from Borja et al. (2011) showing the method of integrative assessment of biodiversity for the Basque country.

For the Basque Country, data on zooplankton, macroalgae, macroinvertebrates, demersal fishes, sea mammals and seabirds, for the period 2003–2009, and over the whole of the Basque continental shelf, were collated. Details on valuation methodology can be found in Derous and colleagues (2007). As a result a map (Figure 11) was constructed illustrating the spatial distribution of biodiversity quality classes in the study area. Then the percentages of each area that is classified to a certain equivalence value of ecological quality class were calculated. The percentages were then standardized to a scale from 0 to 1 and subsequently multiplied by the equivalence values of ecological quality. Results were summed up for all quality classes and the total sum converted to an EQR value for the whole assessment area.

5.4. Biodiversity & Seafloor integrity

In this paragraph an example of spatial variability according to the habitat types is reported, that focuses on the integration of macroinvertebrates indicators taking into account the spatial variance of the habitat types. Criterion 6.2 of the condition of benthic community under the Seafloor Integrity Descriptor D6 combines: biotic and diversity indices namely diversity and species richness.

The work of Simboura et al. (2012) highlights the spatial and temporal variance of diversity indices and their role in environmental status assessment.

According to Rice et al. (2012), communities with GES are those with a few abundant species and many rare ones. Such communities have high resilience potential to moderate pressures, simply because biodiversity buffers ecosystem processes and through them the ecosystem services that can be used sustainably.

The Shannon-Wiener diversity index, developed from the information theory, is one among the most widely used and tested in various environments.

However, except from disturbance, which affects diversity, the values of community diversity are influenced by the sample size, the sampling methodologies and the species identification procedures. Also seasonal natural variability, and habitat type influence diversity and species richness, which are therefore generally recommended to be used with caution as an ecological classification tool (Reiss and Kröncke, 2005; Salas et al., 2006).

The Shannon diversity index tested in the Mediterranean Ecoregion has been proved to respond not monotonically to pressure gradients (Subida et al., 2012).

The number of species in a benthic community also varies greatly with depth and sediment type. A typical trend exhibited within the Mediterranean is a significant decrease in species number with depth and with the relevant food availability, which may also play an important role on biodiversity levels.

Substratum type is the second most significant factor, after disturbance, influencing the species variety in a given biotope. Different communities (benthic assemblages in certain sediment type/depth) hold different species numbers and it is well established in benthic ecology that the sediment composition and mainly the relative contribution of finer or coarser particles related to homogeneity/heterogeneity of substratum, diversity of microhabitats, retirement of food

resources etc. play an important role in benthic community composition and structure (Simboura et al., 2012).

The work of Simboura et al. (2005) gives a categorization of the habitat or community types, that correspond with the water body typology initially adopted by the WFD for the coastal waters (EC, 2003). This typology was later abandoned as during the inter-calibration exercise it was proved that biotic indices were not type specific (GIG, 2013). However, this typology is useful for assessing the spatial variability of these indices.

To assess the environmental status of the benthic communities and specifically the indicator 6.2.2. (multimetric indices), which combines biotic and diversity indices, the methodology described in Simboura et al. (2012) was followed. It was proposed also as a method for setting the targets and standards of indicator 6.2.2 in the initial assessment of Greece for the Determination of GES, submitted to EC by the Hellenic Ministry of the Environment.

This methodology was based on a biostatistical analysis of 625 benthic samples, taken throughout the Hellenic territory, and the relationship of benthic communities with environmental factors such as depth and type of substratum, which were statistically proved to account for the variance of Shannon diversity and species richness indices.

To test the significance and variation of factors other than disturbance in shaping the values of diversity and species richness, depth and substratum type at each sample data were categorized in pre-defined classes according to the basic benthic bionomic principles for the Mediterranean Sea (Pérès and Picard, 1964; Bellan Santini et al., 1994).

To investigate the variance of these indices in relation to the ecological status of the studied sites, benthic communities were classified into specific 'ecotypes' according to the combination of the type of substratum and depth. Four ecotypes (A, B, C, D) were statistically distinguished combining discrete environmental factor categories. Depth categories distinguished among coastal areas (shallower than 90 m or infralittoral and upper circalittoral zones) and shelf areas (deeper than 90 m, or lower circalittoral) and substrata categories distinguished among heterogeneous and homogeneous sediments.

Phase 1: Setting of reference values or threshold values for diversity indices

A methodology for defining threshold values for diversity indices for GES was applied in Simboura et al. (2012). A box-plot analysis of variance was run for each ecotype, in order to

test the significance of the variance of diversity and species richness among different ecological status classes. In cases of statistically significant differences among the classes of good and moderate, the critical class boundary values were identified as the indicator's (Shannon diversity and species richness) threshold values (Table 9).

Furthermore, as shown on a subsequent elaboration on the issue of setting threshold and reference values for diversity indices and using them for GES assessment, the variance of the diversity indices canbe expressed as Ecological Quality Ratios (EQR) values after setting reference values for each ecotype (Simboura et al., 2014).

For the calculation EQRs for H and S, the reference values for each ecotype were set as:

- ecotype A (coastal muddy) and C (deeper than 90m, muddy): S=40 and H=5,
- ecotype B (coastal, mixed): S=100, H=6.

These values refer to the standard sampling surface for benthos (0.1m^2) (UNEP/MAP, 2004). These reference values were derived from maximum values encountered over the Hellenic seas (Simboura et al., 2012; UNEP/MAP, 2004). Shannon index reference values correspond to the maximum possible diversity for the maximum richness found in certain ecotype data calculated as: $Hmax=\log_2(Smax)$.

Table 9. Reference values and GES thresholds for Diversity and Species Richness for the Mediterranean communities-ecotypes.

	H reference value	H GES Threshold	S reference value	S GES Threshold
ECOTYPE B Infra, upper circa mixed	6	4.5	100	40
ECOTYPE C Lower circa, muds	5	More data needed	40	More data needed
BATHYAL	4.4		30	

Phase 2: Integration of indicators

After setting the thresholds or reference values and Ecological Quality Ratios (EQRs) for diversity indices, an integrative assessment of the condition of benthic communities according

to the MSFD including biotic and diversity indices, is possible. The integration may follow different rules as assessed in Prins et al. (2012).

One of these rules is the Conditional rule followed in Simboura et al. (2012). According to this study, the Bentix index results corresponding to indicators 6.2.1 and 6.2.2 and the diversity and species richness indicators of 6.2.2. were integrated by following a conditional rule of 'at least two indicators meet the standard' or 'pass the threshold'.

Other methods of integration have been applied later by Simboura et al. (2014), integrating indicators for Seafloor Integrity after proposing reference values and boundaries for Diversity indices for the Eastern Mediterranean. It is noted that following this approach, threshold values for GES may be different from the previous approach followed in Simboura et al. (2012), but this approach offers the advantage of a full-scale classification based on Diversity indices.

This method of integration of indicators (within a descriptor) is an adaptation of a similar method applied across MSFD descriptors in the Basque country (Borja et al., 2011).

Borja and colleagues (2011) developed this method for a cross-descriptor aggregation; combining the 11 Descriptors of MSFD based on the WFD, HELCOM and OSPAR experiences. An EQR was calculated for each indicator of the various MSFD Descriptors, with the EQR for the whole descriptor being the average value of the EQR of the indicators. Then, by multiplying the EQR with the percent weight assigned to each descriptor (and summing up to 100), an overall environmental status value was derived.

This scheme is included among the integration schemes suggested in the context of the MSFD assessment and compiled in Prins et al. (2013) and in Borja et al. (in press) (Figure 13**).**

Qualitative descriptors	Explanation of the indicators used	Reference condi-tions/EQS	Recent trend	Reliability (%)	Weight (%)	EQR	Final environmental status	Final confidence ratio
Biological diversity	Integrated biological value		NA	69	15	0.51	0.08	10.35
Non-indigenous species	Ratio non-indigenous sp.	OSPAR	▲	80	10	0.98	0.10	8
Exploited fish and shellfish			▼	100	15	0.48	0.07	15
	Fishing mortality<reference			100		0.18		
	Spawning stock<reference			100		0.67		
	% large fish			100		0.59		
Marine food webs			▼	70	10	0.40	0.04	7
Human induced eutrophication		WFD	▼	94	10	0.96	0.10	9.4
	Nutrients in good status			100		0.80		
	Chlorophyll in high status			100		1.00		
	Optical properties in high status			100		1.00		
	Bloom frequency in high status			70		1.00		
	Oxygen in high status			100		1.00		
Seafloor integrity		WFD	►	100	10	0.89	0.09	10
	Area not affected			100		0.87		
	% presence sensitive sp.			100		0.98		
	Mean M-AMBI value			100		0.83		
Alteration of hydrographical conditions			►	100	2	1.00	0.02	2
Concentrations of contaminants	High % of sample <EQS Values are 30% of the most	WFD	▼	100	9	0.80	0.07	9
Contaminants in fish and other seafood	affected in the NEA Values are 50% of the most	WFD	▼	30	9	0.60	0.05	2.7
Marine litter	affected in Europe	OSPAR	▲	30	5	0.57	0.03	1.5
Energy and underwater noise	Moderate ship activity	OSPAR	NA	10	5	0.70	0.04	0.5
Final assessment					100		0.68 Good	75.5 High

Figure 12. Example of an assessment of the environmental status, within the Marine Strategy Framework Directive, in the Basque Country offshore waters (Bay of Biscay) (modified from Borja et al., 2011 from Prins et al., 2013)

<u>Notes:</u> EQS: Environmental Quality Standards; EQR: Ecological Quality Ratio, both based on the Water Framework Directive (WFD); NA: not available. Trends: red color, negative; green color, positive (in both cases can be increasing/decreasing, depending on the indicator).

The scheme proposed here follows a similar approach, since it is based on a modular formula assigning weighting scores to each one of the components: one "biotic" component, two "diversity" components and one "size" component if available, corresponding to the indicators 6.2.3 and 6.2.4. Each component in the formula is expressed by EQR, weighted accordingly, and the sum of all weighted values correspond to the final Environmental Status.

The weighting scores were selected taking into account: a) that the Ecological Quality Status (EQS) within the WFD and the Environmental Status (ES) within the MSFD should be harmonized and the two Directives should be fully and seamlessly integrated (Borja et al., 2010); b) the conclusion that at least in the Mediterranean Sea the Shannon diversity shows a non monotonic response to pressure gradients and that the biotic indices are more efficient to assess the EQS (Subida et al., 2012); c) the species richness is a highly variable indicator and shows a less significant correlation with EQS than the Shannon index (Simboura et al., 2012).

The derived formula is:

EQR = (0,6)*BIOTIC+0,2*SHANNON+0,1*SPECIES+(0,1)*SIZE

The final Environmental status (ES) is expressed as an EQR value and classified according to a standard scale:

1=high; 0.8=good; 0.6=moderate; 0.4=poor; 0-bad.

This method has been developed under PERSEUS FP7 project. In this study the above formula has been applied in two study areas in the Aegean Sea (Saronikos gulf and Limnos offshore area) to assess benthic community condition. The benthic indices applied as components in the EQR formula included the species richness index (S) calculated as average per standard surface unit (0.1 m^2), the Shannon-Wiener diversity index H' (Shannon and Weaver, 1963), the Bentix index (Simboura and Zenetos, 2002) (http://www.hcmr.gr) and the size spectra index ISD (Reizopoulou and Nicolaidou, 2007).

The ISD index represents the indicators 6.2.3 and 6.2.4 for the size spectra and size proportions of benthic communities. It is an index tested in transitional waters and now is applied also in marine waters. For the determination of the ISD the individual body size was expressed as body weight (mg). Individual body weight of the animals was obtained after drying at 60 °C for 48 h and weighing at the 0.0001 g level. The polychaetes were removed from their tubes and mollusc shells were dissolved with dilute hydrochloric acid prior to biomass determination. To

examine the distribution of individuals per geometric size classes (class I = 0.1 mg, class II = 0.2–0.3 mg, class III = 0.4–0.7 mg, ... class XII = 204.8–409.5 mg), histograms were plotted presenting the percentage of individuals belonging to each geometric size class for each station. For every size-distribution set, a skewness value was calculated and the ISD classification scheme was produced, by plotting the whole series of the obtained skewness values (Reizopoulou and Nicolaidou, 2007).

Results from the cross-descriptor aggregation formula in the coastal area (Saronikos gulf) showed a very good agreement with the biotic index results, but it seems that in the bathyal and naturally stressed area (Limnos isl.) the size indicator ISD index has a special advantage.

Bathymetric gradients are associated with increasing pressure and decreasing food availability in the deeper sediments, and thus benthic populations develop adaptive mechanisms to extreme conditions. Adaptations may occur at physiological and structural organization levels, thus further investigation is needed in order to incorporate the effect of natural variability in functional indices based on body-size spectra such as ISD.

Results showed that the integrated index succeed in detecting the natural stress. The ISD index appeared not to be affected much by natural stress as seen by the fact that it renders good status in most naturally stressed sites, and only in excessive stress (deepest bathyal station) it renders a borderline moderate status. However it must be taken in account that is an index developed and applied in transitional water systems, where a strong natural stress is the governing factor. In these systems the strong natural (temporal and spatial) variability often overlap the anthropogenic disturbance and indices are expected to demonstrate human disturbance and not natural stress. A slight modification (reduction) of its good to moderate boundary could also incorporate extreme cases of natural stress. On the other hand, the biotic index Bentix is affected by natural stress and has lower values than the typical border between good and moderate classes, normally classifying naturally stressed stations in moderate status. To accommodate these cases of natural stress, the method is providing a modified adapted boundary (3 instead of 3.5). Regarding other biotic indices tested in the area, AMBI and its multivariate M-AMBI are similar to ISD and do not seem to be affected much by natural stress, not changing status in naturally stressed stations.

Another option for integrating different elements, is the decision tree of Borja et al. (2004) (further updated in Borja et al., 2009), where it integrates biological elements are integrated

together with hydromorphological and physico-chemical elements (including pollutants) into a unique quality assessment within the Basque Country using only WFD elements (Figure 13**).** This decision tree (Borja et al., 2004; 2009) was applied with some modifications for integrating biological and physicochemical elements combining various WFD and MSFD biological and eutrophication indicators based on the results of the WFD monitoring in Hellenic coastal waters (Simboura et al., accepted). In this paper, data were also classified into ecotypes taking into account the variance of some indicators according to the habitat characteristics.

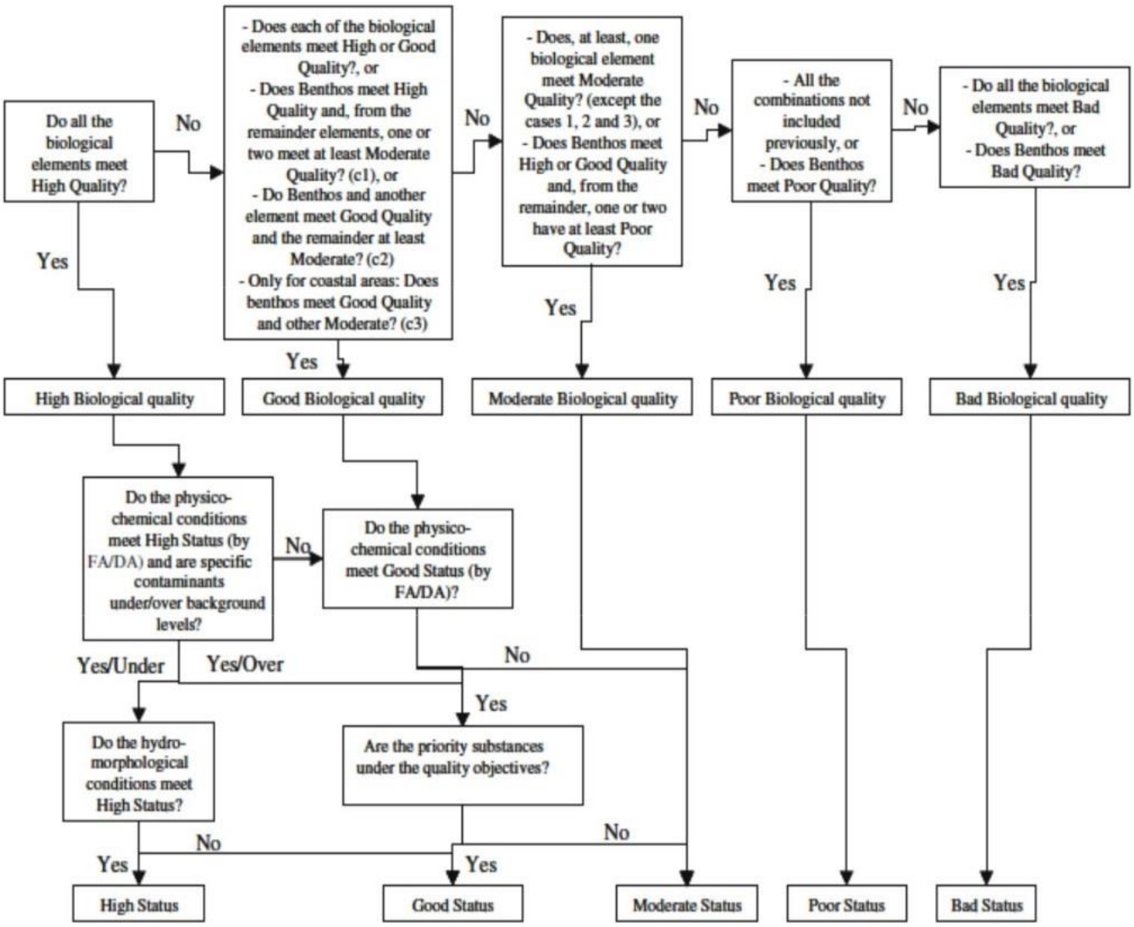

Figure 13. Decision tree to integrate ecological status from Borja et al., 2004.

5.5. Eutrophication & Seafloor integrity

This section presents an example of a case-study (based on the Romanian IA) on the eutrophication in the Black Sea. Black Sea has special natural features and a hydrographical basin six times higher than its surface. Therefore, it has been subjective to anthropogenic pressures and pollution sources (BSC, 2008). Until the 60s, the Black Sea was known like one of the most productive seas, with luxuriant pelagic fauna, being an example of natural eutrophic ecosystem due to permanent Danube's nutrients input. Unluckily, with anthropogenic activities enhancement, increased use of fertilizers, wastewater discharges, detergents, etc, nutrients regime has undergone significant changes. These changes were observed into the nutrients river input, which significantly increased (Cociaşu et al., 2008) and led to alterations in the North-Western Black Sea ecosystem. Thus, at the beginning of the 80s, phytoplankton has been developed excessively resulting into annual intense blooms having extended duration and frequency. During 1983-1988 more than 20 blooms took place due to 8 species, of which 5 (*Prorocentrum minimum, Skeletonema costatum, Skeletonema subsalsum, Eutreptia lanowii, Emiliania huxleyi*) presented the highest densities known until then at the Romanian coastal area. Mean phytoplanktonic biomass was 4,777 mg/m^3, 10 times more than that of 1959-1963. The algae species with more than 100,000 cells/L abundance increased to54 in the period of 1983-1988 from 34 in the 1960-1970. Since then, the effects of eutrophication had appear such as decreased transparency, organic matter decomposition, oxygen depletion with a seasonal hypoxic or even anoxic bottom. This effects have transformed the North Western part of the Black Sea into a highly eutrophic one. In the early 90s decreased nutrients inputs were found indicating the first recovery signs (decreased phytoplankton blooms, improvement of bottom oxygen regime, increased benthic macro fauna). Thereby, in 2005, the North Western part of the Black Sea seemed to be a highly disturbed ecosystem, but relatively functional. Malfunction symptoms, like incapacity of recycling high riverine atter input or biological activity or dominant monospecific phytoplankton blooms, were still evident. Black Sea coastal and shelf waters were still predominantly eutrophic (BSC, 2008). Recently, based on the IA (Lazar et al., 2013) the emphasized spatial and seasonal variability and the extreme phenomena inthe NW Black Sea coast makes the current eutrophication state definable as a moderate - good equivalent of a eutrophic - mesotrophic state, which under the action of climatic factors and a more pronounced human impact in the coastal zone, can easily convert to extreme states, unsatisfactory (hypertrophic) or very good (oligotrophic) conditions occasionally encountered

in the waters of the NW Black Sea, often seasonally.

Step 1: Determination of eutrophication causes: the spatial and temporal distribution of nutrients

Nutrients are chemical components involved in the photosynthetic production of the organic matter. Traditionally, the word was attributed to the inorganic phosphorus, nitrogen and silica compounds, but a great number of seawater major constituents and oligoelements are also nutrients. The actual assessment is based on phosphorus and nitrogen inventories, elements efficiently extracted from seawater which are included in the cells' composition, tissues and extracellular structures of the marine organisms. Some of them are several times regenerated in the water column, while some of them are settled. The nutrients flux is generally less efficient than gravity, thus their concentrations increase with depth. Data used for this step have been previously reported in section 3.3.2. of this Guideline.

Step 2: Direct effects of eutrophication

The distribution and dynamics of phytoplankton abundances show significant temporal and spatial variations, under the influence of natural and anthropogenic conditions (Figure 14). The dominant factors of these variations are represented by the Danube influence on the river mouth area or the effects of various anthropogenic pressures exerted on the shallow areas from the southern littoral.

In terms of numerical abundances, the northern coastal waters were the richest found under the direct influence of the Danube followed by the central zone. During almost all of the study period, the phytoplankton communities from the southern area were quantitatively much more reduced. Only in September, when the second abundancet peak was identified, the southern waters had communities as rich as those in the northern and central waters.

The pattern of temporal and spatial variation differs in such a way that spring is characterized by the poorest communities (generally mean abundances lower than 10^6 cells/L), excluding the years 1999, 2006 and 2007, with important blooms of diatoms *Skeletonema costatum*. The summer period is characterized by mean densities higher than those found in other seasons, recorded mainly in the northern zone but also in the central one, with outstanding changes in 1997, due to the diatoms with high mass growth potential such as *Cyclotella caspia* and *Chaetoceros socialis*. The autumn is characterized by numbers close to those of spring, with the lowest values of mean densities registered in 2000 (lower than $300 \cdot 10^3$ cells/L) in all sectors.

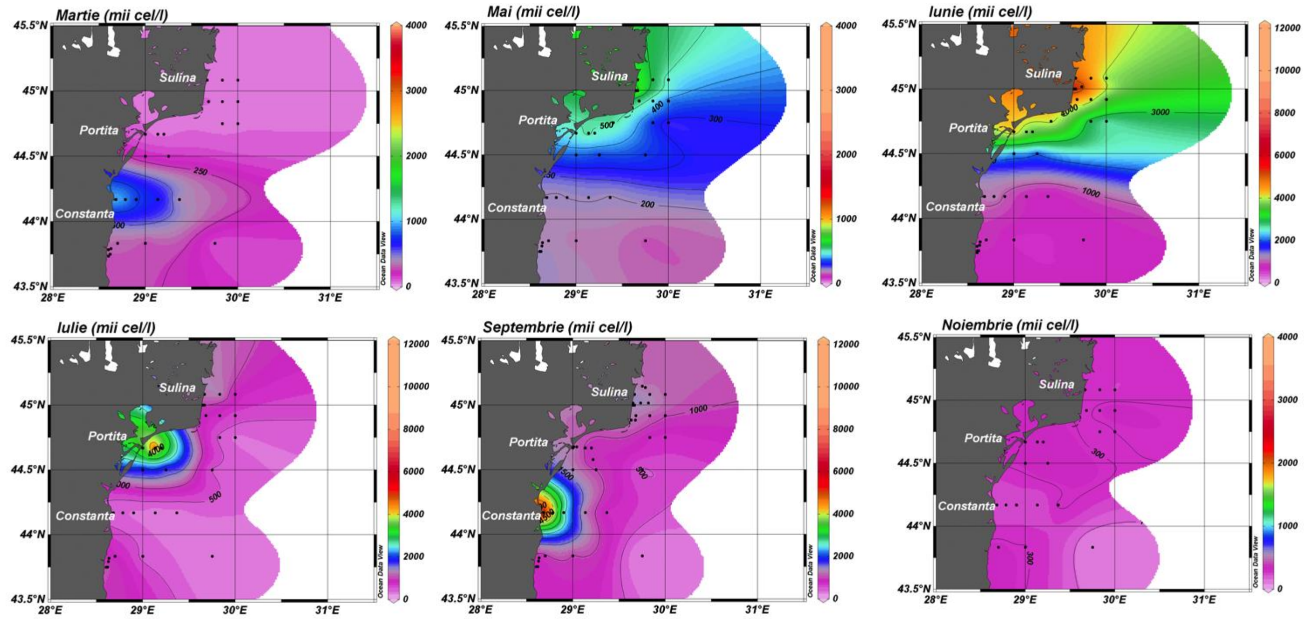

Figure 14. The distribution of mean monthly phytoplankton densities (thousands cells/L) from the Romanian littoral waters, during 1996-2007 period.

The vertical distribution recorded during the noted for 1996-2007 period confirmed the previous results. Generally, the largest abundances of phytoplankton were located in the sub-surface layer, up to a maximum depth of 20m; both the abundances and also the number of species were decreasing towards the deeper layers. Due to water masses movements, some exceptions from this pattern were found, for instance, at 30m depth abundances were found seven times higher than the mean values from the upper layers.

i) Phytoplankton blooms

For the Romanian waters, it was assumed that a bloom is an agglomeration of more than 1 million cells per liter. During the 1960-2007, when the observations on the evolution of bloom events in the Romanian Black Sea coast were performed, 73 species produced densities higher than 1 million cells per liter, with the highest number of blooming species belonging to Bacillariophytes (31), followed by Dinoflagellates and Cyanobacteria, having 12 and 13 species, respectively. On the other hand, six species out of the 73 specieshave produced such densities in each of the five decades (*Cyclotella caspia, Pseudonitzschia delicatissima, Nitzschia seriata, Skeletonema costatum, Leptocylindrus danicus, Prorocentrum minimum*), six occurred in four decades (*Skeletonema subsalsum, Chaetoceros socialis, Cerataulina pelagica,*

Heterocapsa triquetra, Emiliana huxleyi, Eutreptia lanowii), eight in three decades, 12 in two decades, and 43 in only one decade.

Only six species (*Cyclotella caspia, Skeletonema costatum, Prorocentrum minimum, Emiliana huxleyi, Eutreptia lanowii, Chromulina* sp.) achieved extremely high abundances, higher than 100 million cells per liter, all of them among 1981 and 1990, and thus this period considered as the most intense period of eutrophication. An exceptional situation represented by the small-sized species *Microcystis orae*, which produced two bursting blooms, higher than 200 million cells per liter, after 1991, recording the period when the pressure exerted by eutrophication began to decrease (Table 10).

Table 10. List of the phytoplankton species producing densities of more than 100 million cells /liter in the Black Sea Romanian coastal waters, during 1960-2007. Data are expressed as concentrations $*10^6$ cells/L.

SPECIES	1960-1970	1971-1980	1981-1990	1991-2000	2001-2007
Bacillariophyta					
Cyclotella caspia	28.072	26.490	**300.000**	10.460	78.600
Skeletonema costatum	18.080	97.360	**141.400**	34.170	37.280
Dinoflagellata					
Prorocentrum minimum	50.814	196.970	**807.600**	93.720	8.930
Cyanobacteria					
Microcystis orae				**204.750**	**271.950**
Chrysophyta					
Emiliania huxleyi		1.230	**291.200**	6.650	1.080
Chromulina sp.			**1.000.000**		
Euglenophyta					
Eutreptia lanowii		34.950	**108.000**	29.700	7.450

The bulk of the species responsible for the blooms appearances are those producing a high abundance only one season, with maximum populations during the summer months, even though some of them (*Cerataulina pelagica, Prorocentrum minimum, Nitzschia closterium*) are encountered throughout the year.

During spring, the cells of *Skeletonema costatum, Nitzschia seriata, Pseudonitzschia delicatissima, Heterocapsa triquetra* and *Dinobryon* sp. are abundant, but during autumn abundant are the species of *Cerataulina pelagica, Leptocylindrus minimus, Thalassionema nitzschioides*.

A number of species burst in two seasons: the freshwater diatoms *Skeletonema subsalsum, Stephanodiscus hantzschii* and the marine water species *Thallasiosira parva* and *Thallasiosira subsalina*, two forms of *Chaetoceros* – *C. socialis* f. *vernalis* and f. *autumnalis*, the cyanobacteria *Oscillatoria* sp. and the Euglenophyte *Eutreptia lanowii*.

Blooms of harmful algae (HABs) represent a particular case, which is also an indicator forMSFD. At the Black Sea Romanian littoral, most of the species causing harmful algal blooms (i.e. *Prorocentrum minimum, Cerataulina pelagica, Emiliana huxleyi* and *Pseudonitzschia delicatissima*) generate physical damages to the ecosystem, by reducing the oxygen concentration up to hypoxic or anoxic conditions, leading to mass mortality of benthic or nectonic organisms.

Another category of HABs involves species that produce toxins, such as domoic and okadaic acids, yessotoxin or azaspiracids, with toxic effects on marine organisms, as well as on humans through the consumption of contaminated organisms. In fact, in a list of toxin producing species prepared by IOC/IPHAB, a number of those exist in Romanian coastal waters too, like, *Amphora coffeaeformis*, *Pseudonitzschia delicatissima*, *Nitzschia pungens* var. *atlantica* and *N. seriata* (that are domoic acid producers), *Dinophysis acuminata, D. acuta* (okadaic acid producers), *D. caudata, Protoceratium reticulatum* (yessotoxin producers), and *Peridinium crassipes* (azaspiracid producers).

The Cyanobacteria produce two forms of toxins, namely neurotoxins and peptide hepatotoxins. The three genera of freshwater Cyanobacteria, reaching the Black Sea *via* the Danube waters, among them the *Microcystis*, *Anabaena* and *Oscillatoria*, produce toxins called microcystins. The species *Microcystis orae* is a benthic species that sometimes, after severe storms, can reach plankton, where, under favorable conditions (high temperatures, calm atmosphere), multiply up to huge abundances. It happened in the summer of 2001, in the waters off Constanta, when their density reached to $264 \cdot 10^6$ cells/L.

Some blooming microalgae species are harmful due to the production of large amounts of dimethyl-sulfoniopropionat, a metabolite that is found mainly in marine phytoplankton (*Emiliana huxleyi, Prorocentrum minimum, Scrippsiella trochoidea* etc.), but also in macrophytes, and in some species of terrestrial and aquatic vascular plants (Charlson, 1987, quoted by Moncheva and Krastev, 1997).

The fact that at the Black Sea Romanian coast, cases of poisoning of marine organisms or humans, resulting from algae toxins, have not yet being identified, is due to the reduced research

on this issue, and because post-mortem analysis of marine organisms is never carried out, while dead organisms thrown on Romanian beaches.

ii) Blooms produced by *Prorocentrum minimum*

P. minimum is one of the dominant forms in the Romanian littoral waters during the summer season, for 7 decades of the last century, resulting in remarkable blooms from 1962 to 1969. The ample increases of this species, in three consecutive years during the summer months (1974-1976), exceeded far beyond production of any other species, up to date.

In the decade 1980-1990, due to higher concentrations of nutrients and the organic matter quantity in the northwestern waters of the Black Sea, an increase in the frequency and magnitude of algal blooms, took place. Thus, 15 microalgae were involved in 46 monospecific blooms, having a variable magnitude and duration. One of the species reaching the exceptional densities recorded at the Romanian littoral till then (Bodeanu, 1992; 1993) was *P. minimum*, whose density attained $807.6 \cdot 10^6$ cells/L, on July 1987, and remains the highest value ever recorded by a species at the Romanian shore (Table 10).

After 1991, some species that bloomed in the past decade, are not found any longer in the list of the blooming species. A number of species that produce densities higher than $7 \cdot 10^6$ cells/L is reduced to seven, but five of them, among which *P. minimum*, had densities much lower than in the previous decade (Figure 15). The highest density of *P. minimum* reached only $93.7 \cdot 10^6$ cells/L, much below the productive potential demonstrated by these species until 1991 (Boicenco, 2010).

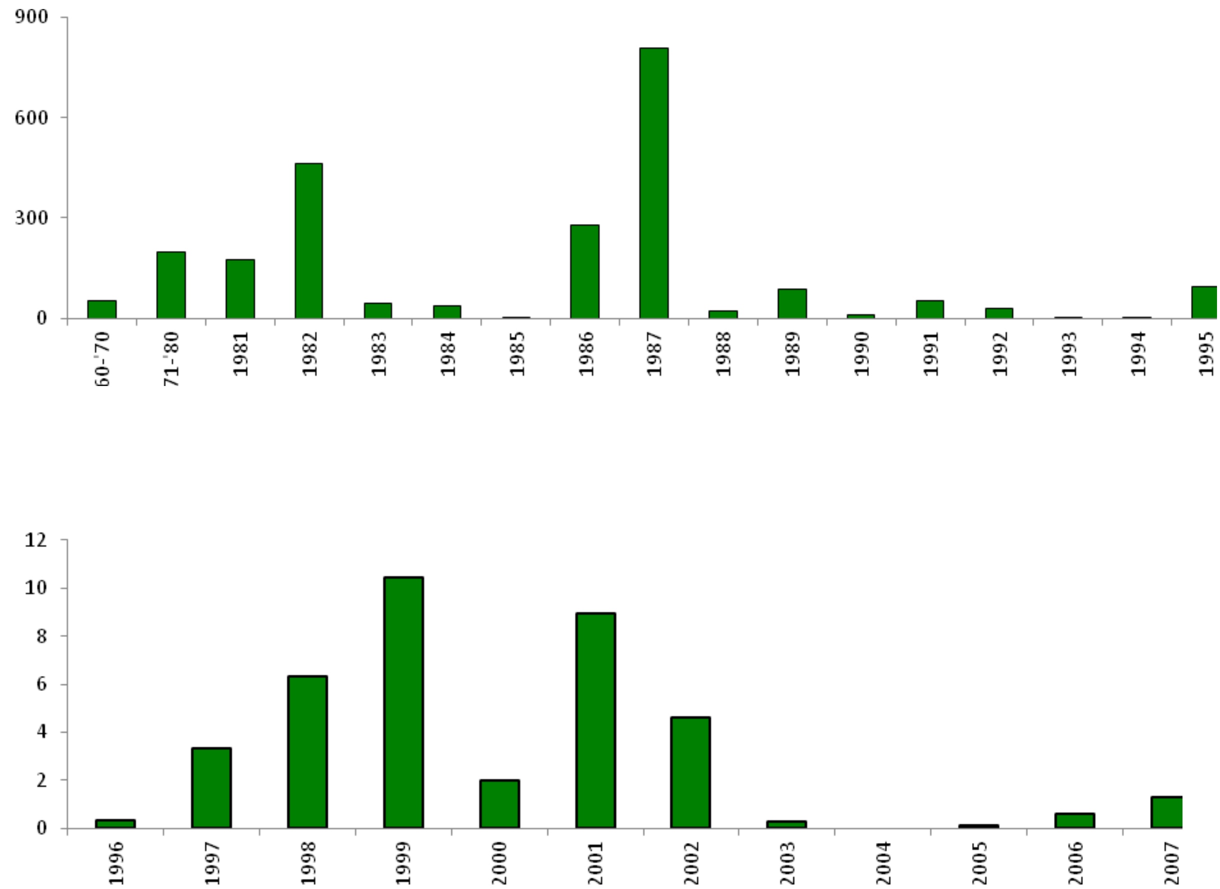

Figure 15. Multi-yearly evolution of maximum values recorded by Prorocentrum minimum, during 1960-2007. Data expressed as x106 cells/L.

In the period of 1996-2007, the *P. minimum*'s blooms did not exceed 10**X**10^6 cells/L, neither in the waters of Mamaia Bay or shelf waters. In each time, the species participated in mixed blooms, as dominant species or as companion species, unlike the monospecific blooms characteristic for the intense eutrophication period (Boicenco, 2010). The changes occurred in the phytoplankton community, in the years after 1994, and continued until today, were: reduced total phytoplankton abundances, decrease in the appearance of monospecific and mixed bloom events, and increase in the proportion of Diatoms in the phytoplankton communities. Comparatively with the earlier periods, the share of Dinoflagellates decreased, but can still reach up to 40% of total phytoplankton in the summer months. Compared with other sectors of the Black Sea, at the Romanian littoral, the Dinoflagellates reveal the highest developments, yet. These improvements appear to be fragile, since the exploitation of environmental resources (agriculture, fishing and sailing) increases with economic development in the post-soviet countries.

Step 3: Macroalgae and seagrass

i) Qualitative structure of macroalgal community

Algal vegetation is subject to human impact and is sensitive to unfavorable environment, which led to major changes over time in terms of macrophyte communities. Eutrophication deeply affects phytobenthos qualitative structure, since the substrate that remained unpopulated following the disappearance of perennial species, was later populated by opportunistic species with fast development cycle and with a wide ecological plasticity (genus *Ulva, Cladophora, Ceramium*).

Currently the number of macroalgae species identified at Romanian seaside is much lower than in the 60's (Figure 16), a decrease due to the synergistic action of natural factors (freezing, light deficiency, high levels of turbidity) and anthropogenic influence (cliffs arrangements, building dams, harbour excavations) (Bologa and Sava, 2006).

In recent years, however, there is a slight increase in the species number and a recovery of key species for the marine ecosystem (*Cystoseira barbata, Zostera noltii, Lomentaria clavellosa)*, but both qualitatively and quantitatively dominants remain the opportunistic species.

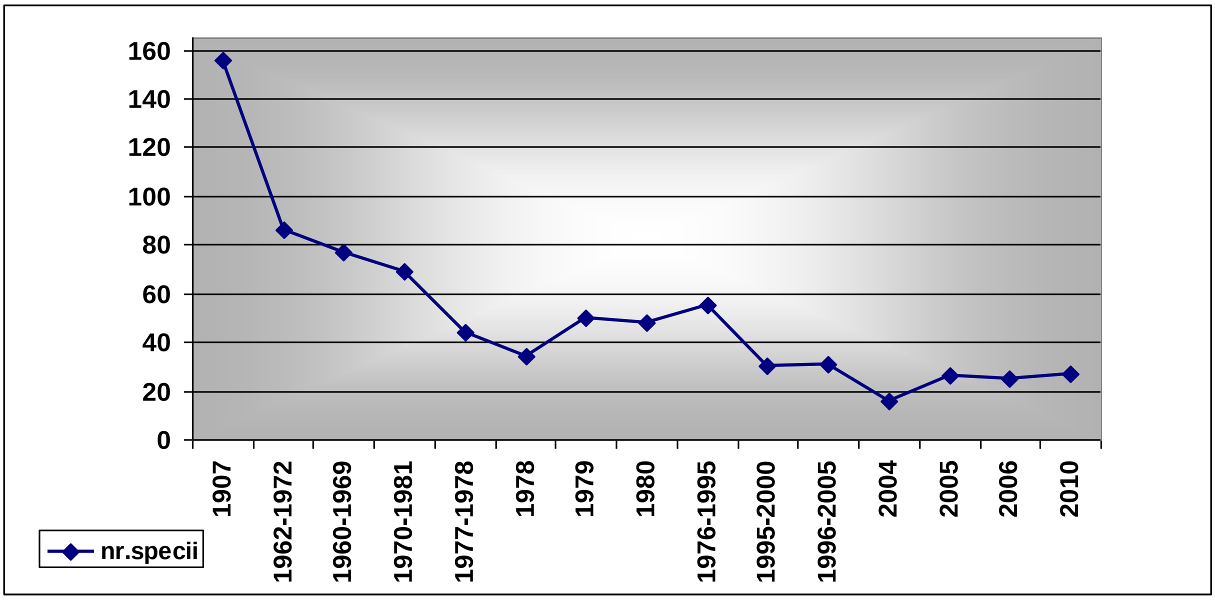

Figure 16. Phytobenthos qualitative evolution at the Romanian Black Sea coast over decades. Data are reported as number of species per year.

Currently, no one can refer on agglomerations of perennial algae of the genus *Phyllophora*, which formed considerable stocks in the past, but only few specimens scattered in the northern part of the Romanian seaside.

Also, perennial brown algae *Cystoseira* does not form fresh biomass estimated at several tons such as in the 60s, but is in the process of regeneration, currently being identified along the coastal strip Mangalia Vama Veche having high specimens forming well-developed fields with epiphyte flora and associated fauna, with high wet biomass developed by this species at depths of up to 3 m. In the 60s, *Cystoseira* was frequently reported in southern Romanian seaside points at depths up to 6 m (Bavaru, 1978). Species decline was largely due to sea freezing in the years 1971-1972, 1984-1985, when much of the population of *Cystoseira* (*C. barbata and C. bosphorica*) was affected (studies showed a destruction in 80% of the stock) (Figure 17).

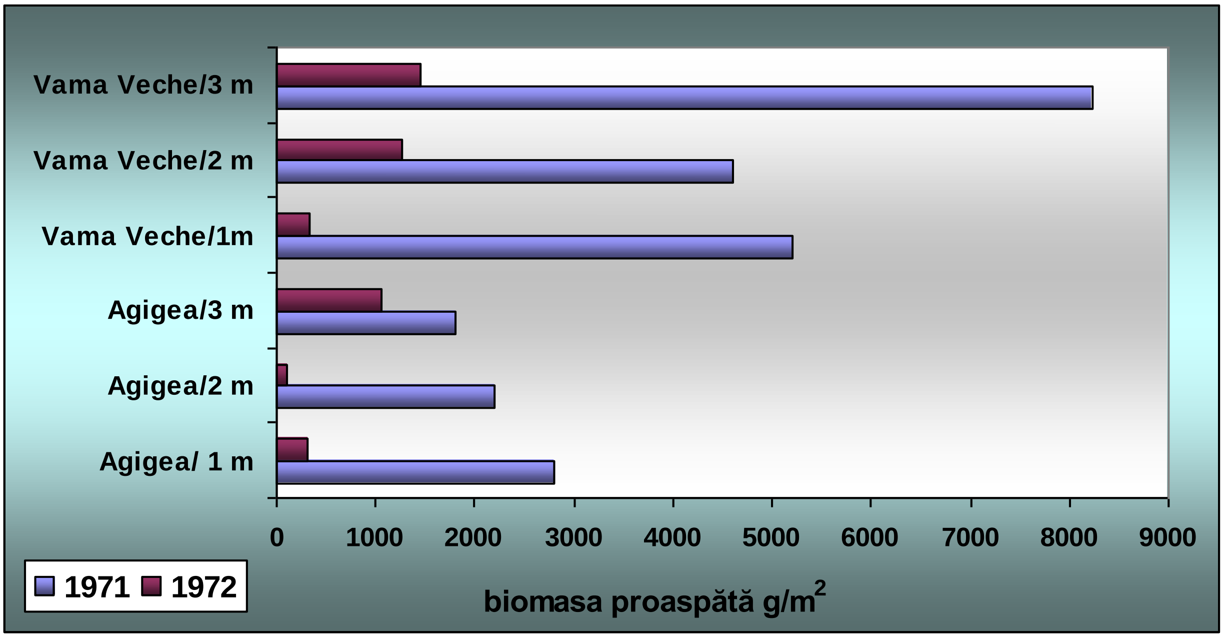

Figure 17. Decline of biomass of Cystoseira population due to sea freezing in the years 1971 – 1972.

Along the coastal strip Mangalia - Vama Veche there is a high productivity, mostly due to perennial species, good water oxygenation and higher specific diversity.

After applying the index EI-EQR (Ecological Quality Ratio) for Romanian seaside (index calculated for the Water Framework Directive), which aims to characterize the ecologically state of each station analyzed, it can be seen that the highest value of the index is at station 2 Mai, where it is considered to be at very good ecological status, followed by Mangalia and

Vama Veche, with a good ecological status. Restoration of the 2 key perennial species (*Cystoseira barbata* and *Zostera noltii*) for the marine ecosystem within these locations vs the lack of them from Eforie South and Costineşti determined that these areas have lower ecological status. In this area during summer only opportunistic species of the genus *Cladophora* are being present (Figure 18).

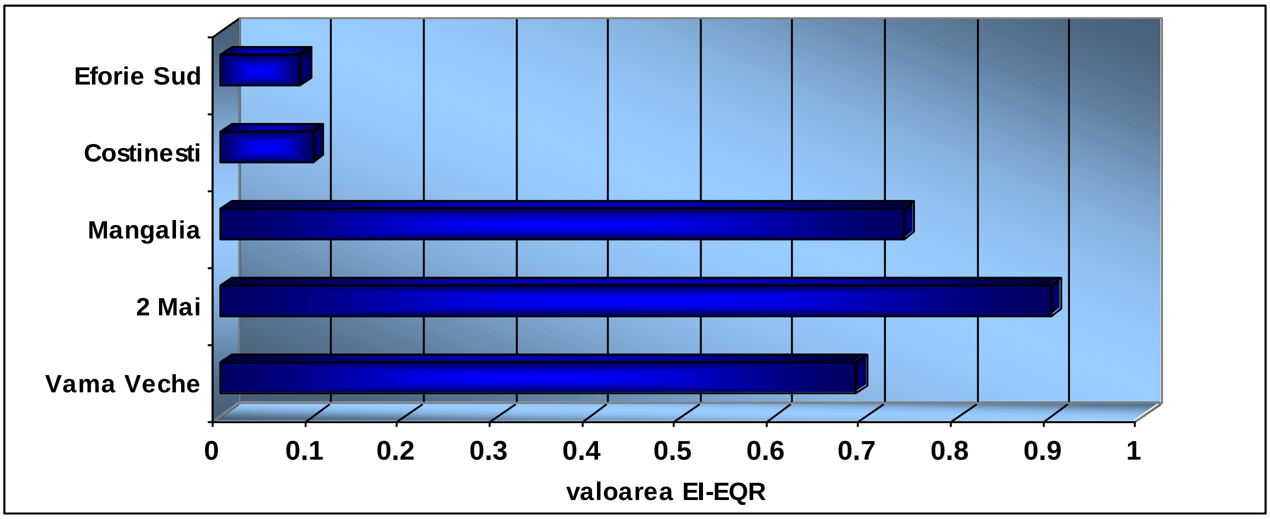

Figure 18. EI- EQR index value for the stations from the southern Romanian coast.

ii) Distribution of macroalgae - seasonal and annual variation

In the winter, brown algae are regularly present including the: genus *Ectocarpus* (*E. siliculosus* and *E. confervoides*), *Scytosiphon lomentaria* and *Punctaria latifolia*, *Porphyra leucosticta* (Rhodophyta) and *Ulothrix* and *Urospora penicillifomis* species (Chlorophyta). These species dominate the shallow hard substrate until early May, after some species as from genus *Scytosiphon, Ectocarpus* retreating to deeper waters where there are still favorable conditions for their development, namely a lower water temperature. During the warm season, green algae of the genus *Ulva* and *Cladophora* and *Chaetomorpha aerea* grows abundantly, and due to the waves and the storms they are separated from the substrate, and remain floating in the water. They periodically form thanatocoenosis - generating discomfort on the beach. Among red algae, the species that develop significant biomasses are *Ceramium* (*C. elegans, C. rubrum, C. diaphanum*), *Callithamnion corymbosum,* due to their ability to reproduce rapidly and their opportunistic character (Figure 19).

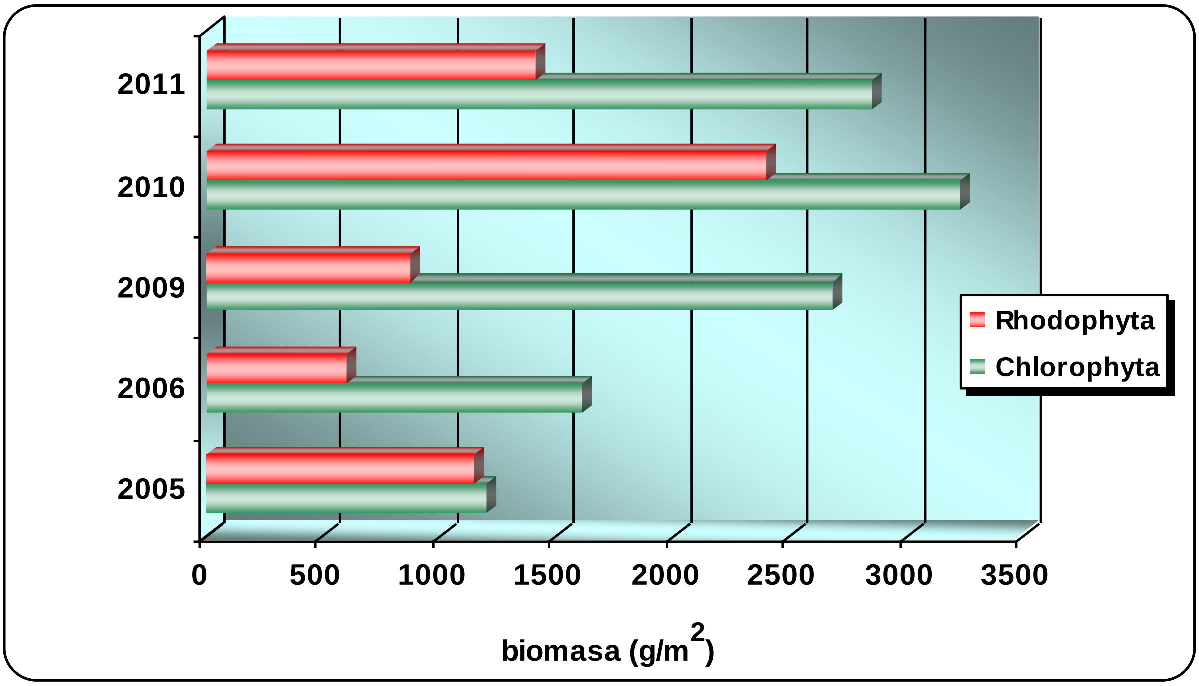

Figure 19. Dominance of opportunistic species in summer.

Drastic reduction of *Cystoseira* fields entailed the extinction of epiphytic species that used elastic substrate for their own development – genus *Kylinia*, *Sphacellaria cirrhosa, Feldmannia irregularis, Stilophora rhizoides, Corynophlaea umbellata, Cladostephus verticillatus*, *Dermatolithon cystoseirae* - which resulted in a higher degree of complexity of algal substrate, essential for fixing of diatoms epibionts and for harboring vagile, climbing or fixed fauna (Müller et al., 1969). Progressive impoverishment of *Cystoseira* fields resulted in fauna depletion, including ichthyofauna.

iii) Macroalgae and other cryptogame communities

The development of macroflora algal was affected by hydro technical works and construction of the new port of Constanta, where much of the stone surfaces were clogged, which reduced the area of perennial macrophytes *Cystoseira, Laurencia* and *Zostera*, especially in the south part of Constanta. Due to clogging and reduced water transparency (through phytoplankton blooms and increased suspension quantities), several species of macrophytae algae disappeared gradually from northern to southern coast (*Dasya pedicellata, Chondria tenuissima, Chondria dasyphylla, Phyllophora nervosa*) from rocky substrate, genus *Laurencia* (*L. pinnatifida, L. coronopus, L. obtusa, L. paniculata*) and a series of species of genus *Polysiphonia.* Hard

substrate clogging reduces populations of perennial species but eutrophication favored the rapid development of seasonal species and those with short life cycle. *Lomentaria clavellosa* considered extinct until it recently reappeared at the Romanian seaside at 2 Mai and Costineşti. In the past, this red algae had large dimensions and formed *Lomentaria clavellosa* – *Antithamnion cruciatum* association, which marked, at the Romanian coastal waters, a limit of development for fixed algal macrophyte vegetation and was enriched with seasonal elements, increasing specific diversity (Bavaru, 1978). Marine phanerogam *Zostera noltii* was reported in the early 2000 only as stranded species after storms in Mamaia resort, Eforie South and Agigea. Today it forms large meadows in Mangalia and northern coast at Năvodari. *Zostera noltii* is an important species for the marine ecosystems as it serves as a biotope for many species of invertebrates and fish, which find a place for feeding, reproduction and defense that also helps in fixing the substrate and improving the water quality. Perennial species *Corallina officinalis*, potentially used in the pharmaceutical industry due to its vermifuge properties, can be identified in Vama Veche attached to the hard substrate, where it is considered that the water is of high quality and allowed its restoration. Also perennial algae *Hildenbrandtia prototypus* was reported on the shallow rocks and shells of Rapana. Although the number of macroalgal species currently existing on the Romanian seaside does not compare to the quality before the onset of eutrophication, the restoration of certain species is a positive sign for the marine ecosystem and with time could lead to the recovery of the species that formed epiphytic flora associated to these key species.

Step 4: Indirect effects of eutrophication

Extreme phenomena signaled over the study period

During 2006-2011, the DO extreme values found in the marine waters, were the most stratified. Thus, the minimum, 69.2 µM, was determined at the end of the warm season at the station of Mangalia at 30m (depth 20m, September 2010). Following this hypoxic event fish mortalities were not reported. The maximum, of 732.9 µM, was found at Sulina at 10 m (surface, April 2007) due to the magnitude of the blooms and the low temperatures. In the same time, the hypoxic events were recurrent. Thus, on Est Constanţa transect, very low DO concentrations in 2009 and 2010, were found which have not being reported since 2001 (Figure 20**).**

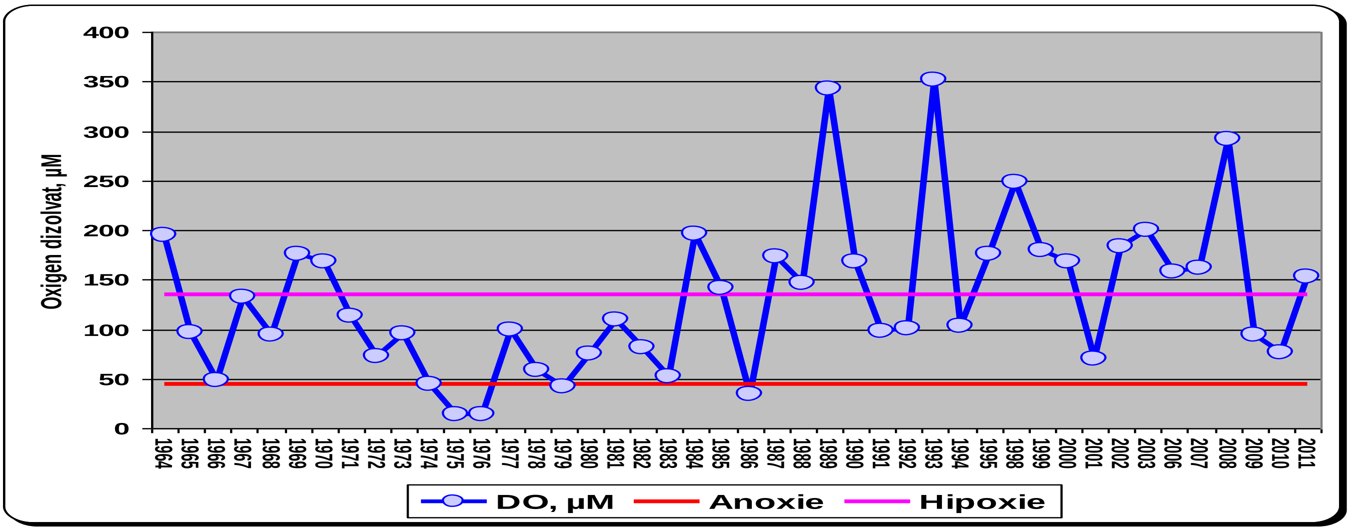

Figure 20. DO minimun concentrations, 1964-2011, Est Constanţa.

One of the lowest DO value that of 75.9µM, was observed during the cruise on July 2010, at 10 m depths. The phenomenon that was observed nearby the coast was a consequence of the water column DO consumption in the oxidation of organic matter. The significant organic matter quantity resulted from the algal blooms recorded in early July, which led also to high ammonia concentrations in the same station. At the same time, an upwelling favored by the winds regime was observed at the end of July. Thus, the water masses near shore moved offshore by being replaced by the masses from the inferior layers of the shallow area (10-20 m), that are colder, more saline with an oxygen deficit, leding to a strong, but episodic hypoxic event. On long term, a decrease trend of the variability, both in the warm and cold season, was outlined. The concentrations were high in the surface layer, 0-10m, with the stratification being more pronounced during spring and autumn. The hypoxic events, which are frequent in the intense eutrophication period, recurred exclusively in the warm season, under the combined influence of the oxidative consumption and the climatic factors. The water temperature, the balanced photosynthesis production – respiration and mixing phenomena, influences the DO content of the Romanian Black Sea waters. The Danube's input influences episodically the DO levels during the phytoplankton development. Seasonally, the DO concentrations vary distinctly between 200.0 – 405.0 µM on a long term, outlining the decreasing trend of this variability due to increased temperatures in both warm and cold seasons. In the water column, concentrations are high in the surface layer, at 0-10 m, the stratification being more pronounced during spring and autumn. The hypoxia, extreme phenomenon in the study period, is found exclusively in the

warm season and has an episodic character. Thus, permanent hypoxic areas are not found, the phenomenon being more influenced by climatic factors than biological ones.

6. QUALITY ASSURANCE, QUALITY CONTROL & GOOD PRACTICES

The definition of clear and applicable Quality Assurance (QA) and Quality control (QC) procedures represent the starting point in monitoring in order to ensure the quality of the collected data and to give to the stakeholders the chance to produce real and solid predictions, as well as models on the actual environmental status of the Marine Ecosystems and its spatial and temporal evolution. The level of accuracy for each variable and indicator have to be well defined considering all the steps of the data acquisition process from the sampling to the data analysis and restitution.

While global monitoring plans, aimed to collect information for long-term, large-scale forecasts may constitute a baseline, flexibility and decentralization is necessary to adapt monitoring plans on local/specific pressures and needs. However, the fragmentation of the monitoring operations between different local agencies generates difficulties in a global analysis. A central organization, able to provide common principles and standards, is needed to 'frame' local strategies in a larger design. Only to give an example, the protocols of the CFP acoustic and demersal surveys are already standardized among countries, even those of the non-EU members.

On a general basis, performing samplings following well recognised and widely approved criteria on an international basis, have to be preferred to self-defined approaches as well as for the followed analytical method. High quality procedures as well as “Chain of Custody” of the collected samples from the sampling site to the laboratory of analysis, should be developed.

The use of standardized and international recognized methods for *in situ* and laboratory analyses published by the scientific literature or laboratory-adapted protocols have to be considered to be used within each state, to reduce errors and to get comparable results among states. Laboratory should be able to prove that could adopt QA/QC protocols and also prove their efficiency by participating frequently to both national and international intercalibration exercises that could represent a key element to reduce errors. Intercalibrations should be performed for each considered indicator.

Integrated environmental monitoring involves the cross-scale cooperation and communication between different subjects. Monitoring plans should at the same time be: 1) focused at the local scale, at which management decisions are made and remedial action are taken, 2) able to provide insights for a long term, large scale forecast. It is important to investigate the possibility for (cross-scale) synergies between monitoring on different purposes. Also different MSFD Descriptors require the same or similar data, allowing a considerable reduction in the effort through integration. Member States, particularly the ones sharing the same marine area, could possibly arrange joint monitoring surveys, in order to share and minimize the costs and ensure common data acquisition done in a comparable manner thus allowing them to come to a comparable assessment and classification of their waters. The dynamic evaluation of the ecosystem health must consider scale dependences. While local observations offer an accurate but partial (i.e. referred to a specific context) representation of the ecosystem responses, forecasts focused on large scale tend to overcome peak events that, even if confined to a limited amount of space/time, could still be relevant for local human communities (e.g. relationships between global eutrophication of the sea and local anoxic crisis).

Data availability and its collection constitute a potential major obstacle to the marine environmental assessment, setting targets and trends for monitoring. Currently, most of the methods in use require gathering of detailed information by using direct observations or sampling methods. Such approaches often provide adequate information for coastal areas but offshore detailed information is usually scarce or absent. The costs and scales required to provide detailed information throughout our seas severely constrain the approaches that require detailed evidence-based information. New broad-scale methods and methods that use surrogate information about the resource, are needed.

Quality assurance and quality controls and good laboratory practices concerning data acquisition on the biogeochemistry are widely treated in the following sites:

http://www.go-ship.org/
http://www.jcomm.info/index.php?option=com_oe&task=viewGroupRecord&groupID=295
http://www.pmel.noaa.gov/pubs/PDF/hood3609/hood3609.pdf

Concerning samples collections, samples treatments, laboratory procedures, quality assurance and controls and data processing for nutrients and chlorophyll determinations, vast details are available at the following web site: http://www.epa.org

Concerning taxonomic data (Descriptor D1), methodological problems are principally referred

to the species that is the object of interest. Monitoring of marine mammals species, reptiles, seabirds, phytoplankton, phytobenthos, fishes or macrozoobenthos requires very different approaches due to the species themselves. Furthermore, for obtaining the same results, different methods or technologies are often available (i.e. satellites, ships, visual-census, samplings etc.) that are associated to the different methodological problems and errors.

7. CONCLUDING REMARKS

This Guideline is developed on the basis of the recent literature collected concerning sampling cruises, models and European Project outputs.

The general conclusion is that the actual knowledge on spatial and temporal assessment and distributions of variables and indicators for the considered Descriptors is not fully described yet. Mammals, Birds, Reptiles (D1, D4, D6) are poorly represented and there is a general lack of homogeneous and complete information on their actual assessment. Descriptors D1, D4, D6 – Phytobenthos and D5 – Eutrophication show, on a general basis, the highest sampling efforts and spatial coverage in the Mediterranean Sea and Black Sea regions.

Actually a large heterogeneity on the variables and indicators measured for each Descriptor, on the density of sampling stations and on the frequency of sampling, is reported.

Neither the density of stations in the different habitats or water masses to be monitored and variables measured in each one, nor the sampling frequencies, are equivalent among Mediterranean countries. This is the case even among EU member states, evidenced by the low correlations among variables and indices used to define the actual assessment. Percentages of overlap among Descriptors vary among Countries ranging from a very low to a good overlap. D1, D4, D6 – Fish has no overlap with other Descriptors. Fortunately this actual gap is quickly going to change. For example in Spain, large Common Fisheries Policy funded survey started being used for answering not only Descriptor D3, but also Descriptor D4 and Descriptor D1, and even to carry out hydrographical sampling and sampling for contaminants in biota and sediments, and marine litter, that have been used a long time now for monitoring seabirds.

The sampling effort (number of sampling stations per square km) used by each Country is notably different and it is frequently very low for distances from the coastline higher than 1

NM. In particular, on a general basis, sampling effort tends to be reduced by an order of magnitude of 10 from 1 NM to 12 NM and of another order of magnitude of 10 from 10 NM to >12 NM. Variables and indicators used by each Country for each Descriptor are also notably different with some specific frequencies.

As a consequence, due to the gaps evidenced in this Guideline, the assessment of spatial and temporal variability for the variables and indicators considered is incomplete. The actual knowledge on spatial and temporal natural variability will be notably improved by the increase of sampling efforts and spatial overlaps that will be performed in the next six years from the commencement of the Countries MFSD monitoring.

Furthermore, an increase of overlap among variables and indicators used for the same Descriptor by all the EU Countries, is also expected, with the improvement of intercalibrations among countries.

A key aspect concerning the Descriptor D5 is represented by the necessity and difficulties to incorporate and control spatial and temporal variability in the Environmental Impact Assessment, in the Ecological Quality Assessment and in the establishment of reference conditions. Design of the monitoring programmes should cope with natural variability of the considered variables and indicators.

Regarding physical oceanographic variability, there are still too many open questions. In fact, the steady state of the system is unknown, and to the best of actual knowledge and technology, it is not yet possible to select better analytical tools able to differentiate or excluding natural variability from anthropogenic changes and to select adequate indicators to detect and differentiate natural *vs.* human induced changes. To give a practical example only the definition of riverine inputs means the employment of many researchers for several years.

The major problem in phytoplankton monitoring is the implementation of relevant methodological and advanced technological, cost efficient approaches for proper biodiversity assessment to cope with the high temporal-spatial variability. The naturally inherent dependency on multidimensional environmental factors makes the efforts for an integrated monitoring, crucial especially those of relevant physical, chemical and biological parameters that are complementary to phytoplankton monitoring. Under the global climatic changes a proper definition of baseline state (conditions) is critically important. Even if the Member states

could succesfully implement the measures for eutrophication reduction, an expansion of species due to temperature changes (areal expansion) not present earlier in the basin (xenodiversity) could represent a threat for increased frequency of toxic blooms events to occur.

Lowered scales, both spatial and temporal, must be taken into account to try to minimize their effect through sampling methods standardization (i.e. at the same month, hour of the day etc.). Incidentally, this is also one of the crucial problems, how to integrate evaluations of GES at the Demarcation macro scale based on a monitoring adapted to detect problems at lower spatial scales, at which in many cases the anthropogenic pressures occur.

On a general basis, the development of well-sized monitoring plans able to cope with natural variability aims to reach the following targets:

- To select better analytical tools to differentiate or exctracting natural variability from anthropogenic changes;
- To clearly define scales of interest: Administrative *versus* process scales;
- To advantageously consider spatial and temporal scales of variability of variables and indicators of interest and to adapt from the observation scales to the processes ones. Scales are quite different related to the marine zone of interest: for example pelagic and benthic processes occur at very different spatial and temporal scales;
- To consider that processes occur at multi-scale levels;
- To select adequate indicators for detecting and differentiate natural versus human induced changes and demonstrating clear response to pressures;
- To select proper indicators implementing Biodiversity Ecosystem Function (BEF) and Biological Trait Analysis (BAT) concepts, reporting confidence and uncertainty of the assessment;
- To include multiple approaches. To perform monitoring using a multi-layer approach could allow reducing mistakes arising from the sampling and methodological limits and furthermore allow the problems reduction within the comprehension of mechanisms to the anticipation of the consequences;

- To improve, encourage, support and ask for a coordination of the monitoring actions at both European and National levels. This is a key aspect to build a knowledge background, to develop predicting models and to increase our capability of anticipation.

Additional recommendations

Rec. numb.	Descriptor	Parameter	Recommendation Type	Notes
1	General	All	Political	The advent of co-management structures and conscious boundary management that includes knowledge co-production, mediation, translation, and negotiation across scale-related boundaries may facilitate solutions to complex problems that decision makers have historically been unable to solve.
2	D5	Chlorophyll-*a*	Methodological	Integrate monitoring programmes with new technologies suitable for surface waters as well as remote sensing analysis to evaluate critical areas to be monitored in detail. Select methods that allow to ensure a good reproducibility of data. The use of field-probe sensors should be preferred compared to the laboratory analysis.
3	D5	TN, TP, TNOx, PO_4	Methodological	Manage samples following the Good Laboratory practices (i.e. ISO or US-EPA Guidelines) regarding their collection and storage. Perform laboratory analyses as soon as possible and before the maximum time indicated in GLP for these indicators
4	D5	NH_4^+	Methodological	Collect samples in a dark glass bottle ensuring to fill it completely to avoid gaseous phase losses. Close the bottle mouth with a pressure seal. Perform analyses immediately after sampling. The use of field-probe sensors should be preferred compared to laboratory analysis.
5	D5	SiO_4	Methodological	Silicates could be released by glass bottles, use appropriate bottles for sampling.
6	D5	Dissolved oxygen	Methodological	Select methods that ensure good reproducibility of data. The use of field-probe sensors should be preferred comparede to the laboratory analysis.
7	D5	Color Transparency	Methodological	Inter-calibration exercises are needed to standardize quantification methods for these indicators that are highly affected by the operator's errors.
8	D5	Turbidity	Methodological	Select methods that allow ensuring a good reproducibility of data. The use of field-probe sensors should be preferred compared to the laboratory analysis.
9	D5	Temperature, Salinity, pH	Methodological	Select methods that allow ensuring a good reproducibility of data. The use of field-probe sensors should be preferred compared to the laboratory analysis.
10	D1	Phytoplankton	Methodological	Further develop and implement operational advanced technological approached for phytoplankton biodiversity assessment and adequate monitoring of the high natural time-spatial community variability (remote sensing, in situ sensors and genetic methods)
11	D1	Phytoplankton	Methodological	Develop relevant methods for the detection of potentially toxic and non-native species and assessment of the ecosystem effects.

Acknowledgements

Writing these types of documents needs to collect, treat and elaborate a huge quantity of literature figures collected by very different sources: databases, grey literature, published scientific articles, public reports etc. Authors are grateful to all contributors to this work and, in particular, they would like to thank PERSEUS project participants, and MYOCEAN project participants for their contributions.

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ANNEX I - GLOSSARY

A list of terms and definitions used in this guideline are reported here.

Term	Definition	Reference
Biocenosis	Interacting organisms living together in a habitat (biotope)	Möbius,1877
Biodiversity or Biological diversity	The variability among living organisms from all sources including, inter alia, [terrestrial,] marine [and other aquatic ecosystems] and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems". Convention on Biological Diversity. Loss of Biodiversity is defined as any human-induced deterioration that could significantly reduce diversity in ecosystem acting at different levels of the hyerarchical scale (i.e. genetic, species, communities, habitat, ecotones, ecotypes, ecosystem losses).	CBD, 1992
Coastline	A coastline or seashore is the area where land meets the sea or ocean. The coastline determination is affected by the Coastline paradox.	Burke et al., 2001
Coherence	Monitoring programmes established for the national part of a marine (sub)region are compared to those within the whole marine (sub-)region or across the EU.	Art 5.2; 11.2; 12; 13.4; 16
Compatibility	Monitoring programmes established for the national part of a marine (sub-) region within areas with the assessments and monitoring of other Community legislation	Art. 11.1
Consistency	Monitoring and assessment methods to be designed so as to facilitate comparability of monitoring results, hence providing data fit for aggregation across Member States sharing the same marine (sub)region and across different scales	Art. 8; 9.3
Ecosystem	"means a dynamic complex of plant, animal and micro-organism communities and their non-living environment interacting as a functional unit".	Art. 2 Convention on Biological Diversity (United Nations, 1992)
Ecosystem Approach	The ecosystem approach is a strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way. Thus, the application of the ecosystem approach will help to reach a balance of the three objectives of the Convention: conservation; sustainable use; and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. An ecosystem approach is based on the application of appropriate scientific methodologies focused on levels of biological organization, which encompass the essential structure, processes, functions and interactions among organisms and their environment. It recognizes that humans, with their cultural diversity, are an integral component of	Sixth Meeting of the Conference of the Parties to the Convention on Biological Diversity the Hague, Netherlands. 7 - 19 April

	many ecosystems.	2002, COP VI Decision, VI/12
Environmental factor	Any factor measurable in the environment that could induce variability.	Tables 1 Annex III (Art. 9.1)
Eutrophycation	Eutrophication is the result of import–driven enrichment by nutrients – primarily N and/ or P– in a water body which modifies ‘pristine’ seasonal cycle, allowing a greater annual primary production with potential algal blooms	Ferreira et al., 2011
Good environmental status (GES)	Environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for uses and activities by current and future generations. Concerning Descriptor D1, GES is acheived if “Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions”; Concerning Descriptor D5, GES is acheived if “Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters”; Concerning Descriptor D6, GES is acheived if “Sea floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected”.	MFSD (Art. 3.5); Cochrane et al., 2010
Human exploitation	Human exploitation human uses of environmental resourcesare considered possible if shall not compromise maintenance of biological diversity.	(Art. 3.5)
Indicator	An indicator provides a measure in relation to a particular component or multiple components.	
Legitimal uses	Allowed uses by the National and International Laws and Directives	
Level	The units of analysis that are located at different positions on a scale.	Gibson et al. (2000)
Marine Zones	Spatial zones in marine ecosystems. The definition of Geographical limits in marine ecosystems represents itself a strong approximation. In fact boundary in sea are not so marked as in terrestrial ecosystems. Otherwise, MFSD defines three principal zones for marine ecosystems: Coastal area < 1 nM from the coastline; Nearshore area 25<X<1 nM zone; Off-shore area >25 nM from the coastline.	
Natural level	The value of a variable or indicator at a specific point in time that it is within the natural (not directly or indirectly due to human activities) range of variation. This range of fluctuation in space or time could be considered as a baseline level.	
Patchiness	Spatial distribution characterized by relatively uniform and homogenous area separated by gaps	
Pressure	Contamination by hazardous substances; underwater noise; Removal of target species (e.g. fishing), introduction of	

	pathogens (e.g. from sewage); Nutrient enrichment, introduction of non- indigenous species; Physical disturbance of seabed (e.g. trawling); Land claim; placement of structures on seabed; dumping of dredge spoil	
Pressure	As pressure we mean and human-due activity that throughout different mechanism (physical, chemical or biological) induces significant effect on any part of the ecosystem.	Robinson et al. 2008b Human pressures are detailed in MSFD Tables 2 Annex III (Art. 9.1)
Process	Sequence of interdependent and linked procedures which, at every stage, consume one or more resources (employee time, energy, machines, money) to convert inputs (data, material, parts, etc.) into outputs. These outputs then serve as inputs for the next stage until a known goal or end result is reached.	
Scale	Spatial, temporal, quantitative, or analytical dimensions used to measure and study any phenomenon	Gibson et al. (2000)
Spatial variability	Variation of a considered indicator or Descriptor in space. Spatial variability could be intended as vertical variability (water column) and/or horizontal variability (along bidimensional space)	
Temporal variability	Variation of a considered indicator or Descriptor during time. On a conceptual basis, temporal variability ranges within fraction of seconds and infinite. Nevertheless, in this Guideline only temporal variability that are of some concern to the MSFD (day-decades) are considered.	

ANNEX II - ABBREVIATION LIST

The abbreviation used for the terms is reported in an appearence order.

Term	Definition
MSFD	Marine Strategy Framework Directive
GES	Good Environmental Status
MS	Member States
RSCs	Regional Seas Conventions
EcAp	Ecosystem Approach
NM	Nautical Miles
WFD	Water Framework Directive
PoMs	Programme of Measures
CFP	Common Fisheries Policy
EE	Ecosystem engineers
UNEP	United Nations Environment Programme
IUCN	International Union for Conservation of Nature
BEF	Biodiversity Ecosystem Functioning
HAB	Harmful Algal Blooms
STX	Saxitoxin
CIL	Cold Intermediate Layer
ES100	Expected Species number per 100 speciements
OTU	Operational Taxonomic Units
DO	Dissolved Oxygen
EMED	Eastern Mediterranean
WMED	Western Mediterranean
MTHC	Mediterranean Thermohaline Current
LIW	Levantine Intermediate Water
DIP	Dissolved Inorganic Phosphorous
n	Number of samples to which results are referred to
TOC	Total Organic Carbon
PBs	Phytoplankton Blooms
EQR	Ecological Quality Ratio
EI-EQR	Evalution Index - Ecological Quality Ratio

ANNEX III – SOME PRACTICAL EXAMPLES ON NATURAL VARIABILITY OF D1, D5, D6

This annex collect some practical examples on natural variability of some of the indicators and variables used for Descriptors D1, D5, D6, in the light of the MSFD. Reported data or images are collected by literature or public sources that are extensively cited in the text.

III.1. Descriptors D1– Biological diversity

III.1.1. Seabirds

Seabirds are the most threatened group of bird species (Cecere et al., 2012; Croxall et al., 2012) and are protected under the Barcelona Convention (2009/147/EC). Among associations working to protect seabirds, BirdLife International, that aims to identify key areas for the conservation of bird species, has collected throughout standardized methods, using on vessel-based survey and data tracking (BirdLife International, 2010a), several data concerning the network of such marine environments essential for them (BirdLife International, 2010b).

In the Mediterranean, a general consensus has been accepted for the conservation of this relatively sensitive and heavily influenced sea region. According to Appendix II of the Barcelona Convention, an action plan has been prepared for 15 threatened marine bird species (UNEP MAP RAC/SPA, 2003). Recent researches have been performed to estimate, on the basis of harmonized monitoring protocols, the number of individuals, breeding pairs and the extension of feeding areas and migration routes. Even if some recent data are available on temporal variability, significant differences concerning monitoring protocols during this time, are reported. Thus, within the observed temporal variability from the results it is very difficult to separate natural fluctuations from human induced ones.

Concerning the number of individuals, the seabird Scopoli's Shearwater (*Calonectris diomedea*) community was composed in 2009 by 10,000 breeding pairs in the Mediterranean (Baccetti et al., 2009). However, the largest population of *C. diomedea* was previously estimated on Zembra Island (Tunisia) at 15,000-25,000 pairs. Moreover, a distance-sampling survey conducted in 2009 and 2010 resulted in a new estimate of 141,780 (95 % CI: 113,720-176,750) breeding pairs (Defos et al., 2012). Actually a recent estimation of breeding population for the Mediterranean is from 57,000-76,000 to 179,000-193,000 breeding pairs. For this species, incubation occurs in June and chick rearing occurs from July to August (Cecere et al.,

2012). One of the biggest breeding colony (about 400 breeding pairs) is reported to be settled in the Adriatic Sea, Tremiti Archipelago (Baccetti et al., 2012).

C. diomedea revealed inter-individual variability in migratory behavior (Péron et al., 2012). At large and meso-scales, seabirds can move directly from the breeding site to the foraging areas that could better improve their fitness (Weimerskirch, 2007). It has been proved that the used area could vary in its extension with the reproductive period and the colony geographical position. A recent research evidenced that during chick-rearing, birds from Linosa used larger areas than those used during incubation and by birds owing to the same species, from Tuscany Archipelago or Tremiti Islands (Cecere et al., 2012) and that during the reproduction *C. diomedea* can alternate short trips for chick provisioning with longer trips for self-provisioning (Magalhaes et al., 2008). The use of smaller feeding area seems to be related to the opportunity to find easier needed feeding resources (Cecere et al., 2012). Obviously all of these genetic or feeding migration patterns should be considered when designing monitoring surveys for estimations of population abundance.

Concerning body mass, this species is colonial and characterized by a high reproductive investment (up to eight months), including a long incubation period (54 days) and a long phase of chick rearing (90 days). The energetic demands might vary largely during the breeding season and can be different between females and males (Navarro et al., 2007). A recent study performed on this species evidenced that body mass ranged on average between 711 g (May) – 640 g (June) for males and between 560 g (May) – 600 g (June) for females (Becciu et al., 2012). Furthermore, the sharing of the parental care resulted in a similar energetic expenditure in both parents during the whole breeding period, except for the period around the onset of the incubation. The marked decrease in body mass observed in males during this phase could be related to higher costs encountered, thus supporting the hypothesis of reproductive stress (Becciu et al., 2012) proposed by Moe and colleagues (2002).

The species Yelkouan Shearwater (*Puffinus yelkouan)* is endemic to the Mediterranean too. The global population was estimated at 15,337-30,519 breeding pairs and 46,000-92,000 individuals (Derhé, 2012) and the largest world colony of this species is breeding at Tavolara (Sardinia, Italy). Even more recent results evidence a number of individuals ranging between 9,991-13,424 breeding pairs (Zenatello et al., 2012). However, very high non-breeding season numbers reported in the Bosporus suggest that it is likely to be a large percentage of non-breeding birds in the population and estimates of breeding numbers at colonies may be

underestimated. The same study reports that feeding areas of breeding adults stretch for some hundred kilometres and are mostly located at coastal gulfs of N and W Sardinia and SW Corsica, between Oristano, Ajaccio and the Maddalena archipelago. Feeding routes were evaluated in a recent research (even if data have no statistical significance) proving that individual birds do not follow set routes in subsequent years (Borg et al., 2012).

The life cycle and dynamics of fish species are explaining the abundance of *P. yelkouan* in the Black Sea (Nankinov, 2001) and hence the timing of their passage through the Bosphorus, that includes a significant proportion of the world population (Şahin et al., 2012).

Studies on Mediterranean Shag (*Phalacrocorax aristotelis desmarestii*) evidence that there is a large aggregation (individual numbers) of forages settling in the Trieste Gulf in late summer and autumn, with a counted number of 2,000−4,000 birds, which is more than half the entire breeding population in the Adriatic (Škornik et al., 2012).

A recent study reported for the species of Audouin’s Gull *Larus audouiniii* show a notably decrease in Greece. This species droped from 700-900 breeding pairs (1995) to 350-500 breeding pairs in 2010 (Saravia Mullin et al., 2012). In this case, an estimation of predation rates evidenced that rats are predating up to 14% (potentially up to 23%) of eggs and chicks. Gulls are predating up to 9% of eggs and chicks. Raptors can locally predate up to 100% chicks and even adult birds.

In Table 11 , recent data on spatial and temporal variability are summarized for the species that are discussed here.

Table 11. Recent available data on spatial and temporal variability for seabirds'indicators

***Calonectris diomedea* (Scopoli's Shearwater, Common name)**						
	Spatial variability		Temporal variability			References
N. individuals	n.a.	n.a.	10,000 (2009)	n.a.	n.a.	Baccetti et al., 2009
N. breeding-pairs	n.a.	n.a.	n.a.	14,780 (2010)	193,000 (2012)	Defos et al., 2012; Cecere et al., 2012
Areal extension (ha)	26,600 173,000 (High feeding resource areas)	432,000 4,720,000 (Low feeding resource areas)	173,000 (incubation) 26,600 (chick-rearing)	3,529,000 (incubation) 4,720,200 (chick-rearing)		Cecere et al., 2012
Body size (g)			640 (male) 560 (female)	711 (male) 600(female)		Becciu et al., 2012
***Puffinus yelkouan* (Yelkouan Shearwater, Common name)**						
N. individuals	46,000	92,000	n.a.			Derhé, 2012
N. breeding-pairs	15,340	30,500	n.a.			Derhé, 2012
Areal extension (ha)	High spatial variability of routes	n.a.	High temporal variability of routes			Borg et al., 2012
Body size (g)	n.a.	n.a.	n.a.			
***Phalacrocorax aristotelisdesmarestii* (Mediterranean Shags, Common name)**						
N. individuals	2,000 (Adriatic)	4,000 (Adriatic)	n.a.			Škornik et al., 2012
N. breeding-pairs	n.a.	n.a.	n.a.			
Areal extension (ha)	n.a.	n.a.	n.a.			
Body size (g)	n.a.	n.a.	n.a.			
***Larus audouiniii* (Audouin's Gull, Common name)**						
N. individuals	n.a.	n.a.	n.a.	n.a.		
N. breeding-pairs	n.a.	n.a.	700-900 (1995)	300-500 (2010)		Saravia Mullin, 2012
Areal extension (ha)	n.a.	n.a.	n.a.	n.a.		
Body size (g)	n.a.	n.a.	n.a.	n.a.		

Notes: n.a. = not available.

III.1.2. Reptiles & Marine Mammals

Reptiles and Marine mammals have been observed in Mediterranean Sea since time immemorial. Nevertheless, an organized data collection on their distribution and location of nesting areas (for reptiles) is not yet completely available and a general condition of "data deficient" for the definition of the Mediterranean status is reported by the International Union for Conservation of Nature (IUCN, 2012).

The principal problem that must be addressed to monitor these species is related to the fact that they are great migrators (as well as birds) and monitoring strategies able to reconstruct their spatial and temporal migrations became available only in recent times, with the introduction of the telemetry approach. Due to a general lack of data, the reconstruction of their natural variability is quite difficult to perform, since actual available data on their distribution are for sure the result of both natural and human induced pressures.

Reptiles

Available data on reptiles, as number of individuals, breeding areas and number of eggs per nest, are in a considerable amount obtained by voluntary work (Casale and Margaritoulis, 2010; IUCN, 2012). Some available data on genetic of the reptile populations and body size are reported in the present annex.

The presence of a small number of individues of *Caretta caretta* and *Chelonia mydas* nesting on the Mediterranean coast of the Sinai Peninsula, was reported, primarily to the east in the region surrounding the resort town of El Arish (Clarke et al., 2000). For this species, a population substructure with reduced gene flow among groups of rookeries, such as Greece, Cyprus, Turkey and Israel, was evidenced (Casale and Margaritoulis, 2010 and citations therein).

Major concentration of *C. caretta* nesting areas are settled along beaches from Greece, Turkey, Cyprus and Libya, while for *C. mydas* the great concentration of nesting sites are recorded in Turkey and Cyprus (Figure 21 from http://www.euroturtle.org/index.htm).

No nesting sites are documented for *Dermochelys coriacea* even if this species has been seen in the Mediterranean all year-round (MEDASSET, www.medasset.org). In Figure 22recent and former distributions are represented (Coll et al., 2010).

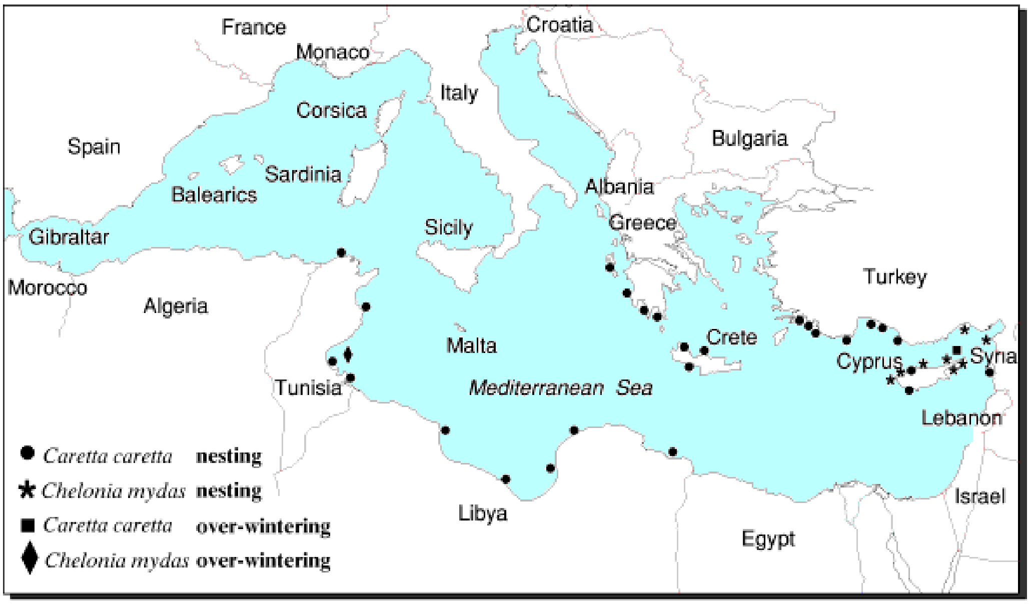

Figure 21. Seaturtle distribution in the Mediterranean Sea. Occasional records are not reported in this figure.

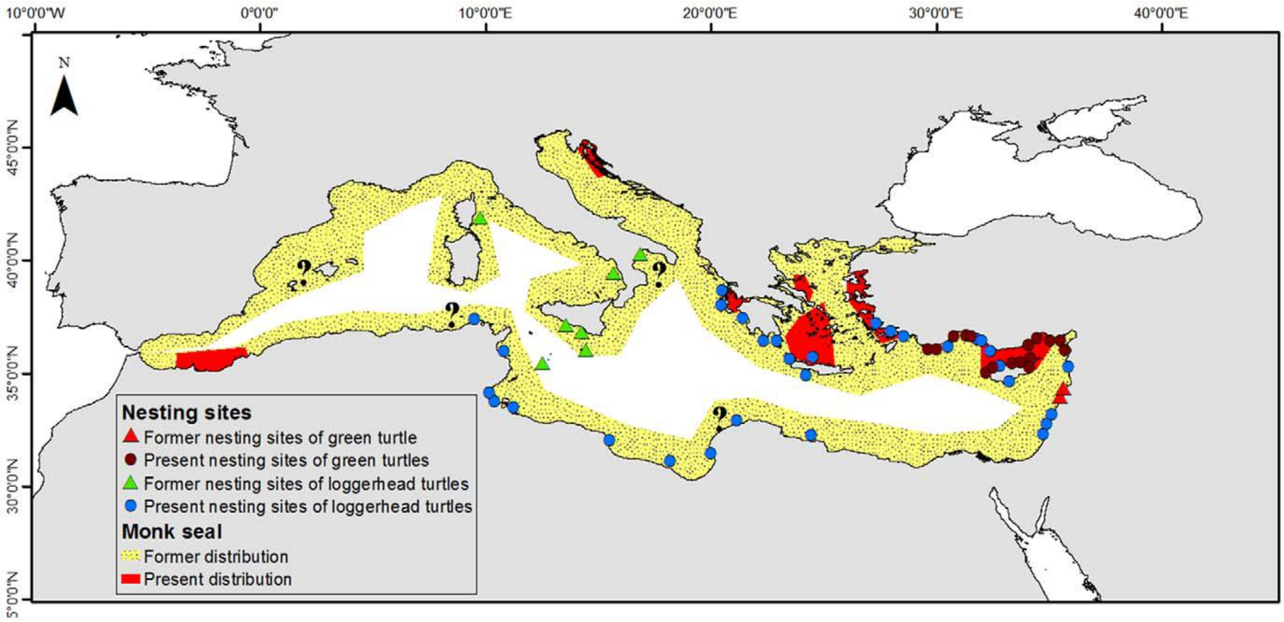

Figure 22. Seaturtle distribution in the Mediterranean Sea and temporal evolution by Coll et al., 2010. Nesting sites for loggerhead turtle and green turtle. Green and red triangles, respectively, are the former nesting sites for loggerhead turtle and green turtle.

In Table 12, a summary related to the abundance and the number of nesting sites is reported for the three species of reptiles, present in Mediterranean Sea and/or Black Sea (IUCN, 2012).

Table 12. **Abundance of individuals and nesting sites for Reptiles.**

Species	Abundance	Nesting sites in Mediterranean Sea	Nesting sites in Black Sea	IUCN Concern (IUCN, 2012)
C. caretta	Common	7,200 /y (2010)	No nesting area	Endangered
C. mydas	In decline	1,500 /y (2010)	No nesting area	Endangered
D. coriacea	Rare	No nesting area	No nesting area	Critically Endangered

In Figure 23, Trends of abundance of specimens in sea and of nests per year are reported for *C. caretta* and *C. Mydas* (extracted by Casale and Margaritoulis, 2010).

Some interesting data on seasonal frequency and size class frequency have been reported for *C. caretta* (adapted by Casale and Margaritoulis, 2010). Thus, in Albania during 2002-2006 about 100 speciements per month were recorded from May to July, 80 specimens per month from August to September and <20 specimens per months from December to April. These seasonal differences must be taken into account for a proper interpretation of the monitoring programs results. Concerning body size, the size classes with the highest frequency were 50-59 cm and 60-69 cm of curved carapace length (Casale and Margaritoulis, 2010).

		Caretta caretta		*Chelonia mydas*	
		Long term	Recent	Long term	Recent
Albania		Increased (S)	Increasing (S)		
Algeria*					
Bosnia and Herzegovina*					
Croatia			Decreasing (S)		
Cyprus					
	Region A		Stable (N)		Stable (N)
	Region B	Decreased (N,S)	Increasing (N)	Decreased (N,S)	Stable (N)
Egypt		Decreased (N,S)	Decreasing (S,N)		Decreasing (S)
France					
Greece		Decreased (N,S)	Stable (N)		
Israel		Decreased (N)	Increasing (N)	Decreased (N,S)	Stable (N)
Italy		Decreased (N)	Decreasing (S)		
Lebanon					
Libya					
Malta		Decreased (N,S)			
Monaco*					
Montenegro*					
Morocco					
Slovenia					
Spain					
Syria				Decreased (N) (a)	Increasing (S) (a)
Tunisia					
Turkey					

*Countries without a dedicated chapter. Blank spaces: data not available; (a) it is uncertain which species the information refers to.

Figure 23. Temporal trends of abundance (Sea and number of nests). Sea = S, Nest = N.

Marine Mammals

Concerning cetaceans, 23 species of cetaceans (whales and dolphins) have been recorded in the Mediterranean and Black Seas. Data here reported are from IUCN (2012).

Regarding dolphins, the species ***Tursiops truncatus*** is commonly found along many parts of the mediterranean coasts, even if there is no overall estimate of the Mediterranean population that is estimated to be as low as 10,000 specimens. The species ***Ziphius cavirostris*** is regularly found in the eastern Ligurian Sea, the eastern Alboran Sea and the Hellenic Trench, preferring deep water, offshore areas and canyons. The species ***Grampus griseus*** is widely distributed in the Mediterranean Sea, although most frequently sighted in the western basin, Ligurian-Corso-Provençal basin, northern Alboran Sea, southern Tyrrhenian Sea and occasionally the Balearic Sea. The species ***Delphinus delphis*** is considered "endangered" by the IUCN (2012) due to the notably reduction observed (more than 50% in the past 30–45 years). The most common species in the Mediterranean Sea is ***Stenella coeruleoalba***. The species is particularly common in the Ligurian Sea, Gulf of Lion, the Alboran Sea and in the waters between the Balearic Islands and the Iberian Peninsula. The Western Mediterranean subpopulation of Striped Dolphins, excluding that of the Tyrrhenian Sea, was estimated at 117,880 specimens in 1991.

Concerning whales, the species ***Balaenoptera physalus*** occurs mostly in deep offshore waters from Northeast of the Balearic Islands to the Ionian Sea. It is particularly abundant in the Corso-Ligurian Basin and Gulf of Lion. Its current population in the Mediterranean is believed to be close to 5,000 adults. The species ***Globicephala melas*** is principally spread in the western Mediterranean Sea (Strait of Gibraltar, Alboran Sea). The species ***Physeter macrocephalus*** is widely distributed in the Mediterranean Sea, even if a notably decline is reported in the last 20 years.

Concerning seals, sea lions and walruses (Pinnipeds), the Mediterranean Monk Seal (***Monachus monachus***) is the only pinniped species inhabiting the Mediterranean region.This species was once widely and continuously distributed in the Mediterranean, Black and adjacent seas, and in the Eastern Atlantic from Morocco to Cape Blanc. Today it is extinct in the Black Sea and only a few subpopulations survive along some coastal stretches of the Mediterranean. The entire Mediterranean Monk Seal population numbers are less than 600 individuals divided into very small colonies that are probably isolated from each other. The largest subpopulation, comprising of 250–300 individuals, inhabits the eastern Mediterranean (Greece and Turkey), and a few seals still seem to use the waters of Algeria and Cyprus. Sporadic sightings of some

individuals have been reported from other Mediterranean coasts.

Sampling strategies and sampling design should be well sized on the type of migratory routes (coastal; offshore) used by the animals to avoid underestimations. Furthermore, a faster adaptation of monitoring design to cope with changes of migratory routes and abundances should be performed to avoid underestimations, also.

Resident species in Black Sea are represented by ***Tursiops truncatus ponticus***, ***Delphinus delphis ponticus***, ***Phocoena phocoena relicta*** (Dolphins). A summary concerning abundances of Marine Mammals in Mediterranean and Black Sea is reported in Table 13.

Table 13. Abundances of Marine Mammals in Mediterranean and Black Seas (data from IUCN, 2012).

Group	Species	Abundance in Mediterranean	Abundance in Black Sea	IUCN Concern in Mediterranean (IUCN, 2012)	IUCN Concern in Black Sea (IUCN, 2012)
Dolphin	*Tursiops truncatus*	10,000	Absent	Vulnerable	X
	Ziphius cavirostris	Common	Absent	Data Deficient	X
	Grampus griseus	Common	Absent	Data Deficient	X
	Delphinus delphis	Common	Absent	Endangered	X
	Stenella coeruleoalba.	117,880 (1991)	Absent	Vulnerable	X
	Tursiops truncatus ponticus	Occasional	<1,000	X	Endangered
	Delphinus delphis ponticus	Occasional	Unknown	X	Vulnerable
	Phocoena phocoena relicta	Occasional	<10,000	X	Endangered
Whales	*Balaenoptera physalus*	5,000	Absent	Vulnerable	X
	Globicephala melas	260-270	Absent	Data Deficient	X
	Physeter macrocephalus	Common	Absent	Endangered	X
Pinnipeds	*Monachus monachus*	600	Extinct	Critically Endangered	Extint

III.1.3. Pelagic and benthic fish

Changes in some exploited fish populations abundance and structure, both seasonal and interannual, can be driven by anthropogenic pressure, especially direct impact of fishing activities or through more indirect impacts derived from anthropogenic climate change, pollution or essential habitats destruction. However, most of them are attributable to natural causes. Firstly, in addition to the diverse environmental factors determining the geographical distribution of the species (depth, type of habitat, temperature, salinity, chlorophyll, etc.), which must be taken into account for a proper populations monitoring design through a right stratification of the study area, other factors as the timing of the reproductive cycle, the ontogenic variations in spatial distribution or seasonal migrations must be also considered, since they will condition the biomass and structure of fish populations in a given place and time. To describe these intraannual variations is not the goal of the MSFD, but obviously they must be analyzed in order to reveal their causes and hence designing standardized monitoring systems able not only to detect interannual variations but also interpret them properly. In fact, the main ongoing programs directed to fish monitoring in the Mediterranean Sea already take into account some of these aspects, being realized always in the same season and areas throughout the whole region. Unfortunately, this is not enough to totally eliminate the effect of seasonal variability when comparing areas or years, because small changes in the phenology, as temporal variations in spawning peaks or optimal environmental windows for larval survival, or changes in the hydrodynamic scenario leading to variations in the location of settlement areas, can affect largely the obtained results. However, the aforementioned large scale surveys carried out under Common Fisheries Policy in the Mediterranean, as acoustic surveys targeting small pelagic, such as sardine (*Sardina pilchardus*) or anchovy (*Engraulis encrasicolus*), in neritic areas, or bottom trawl surveys focused on the main demersal exploited species, such as hake (*Merluccius merluccius*) or mullets (*Mullus* spp.), covering continental shelves and slopes, allow to minimize the potential biases caused by smaller scale variations. These surveys are not only carried out by EU member states, but also have been realized in some southern Mediterranean countries (i.e. Morocco, Tunise, etc.). This, besides other port sampling or on board observer programs, combined with official landings statistics, allow to fulfil most of the monitoring requirements associated to Descriptor 3, but it is obviously also useful to respond partially to D1 requirements in relation to fishes. Moreover, during the last years the field scientific surveys, both directed to demersal and pelagic resources, have broadened their objectives, analysing the

whole biological communities captured by the sampling gears, even using new sampling devices for complementing the data on the studied communities (plankton and micronecton gears for pelagic, epibenthic sledges for demersal communities. etc.), which make them a good platform for providing information directly applicable to the fulfilment of D1 and D4 monitoring needs. Regrettably, the spatial coverage of these sampling schemes is still not enough to monitor the whole fish communities at marine demarcation scale, since coastal hard bottoms communities, deep benthic communities and, over all, mesopelagic populations occupying the water column in offshore areas, crucial for the whole ecosystem functioning, are only occasionally sampled.

In any case, both the results of routine scientific surveys and landing statistics show that important fluctuations occur in most of the fish exploited populations, are not only attributed to fishing pressure, since in many cases fishing effort have remained almost constant, or varied much less than the fish populations biomass. It is suggested that the main problem for defining the GES in relation to fish populations are not only the insufficiency of monitoring systems and the resulting lack of data, or the possible biases derived from insufficient standardization of sampling methods or interannual phonological variations. To interpret correctly the results of the monitoring, it should be able to disentangle the interannual real fluctuations caused by natural causes from those induced by anthropogenic pressures. This is a difficult and challenging task, because of the heterogeneity and inherent complexity of the processes causing these interannual variations. For example, at spatial scales smaller than the distribution of the monitored whole population, variations detected in a given area can derive from natural changes in the geographical distribution of the species. This effect can be important in these demarcations in which a given species is in the limit of its distributional range. As a case study, in the Mediterranean Sea the changes in the latitudinal distribution along the Spanish coast of the thermophilic *Sardinella aurita* and that of relict boreal species *Spratus spratus*, can be cited. Thus, a few years ago a northern progression of *S. aurita*, reaching Catalonian coasts was detected that was previously not found, and this was attributed to global warming, whereas the *S. spratus* populations were declining in the area, becoming more and more restricted to northern parts of Gulf of Lions (Sabates et al., 2006). However, during the last years, a regression of *S. aurita* in northern areas and a recovery and expansion to the south of *S. spratus* populations has been observed (Iglesias 2011, 2012, 2013).

However, the more relevant fluctuations, affecting directly the interpretation of MSFD monitoring and hence GES evaluation, are not these variations in the geographical distribution, but those important changes in populations biomass derived from variations in recruitment strength produced by natural environmental changes affecting larval survival. This is a well-known worldwide phenomena (Cushing et al., 1996 and references therein). Many cases can be cited for the Mediterranean Sea. As an example of a long term change directly attributed to the climatic variations can be cited in the sharp decline of bluefin tuna between the XVI and XVIII centuries, reflected in the captures carried out by fixed traps (almadrabas) (Ravier and Fromentin, 2004; López González and Ruiz Acevedo, 2012).

Interannual fluctuations in the total abundance and relative proportions of different small pelagic species such as sardine and anchovy have also been recorded throughout the Mediterranean, and in relation to the environmental factors (Martín et al., 2012). In spite the fact that the environmentally driven fluctuations have been better documented for pelagic species, they also occur in demersal stocks, and climatic influence on such variations have also been detected and analyzed in the Mediterranean (Quetglas et al., 2013).

It must be pointed out that a good knowledge of trophic webs (D4) and other biological interactions is also a key element to understand the fluctuations in species abundance or variations in their distribution.

In conclusion, fish populations monitoring programs in the Mediterranean Sea should be primarily designed to cope, through a proper stratification of sampling methods, with the large heterogeneity of habitats and hence the spatial distribution of the diverse fish communities, trying to cover not only neritic areas but also offshore pelagic and deep bottoms domains, using in each case the most adequate sampling techniques. Secondly, to get reliable and coherent time series of data, sampling protocols should be standardized as much as possible, optimizing them through cost/benefit analysis. Finally, the results from these monitoring programs should be always interpreted considering the whole time series and all the available knowledge about natural fluctuations and their causes. It implies that for environmental conditions, monitoring is crucial, and that basic ecological studies directed to unveil the complex relationships between environmental changes, including biological interactions, and population abundance or biomass, should be promoted.

III.1.4. Phyto and Zooplankton

Marine phytoplankton are photosynthetic microorganisms, adapted to live partly or continuously in the water column, where they constitute part or most of the organic carbon available to the pelagic food webs (Reynolds, 2006). Marine plankton are the base of ocean food web, as organic matter producers and an integral part of the biogeochemical cycles, providing an essential ecological function for all aquatic life. Phytoplankton diversity in the ocean may influence the functioning of marine ecosystems through primary productivity, nutrient cycling, and carbon export.

Cloern, and Dufford (2005) conceive phytoplankton diversity as hyperdimensional domain, whereby communities are assembled by selective forces operating on variation in algal size, motility, behavior, life cycles, biochemical specializations, nutritional mode, chemical and physiological tolerances, and dispersal processes. They identified 7 principles in shaping phytoplankton diversity and ecosystem interaction: (1) habitat heterogeneity at all scales relevant to plankton population dynamics, (2) community shifts in response to global climatic cycles, (3) fast and selective predation as a powerful top–down force, (4) turbulent mixing as a physical process that selects species on the basis of their size and form, (5) mixotrophy that allows some algal species to tap organic nutrient pools and function at multiple trophic levels, (6) taxon-specific life cycles including alternating vegetative and resting stages, and (7) the pelagic as an open system where communities are continually reshaped by species immigration.

Availability of light and mineral nutrients (nitrate, phosphate and silicic acid) are the building blocks for new growth and play crucial roles in regulating primary production in the ocean and along with physical processes precondition different patterns of spatial and temporal variability - biogeographic, seasonal, vertical etc. (Falkowski et al., 1998; Barton et al., 2010).

Even if contributing to almost half of the total production, oceanic autotrophs only account for about 0.2% of the total biomass (Field et al., 1998). Phytoplankton biomass (standing stock) is structured by taxonomic, functional and genetic factors, resulting in differences in the distribution over space and time. Biomass does not reflect necessarily the production, because the majority of phytoplankters can be removed by a variety of disturbances, as intense herbivory, or transported away from production sites by currents (Cebrian, 2002). Production and biomass are thus in some way separate ecosystem functions, with production measuring energy and material fluxes and biomass measuring habitat characteristics, thus might not show

the same relationship with diversity (Cermeño et al., 2013). Top-down processes acting through the food web (‘vertical’ diversity) have important controlling influences on biomass, productivity, and composition of pelagic communities (Verity and Smetacek, 1996; Verity et al., 2002) but complementary use of resources and responses to environmental change, as well as interspecific facilitation by species within trophic levels (i.e. horizontal diversity) can enhance the consistency and stability of such basic ecosystem processes as primary production and nutrient cycling.

Phytoplankton community composition play a key role in the pathways and efficiencies of primary producers and energy transfer and the direction of ocean–atmosphere CO_2 fluxes exchange through a number of traits: taxonomy, size, life cycles, energetic value and chemical composition (palatability to the consumers, toxicity) and as such determine the quality of pelagic/benthic habitat conditions (*sensu* MSFD, D1).

Although phytoplankton diversity is extremely important for the stability and functioning of the marine ecosystem and biogeochemical cycles (Ptacnik et al., 2008) the indicator role bear a number of constrains. On one hand our knowledge of marine phytoplankton biodiversity is limited due to both methodological constrains of species identification techniques (Venter et al., 2004), the effort and expense of gaining appropriate data sets by traditional microscopic methods (Irigoien et al., 2004; Cermeño et al., 2013) and mismatches between sampling and the scales of phytoplankton natural variability, for which species identity concepts within Biodiversity Ecosystem Functioning (BEF) concept, are rather vague. On the other hand, mechanisms regulating patterns of phytoplankton biodiversity still remain debated and largely unexplored (Garmendia et al., 2012; Cermeño et al., 2013). Albeit the great effort to explain the factors that determine the distribution, community assembly, blooms, and succession of species the macroecological and morphospecies approaches are not properly scaled to the ecophysiology and niche requirements of the phytoplankton phylogenetic groups and species present (Smayda, 2011); there are no species (the occurrence or abundance of which) that can be used as universal indicators and there is no unique fixed assemblage of species each with its own abundance that is representative enough of a given ecological state of the environment. The insights into the speciation, genetic diversity, and ecophysiology being gained through molecular studies (Rynearson et al., 2006; Härnström et al., 2011) indicate the need to redefine the species behavior of classic interest and apply a deeper conceptual and applied level of

inquiry—a microecological approach.

Litchman and colleagues (2010) proposed trait-based approach as an effective way to link species diversity and community structure in phytoplankton, by providing mechanistic explanations of why certain species are found under given environmental conditions. However how traits evolve in response to different selective pressures (because traits may evolve rapidly owing to short generation times and large population numbers), making microevolutionary processes likely to affect community dynamics, is still poorly resolved (Hairston et al., 2005; Litchman and Klausmeier, 2008). Harmful algae blooms (HABs) are known to have a pronounced impact not only on water quality, but on species diversity, community structure and ecosystem functioning by their traits (best expressed in the toxic species) or by their abundance (hypoxia conditions and associated benthic species mass mortality), impairment of reproduction (chemical biomediation) and many ecosystem functions (GEOHAB, 2001; Paerl and Huisman, 2009). Ecophysiological flexibility in HAB species favours their success in different environments and may help maintain high fitness in a wide range of environmental conditions. For example, de Tezanos Pinto and Litchman (2010) showed that heterocystous nitrogen fixers grown in low N and high light gained dominance because of nitrogen fixation. However, when grown in low light, the traits providing higher fitness were related to light acquisition (low Ik and high relative growth rates at low light) and behaviour (gas vesicles that enable positioning in better illuminated zones). Zimmer and Ferrer (2007) linked chemical defenses, chemical signals, and the keystone species hypothesis stating that the impacts of signal/defense compounds play keystone roles within natural community organization. The presence of saxitoxin (STX) in phytoplankton (genus Alexandrium) is known to determine the habitat and prey choices of higher order consumers, significantly impacting species compositions of coastal ocean communities (Kvitek and Bretz, 2004; 2005). Large, episodic die-offs of predatory fish and mammals also modify primary plant-herbivore relationships, and thus regulate trophic cascades in both benthic and pelagic environments (Myers and Worm, 2003; Bruno and O'Connor, 2005).

However genetic shifts in trait values of a given species can easily occur over relatively short timescales (within a single growing season), often because of clonal selection, as pointed out by Kardinaal et al. (2007). Predation, competition, or changing environmental conditions can exert sufficient selective pressures to cause such shifts. Kardinaal et al. (2007) observed a rapid

decrease (within 30 days) in toxicity of the cyanobacterium Microcystis due to a competitive displacement of toxic strains by nontoxic strains with better competitive abilities for light.

Long-term temperature change in ocean waters associated with climatic trends has been shown to affect phytoplankton abundance (Richardson and Schoeman, 2004), phenology (Edwards and Richardson, 2004) and shifts in taxonomic composition (Leterme et al., 2005). Beaugrand et al. (2010) document that global warming has been accompanied by an increase in the taxonomic biodiversity of phytoplankton and zooplankton in the North Atlantic Ocean on the expense of average size reduction, e.g. these structural modification could result in lowering the total phytoplankton biomass already observed in the Black Sea (Nesterova et al., 2008; Moncheva et al., 2012). Assessment of *in situ* chlorophyll observations since 1899 showed that interannual to decadal phytoplankton biomass fluctuations superimposed on long-term trends, strongly correlated with basin-scale climate indices, whereas long-term declining trends were related to increasing sea surface temperatures (Boyce et al., 2010), suggesting that the global phytoplankton concentration decline need to be considered in future studies of marine ecosystems, geochemical cycling, ocean circulation and fisheries.

Field cruise results indicate clear effects of UV-B and UV-A on the photosynthetic carbon fixation of phytoplankton communities with spatial differences between coastal and open-ocean waters suggesting increasing temperatures and ocean acidification due to global climate change that may exacerbate the detrimental effects of solar UV-B radiation (Häder, 2011).

Microalgae exhibit considerable physiological plasticity of C:N:P in response to nutrient and light conditions (Geider and La Roche, 2002). The "Non-Redfield behaviour" of phytoplankton is based on the unique stoichiometric properties of the different cellular components e.g. the resource (light or nutrients) acquisition machinery such as proteins and chlorophyll, is high in N but low in P, whereas growth machinery, such as ribosomal RNA, is high in both N and P (Falkowski, 2000) that result in three different phytoplankton strategies (Klausmeier et al., 2004): 'survivalist' (high N:P ratio (>30) can sustain growth when resources are low; 'bloomer' (low N:P ratio (<10) adapted for exponential growth) and 'generalist' (N:P ratio near the Redfield ratio). As environmental conditions change, the global mean phytoplankton nutrient stoichiometry could vary over time, potentially modifying current nutrient inventories. N_2 fixation by phytoplankton was estimated to be equivalent to 50–180% of the flux of NO_3 into the euphotic zone (Karl et al., 1997; Capone et al., 2005), demonstrating that a large fraction of

the new production is fuelled by N_2 fixation, while anammox (anaerobic ammonium oxidation), accounted for 24–67% of the total N_2 production in the continental shelf sediments (Thamdrup and Dalsgaard, 2002).

A multi-decadal analysis of Baltic Sea phytoplankton point to median increase of algal biomass per unit TP and TN by a factor of 1.4 and 1.2, respectively, implying that management decisions and associated recovery scenarios should take such shifting baselines into account when assessing the effects of pressure–response relationships (Olli et al., 2014).

In addition phytoplankton biodiversity, abundance and distribution as a multiparametric function of both top-down and bottom up controls (such as hydrodynamics and circulation, light, nutrient availability and stoicheometry, temperature and salinity, and biotic interactions) to which the responses are non-linear (McQuatters-Gollop et al., 2008) underline the complexity in definition and application of robust phytoplankton related indicators (Garmendia et al., 2012) and the ultimate need for filling in the gaps of knowledge on natural scales and mechanisms of phytoplankton variability.

The anomalies in the Danube flow, land-based human induced pressures are considered the main drivers of the inter-annual and seasonal phytoplankton species composition and biomass variations in the coastal area of the NW and coastal-shelf W Black Sea (Vasiliu et al., 2010; Yunev et al., 2005; Yunev et al., 2007; Moncheva et al., 2012), but as suggested by the threshold generalized additive model results (Llope et al., 2010) the combination of climatic influences (indexed by NAO), and grazing pressure could have a strong control on phytoplankton growth (Moncheva et al., 2001). There is evidence that in the open Black Sea the long-term trends in the phytoplankton biomass, chlorophyll-a and primary producers correlate closely with the warm/cold phases of the winter temperature that influence the position and temperature of the Cold Intermediate layer (CIL), the intensity of the circulation and the nutrients transport (Yunev et al., 2005; Yunev et al., 2007; Shapiro et al., 2010; Mikaelyan et al., 2013).

Along with the changes in phytoplankton dominant species since mid 90-ies and bloom reduction in the Black Sea (Bodeanu et al., 1998; Nesterova et al., 2008), after the year 2000, *Emiliania huxleyi* blooms emerge as a robust signature of the annual phytoplankton structure each spring-summer, but the reported underlying conditions that favour its proliferarion are rather contradicting. According to model simulations the species flourish after a diatom-dominated bloom in March and dinoflagellate-dominated bloom in April under nitrogen

depleted conditions wheares the top-down grazing pressure impose the control on timing and intensity of *E. huxleyi* bloom (Cokaca et al, 2001; 2004; Oguz and Merico, 2006). Based on a 40 years long-term data set Mikaelyan and colleagues (2011) found a close correlation between the phosphate content and the size of the coccolithophorids fraction in the total phytoplankton biomass, while Churilova et al., 2014) based on data collected from 1999-2013, reported different pattern of spatio-temporal distribution in the coastal-shelf versus open sea habitats, the latter related to the thickness of upper mixed layer in May-June that depends on the winter conditions of the previous year.

The spatial and temporal structures in plankton ecosystems span many orders of magnitude, but the space–time windows of observation are much narrower; thus, multiscale analysis is needed to reveal macroscopic patterns. Organizing principles of trophic levels, body size, functional attributes, phylogenetic diversity, and elemental stoichiometry should be used to discern patterns of natural variability (Li, 2014).

Summarizing, the variability of the planktonic community is determined by diverse factors (abiotic and biotic). The abiotic factors affect at different scales (Dickey and Bidigare, 2005): from the global scale, where climatic variability originates the physical forcing, to mesoscale structures like eddies and fronts that concentrate biomass and enhance the metabolism and the zooplankton's vertical migration to deep waters; at the microscale the turbulence affects the predator-prey encounter rates. The chemical properties of water masses (i.e. salinity, nutrients, oxygen and CO_2 concentrations), as well as temperature and light, directly affect the planktonic community. In addition, the biomass of the planktonic community is also affected by biotic factors such as food distribution and abundance, by determining their growth rates, and predation that modulates mortality rates. All these, are factors that drive the plankton population dynamics and the communities' structure.

III.1.5. Phyto and Zoobenthos

Concerning **phytobenthos**, natural fluctuations are significantly affected by latitude (temperature and communities), water depth (light availability) and nutrients.

Seagrasses cover about 0.1–0.2% of the global ocean, and develop highly productive ecosystems which fulfil a key role in the coastal ecosystem (Duarte, 2002). As reported by

Duarte (2002) and detailed described in citations therein, "*seagrass meadows generally occupy 0–30 m depth littoral fringes off all of the continents except Antarctica. The global extent of seagrasses, although rather inadequately defined, is believed to be about 0.6-106 km^2, and their net production is about 0.6-1015 gC yr^{-1}, 15–50% of which is allocated to the growth of belowground organs. Because of the large below-ground allocation of production, the generally low use of seagrass production by herbivores, and the low decomposition rates of seagrass carbon, seagrasses store a large fraction of their substantial production, being responsible for about 15% of the carbon storage in the ocean. In addition, seagrasses also on average export 24.3% of their net production to adjacent ecosystems, both to the land and seaward, acting as important trophic links with other ecosystems. In addition to their high primary production, seagrasses perform many other functions in the ecosystem:*

- *Provision of food for coastal food webs,*
- *Provision of oxygen to waters and sediments,*
- *Carbon sequestration from the atmosphere,*
- *Organic carbon export to adjacent ecosystems,*
- *Sediment stabilization,*
- *Prevention of sediment resuspension,*
- *Improvement of water transparency,*
- *Wave attenuation,*
- *Shoreline protection,*
- *Habitat for microbes, invertebrates and vertebrates, often endangered or commercially important,*
- *Trapping and cycling of nutrients*".

Excessive increases in the nutrient loads, both from natural and/or human origin, determine the occurrence of eutrophication consequences in water ecosystems (Morand and Briand, 1996), which produce changes in abiotic matrices such as the water column and surface sediments (Chessa et al., 2005), zoological, and phytosociological assemblages or communities (Orfanidis et al., 2008; Viaroli et al., 2008). Relationships among sediment characteristics (i.e. pH, oxidation-reduction potential, grain–size, nutrients, sulphide) and phanerogams distribution were observed in many studies (Giusti et al, 2010; Renzi et al., 2007; Short, 2007; Van Katwijk and Wijgergangs, 2004; Chau, 2002; Azzoni et al., 2001; Miller and Sluka, 1999; Viaroli et al., 1996; Goodman et al., 1995; Ferrari et al., 1972) evidencing sediment as a key element for the plants establishment, presence and recolonization after the occurrence of environmental crises (Plus et al., 2003). Furthermore, phanerogams actively contribute to the regulation of the

oxidation level in sediments by spreading the oxygen produced by photosynthesis from the rhizosphere (Sand-Jensen et al., 1982; Pedersen et al., 1998) and to the reduction of the system turbidity (Mannino and Sarà, 2006).

A strong relationship among the depth of the lower limit of the *P. oceanica* meadow and the water transparency (also related to water turbidity) is reported in the literature (Pergent et al., 1995). With the exclusion to the erosive limit that is strongly conditioned by bottom hydrodynamic, relationships between transparency and the depth of lower limit are:

- between 0 m and -15 m of depth for very low transparency water;
- between -15 m and -25 m of depth for low transparency water;
- between -25 m and -35 m of depth for transparency water;
- lower than -35 m of depth for very transparency water.

Abundance (density as number of shoots/m^2) of *P. oceanica* meadow is strictly related to the water depth in the sampling site, light intensity, and substratum type. Giraud (1977) classified meadows according to density of shoots as follow:

Class 1	Very dense meadow	> 700 shoot/m2;
Class 2	Dense meadow	400 – 700 shoot/m2;
Class 3	Rade meadow	300 – 400 shoot/m2;
Class 4	Very Rade meadow	150 – 300 shoot/m2;
Class 5	Semi-meadow	50 – 150 shoot/m2;

More recently Pergent-Martini and Pergent (1994) and Pergent (1995) proposed a new classification taking into account both water depth and the number of shoot/m^2 that allow to identify three types of meadows: undisturbed (shoot density is normal or exceptional), disturbed (low shoot density) and very disturbed (abnormal shoot density).

As example, for undisturbed meadows, the number of shoot/m^2 is between 173-397 at 20 m depth (normal meadow) and >397 shoot/m^2 for exceptional dense meadows. On the contrary, at the same depth disturbed meadows and very disturbed meadows show values of 61-173 shoot/m^2 and <61 shoot/m^2 respectively. At 25 meters of depth, for undisturbed meadows, the number of shoot/m^2 is between 116-340 (normal meadow) and >340 shoot/m^2 for exceptional dense meadows. On the contrary, at the same depth disturbed meadows and very disturbed

meadows show values of 4-116 shoot/m^2 and <4 shoot/m^2, respectively. Finally, at 30 meters of depth, for undisturbed meadows, the number of shoot/m^2 is between 70-249 (normal meadow) and >249 shoot/m^2 for exceptional dense meadows. At the same depth disturbed meadows are <70 shoot/m^2, while very disturbed meadows are considered to disappeed at depth higher than 25 meters.

Concerning **benthic crustaceans**, seasonal fluctuations due to molting, feeding and reproduction, and in some cases migrations, require standardizing sampling programs by species that account of their biology and ecology. Seasonal variability in relation to temporal fishery closures used to manage commercial species in some countries must be taken into consideration when designing sampling programmes. Interannual variations attributable to fluctuations in recruitment strength associated with larval survival must be taken into account. Furthermore, because many benthic crustaceans have a metapopulation structure changes in larval supply and connectivity patterns caused by large scale processes (e.g. decadal astronomic cycles (solar activity), others are more irregular but related to climatic indices showing alternate patterns, occasional but repetitive events (El niño) or occasional ones (changes in thermohaline circulation (i.e. great salinity slug) must also be taken into consideration. For commercial benthic species, spatio-temporal trends in fishing effort and of changes in fishery regulations must also be incorporated in interannual monitoring plans.

III.1.6. Biodiversity

In a major review work Coll et al. (2010) assessed the overall spatial and temporal patterns of species diversity and identified major changes and threats of biodiversity in the Mediterranean Sea. Spatial patterns showed a general decrease in biodiversity from northwestern to southeastern regions following a gradient of production, with some exceptions and caution due to gaps in our knowledge of the biota along the southern and eastern rims.

Biodiversity was also generally higher in coastal areas and continental shelves, and decreases with depth. Temporal trends indicated that overexploitation and habitat loss have been the main human drivers of historical changes in biodiversity.

Habitat loss and degradation, followed by fishing impacts, pollution, climate change, eutrophication, and the establishment of alien species were shown as the most important threats and affect the greatest number of taxonomic groups. All these impacts are expected to grow in

importance in the future, especially climate change and habitat degradation. The spatial identification of hot spots highlighted the ecological importance of most of the western Mediterranean shelves (and in particular, the Strait of Gibraltar and the adjacent Alboran Sea), western African coast, the Adriatic, and the Aegean Sea, which show high concentrations of endangered, threatened, or vulnerable species. The Levantine Basin, severely impacted by the invasion of species, is endangered as well.

In the Mediterranean, a northwestern-to-southeastern gradient of species richness was observed in most groups of invertebrate species analyzed, with a highly heterogeneous distribution of species in the different regions. Similar results were found for vertebrate species (Figure 24). There was a decreasing gradient from northwest to the southeast, while the sea around Sicily had the highest richness, followed by other northwestern coastal and shelf areas. The endemic richness gradient of fish species was more pronounced with latitude, the north side exhibiting a greater richness, and the Adriatic appearing as a hot spot of endemism with 45 species per cell. Spatial patterns also showed how most of Mediterranean coastal waters have been colonized by exotic species. The highest richness of exotic species occurred along the Israeli coast.

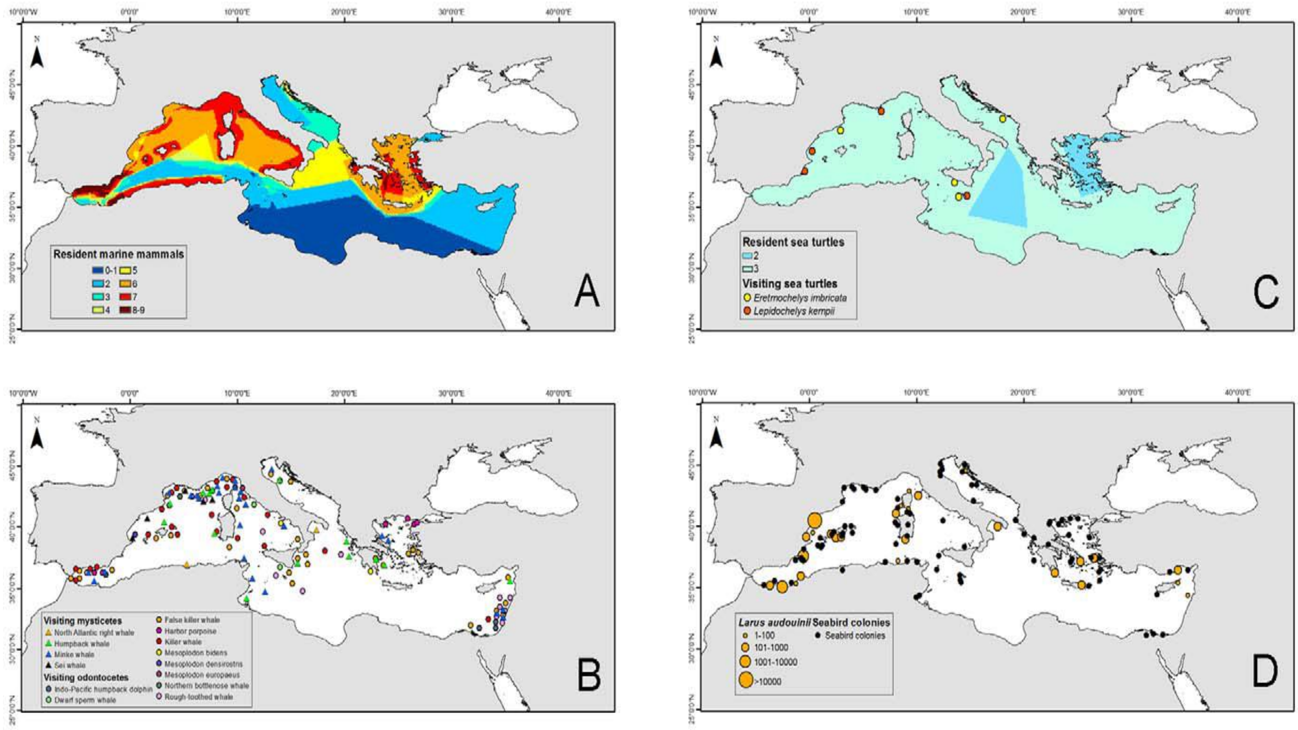

Figure 24. Spatial patterns of vertebrate species richness in the Mediterranean Sea based on superimposed expert-drawn maps (excluding fish species).

Results for the deep sea of the Mediterranean show a clear longitudinal biodiversity gradient that also occurs along the open slopes, where values decrease eastward, from Catalonia to the margins of southern Crete.

Predicted patterns of overall species richness based on AquaMaps showed a concentration of them in coastal and continental waters most pronounced in the Western Mediterranean, Adriatic, and Aegean seas.

The pattern of a generally decreasing diversity with increasing depth was also documented for invertebrate and fish species (Figure 24). In particular, in Figure 24(A) all species are represented while in Figure 24(D) invertebrates are represented.

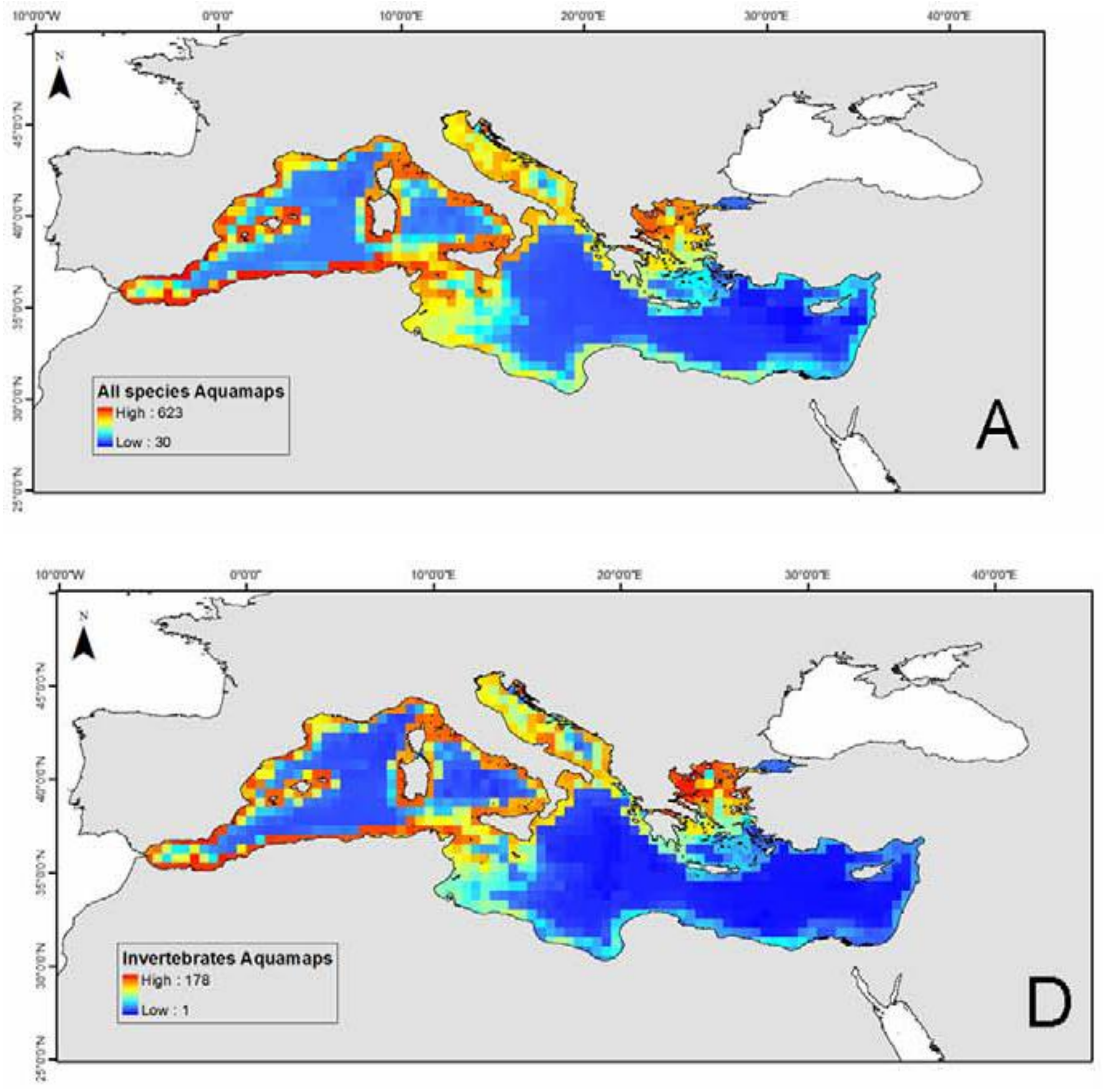

Figure 25. Spatial predicted patterns of species richness in the Mediterranean Sea based on the AquaMaps model. A. All species, D=Invertebrates.

Other studies carried out on depth-related distribution of marine biodiversity in the deep sea of the Mediterranean available form the literature suggest a generally unimodal pattern of species richness, the highest values of which are observed at intermediate depths (about 2,000 m) and lower values at upper bathyal (<2,000 m) and abyssal (>2,000 m) plains.

A recent research performed by Danovaro and colleagues (2010), analysed spatial (longitude and depth) contribute to the species richness and the expected species number per 100 specimens (ES100) in deep-sea habitats are reported in Figure 26.

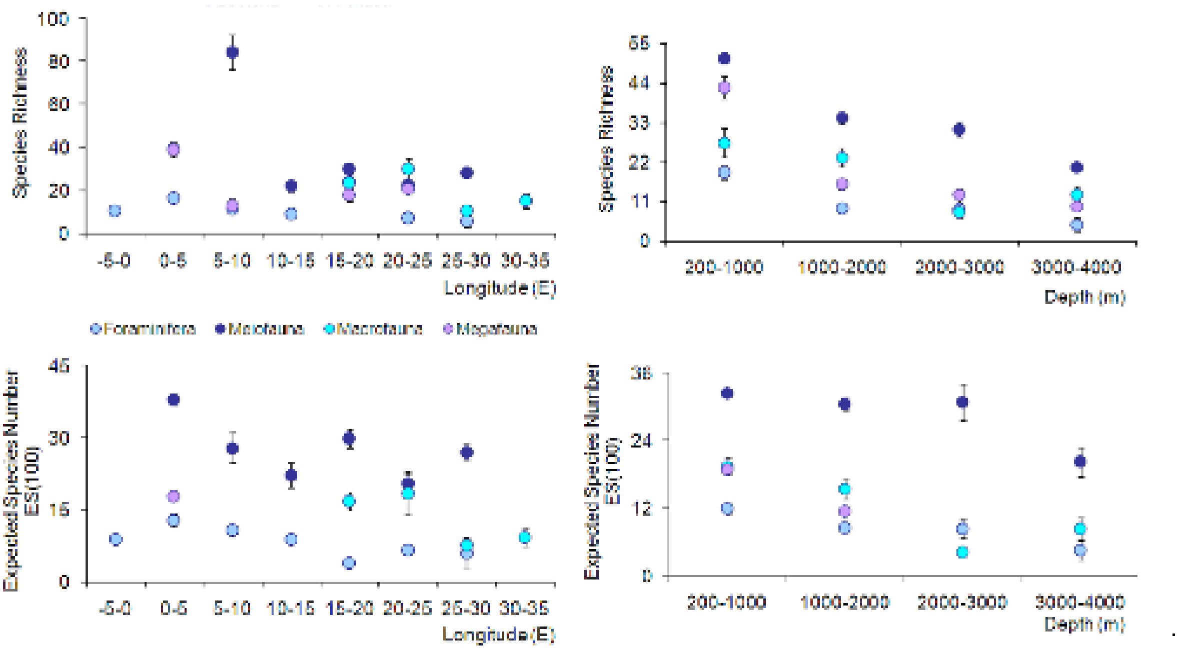

Figure 26. Longitude and depth contributes to the species richness and the (ES100) in deep-sea habitats from Danovaro et al., 2010, modified.

Danovaro and colleagues (2010), also, reported contribute to the total species richness of different deep-sea habitats (slope, canyons, basin, deep-water corals, seamount). This research reported that the expected number of species per 100 specimens attended for different groups shows the following decreasing trend:

- Foraminifera: Slope>Canyons (about 30)>Basin (about 10);
- Meiofauna (Nematoda) Deep-water corals (40-60)>Slope&Canyons (40)>Basin&seamount (20-40);
- Macrofauna: Slope (20-30)>Basin (about 10);
- Megafauna: Canyons (about 200)> deep-water corals (about 150)> Slope (about 100).

III.1.7. Data availability on less considered Taxa

Microbes, jellyfishes, pelagic cephalopods and the range of marine habitat types that occur within the jurisdictional area of the Directive are, also, considered to fall within the scope of the MSFD and are grouped under the Descriptor of Biological Diversity (D1).

Concerning **jellyfish**, different projects show online sights collected by different sources and methods. In some cases privates could contribute to the database by the direct submission of jellyfish sighting sending to the Webmaster the geographical location of the sight and a picture of the jellyfish. As example, the map reported in Figure 27summarizes the latest sightings in the 2014 summer's season and their specific location for the Mediterranean and Black Seas reported by PERSEUS (http://www.perseus-net.eu/en/jellyfish_map/index.html).

Figure 27. An example on PERSEUS jellyfish webmaps (sightings reported by for Summer 2014).

The CIESM website (http://www.ciesm.org/gis/JW/build/JellyBlooms.php) reports interactive maps that can visualize: I) Jellyfish blooms at different times, II) Persistent jelly blooms observed in the time period selected and their persistence (in weeks), III) Data organized by total amount or by species, IV) pilot areas observed at least 15 weeks in the last year. An example is reported in Figure 28.

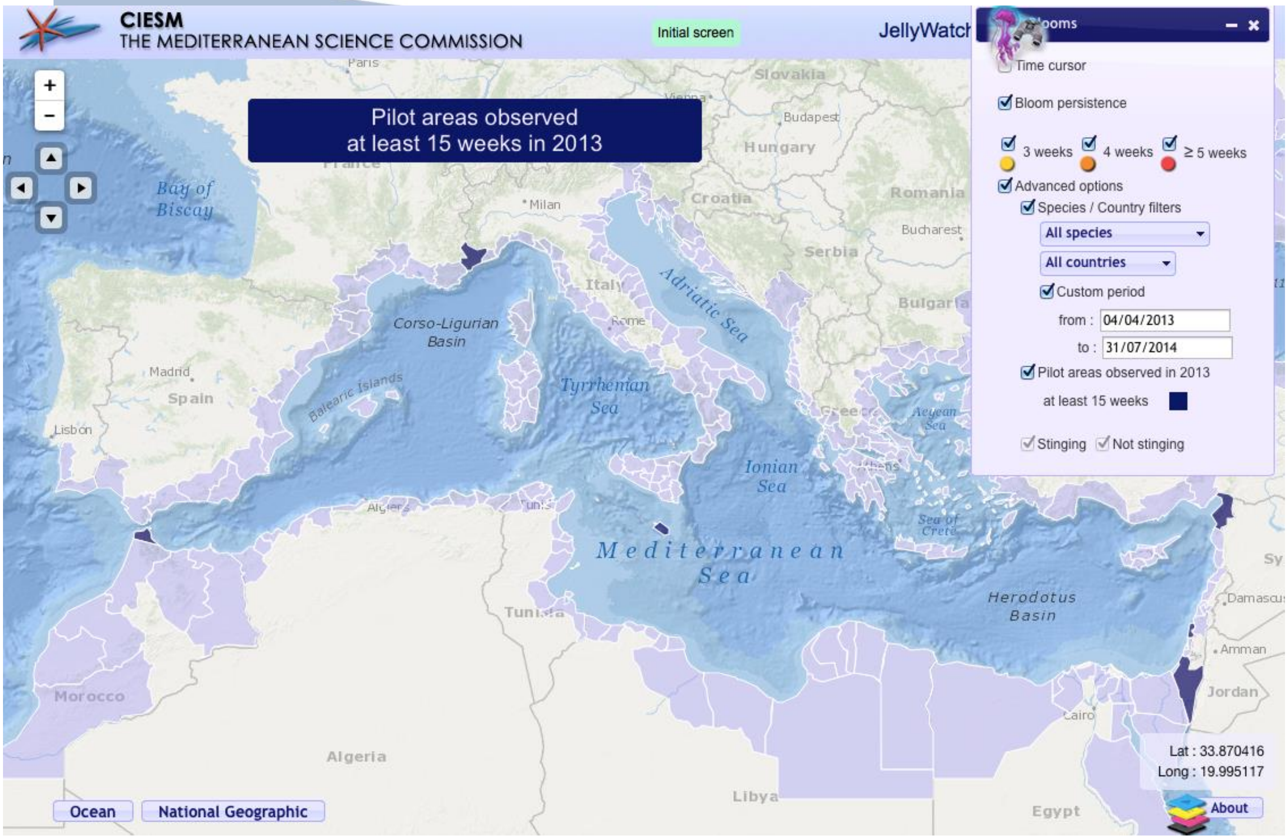

Figure 28. An example on CIESM jellyfish webmaps.

Concerning **microbes**, a recent paper of Danovaro and colleagues (2010) evidenced the spatial contributes (longitude and depth) to the operational taxonomic units (OTU) richness both for Bacteria and Archea (Figure 29).

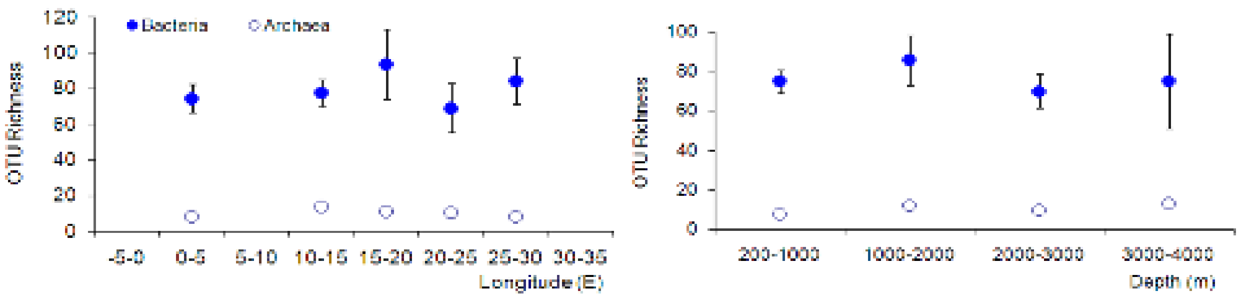

Figure 29. Spatial contributes to the Microbes OTU Richness, by Danovaro et al., 2010, modified.

Data on pelagic cephalopods, ctenophora and the range of marine habitat types that occur within the jurisdictional area of the Directive are only occasional and could not be included.

III.2. Descriptor D6 – Seafloor Integrity

An example of defining spatial scales for the mapping of *Posidonia oceanica* and corralligenous formations taking into account conservation priorities, is reported.

The work of Giakoumi et al. (2013) contemplates the issue of defining spatial scales for mapping three key Mediterranean habitats, i.e. seagrass *Posidonia oceanica* meadows, coralligenous formations, and marine caves, pertaining to D6 and D1 Descriptors taking into account conservation priorities, biogeography and managerial issues.

Different scenarios were determined through a systematic planning approach dealing with large-scale heterogeneity among which the basin scale and the ecoregion scale approaches. In comparison, the ecoregional scenario resulted in a higher representation of ecoregions and a more even distribution of priority areas, albeit with a higher opportunity cost.

The authors suggested that planning at the ecoregional level ensures better representativeness of the selected conservation features and adequate protection of species, functional, and genetic diversity across the basin. Spatial priorities for the conservation of three key Mediterranean habitats (i.e. seagrass *Posidonia oceanica* meadows, coralligenous formations, and marine caves), were determined through a systematic planning approach.

Available information on the distribution of these habitats across the entire Mediterranean Sea was compiled to produce basin-scale distribution maps. Conservation targets for each habitat type were set according to European Union guidelines. Surrogates were used to estimate the spatial variation of opportunity cost for commercial, non-commercial fishing, and aquaculture.

While there are several initiatives that identify priority areas in the Mediterranean Sea, this work approach is novel as it combines three issues:

(a) it is based on the distribution of habitats and not species, which was rarely the case in previous efforts;
(b) it considers spatial variability of cost throughout this socioeconomically heterogeneous basin;
(c) it adopts ecoregions as the most appropriate level for large-scale planning.

Bianchi and colleagues (2012) give an approach to the management of marine biodiversity including Posidonia meadows taking into account spatial scales. This method divides the mapped area in territorial units having different sizes according to the scale adopted. Territorial

units (grid cells) are assigned to one of five classes of evaluation, ranging from high necessity of conservation or protection to non-problematic, unimportant or already compromised (according to the specific map) situations.

Depending on the scale, these maps are suited for territorial planning (small scales, allowing for a synoptic view) or for administration and decision making (large scales, providing detail on local situations and problems) purposes.

Mapping should be periodically repeated (diachronic cartography) to assure an efficient tool for integrated coastal zone management.

The production of maps includes multiple levels of environmental diagnostics, namely:

(i) Morphobathymetry and sedimentology;
(ii) Habitats;
(iii) Natural emergencies;
(iv) Degradation and risk;
(v) Weighted vulnerability;
(vi) Environmental quality;
(vii) Susceptibility to use.

In Figure 30 and Figure 31 are respectively reported the distribution of *Posidonia oceanica* meadows and the distribution of coralligenous formations in the Mediterranean Sea (from Giakoumi et al., 2013).

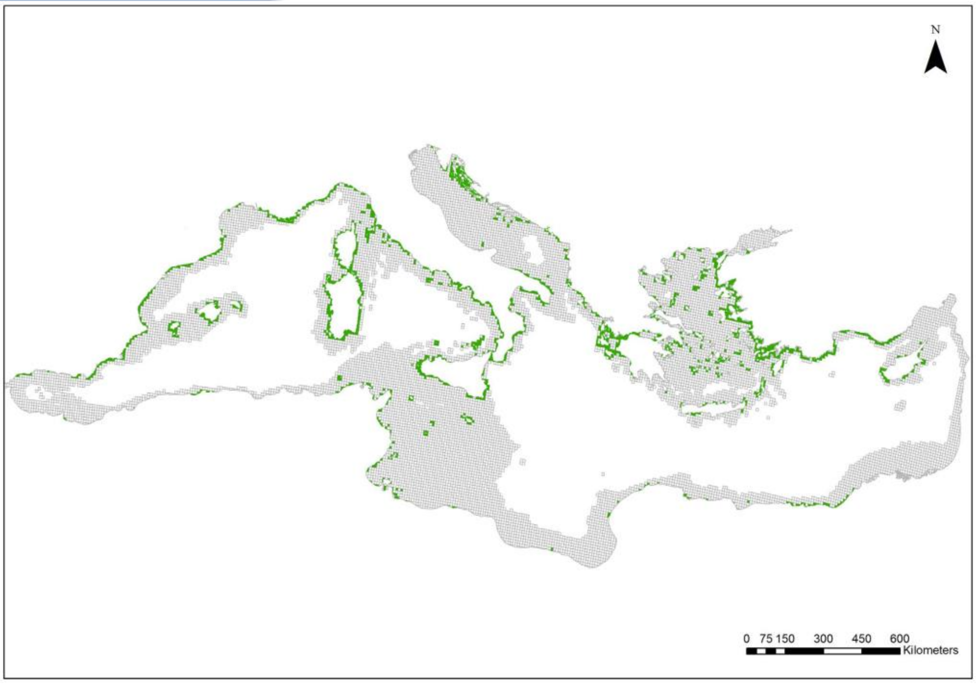

Figure 30. Distribution of Posidonia oceanica meadows in the Mediterranean Sea (from Giakoumi et al., 2013).

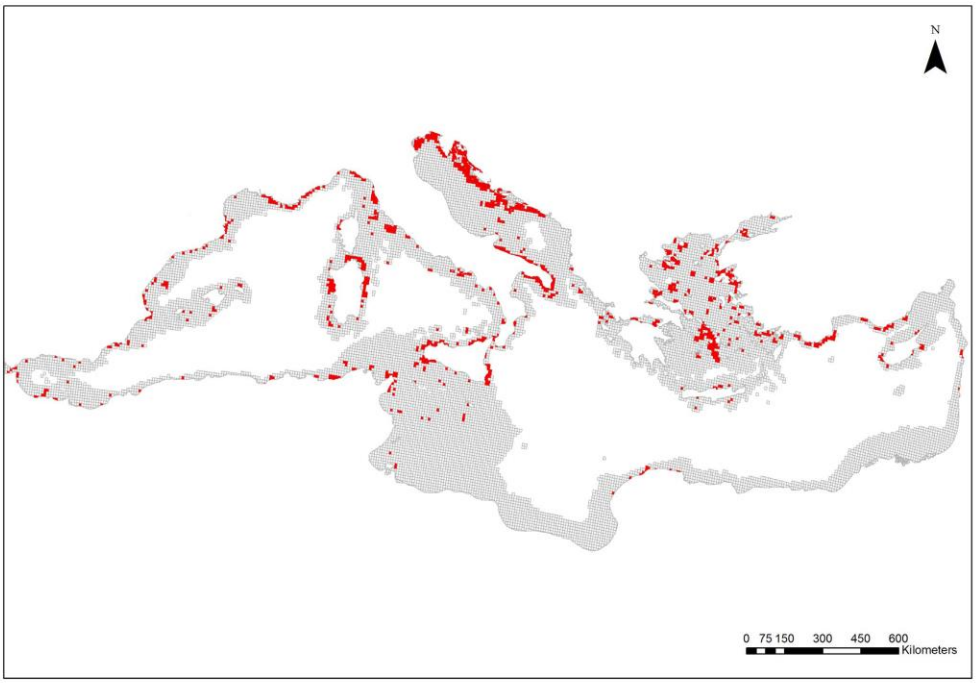

Figure 31. Distribution of coralligenous formations in the Mediterranean Sea. (from: Giakoumi et al., 2013).

III.3. Descriptors D5 – Eutrophication

Eutrophication is a common and well-known phenomenon in coastal waters. Depending on the area, phosphorous rather than nitrogen, or alternate, could represent key limiting nutrients. Thus, their levels have greater importance to understanding eutrophication problems in salt water. Upwelling in coastal systems also promotes increased productivity by conveying deep, nutrient-rich waters to the surface, where algae can assimilate them. Changes in the phytosociological equilibrium related to variations in nutrient loading represent a well-known and critical phenomenon in coastal ecosystems (Duarte, 2002). The ecological cascade induced by increasing levels of nutrients in the water and it is well documented in freshwater, transitional and coastal waters.

Concerning nutrients, despite the high chemical stability of the molecular nitrogen, in seawater is quickly responding to enzymatic activity and thus could appear in any of the nine possible different oxidation states (NO) (from -3 to +5). The reduced nitrogen could be represented by ammonia, $(NH_4)^+$ (NO = -3) and organic compounds. These substances are generally final products of the marine plants or bacteria assimilation and represent approx. 35% of the total nitrogen synthesized in the seawater. The marine nitrogen oxidized forms are nitrite, $(NO_2)^-$ (NO = +3) and nitrate, $(NO_3)^-$ (NO = +5), the latter representing approx. 65% of the synthesized nitrogen forms. Because nitrogen-nitrate is the final oxidation stateis considered that could be naturally present only following oxidative processes. Thus, the inorganic nitrogen forms dominance depends on the redox potential of the seawater. Thereby, as oxygen is higher than more nitrates it will dominate. Additionally, the atmospheric precipitations, the continental drainage and marine animals' migration, excreting nitrogen compounds, are important factors for the nitrogen supply and distribution (Riley and Skirrow, 1965).

Oxygen is the most important of all dissolved gases in the seawater and is easy to quantify it. The dissolved oxygen (DO) concentrations and its influencing factors have a major importance for the marine ecosystems pollution and eutrophication impact assessment because it is necessary both to life and chemical processes of the aquatic environment. DO variability depends on many factors acting antagonistic. Thus, the contributing factors to the waters enrichment in DO are: winds and currents regimes, the contact with the atmosphere acting in the surface layer, as well as the production of DO during photosynthesis. At the same time other factors, more numerous and diverse, act leading to the decrease of the DO level: biological and

chemical processes linked with oxidation (of the reducing agents - H_2S, FeS, of the organic matter, sediments, enzymes, etc.), water masses stratification (Riley, 1971; Horne, 1969; Peres, 1961; Best, 2007).

III.3.1. Mediterranean Sea

A study of spatial and temporal variability of the various variables and indicators (dissolved oxygen, nutrients and chlorophyll concentrations) addressed in the Mediterranean area for Descriptor D5 - Eutrophication, are reported here. Data are collected from the MYOCEAN platform (http://www.myocean.eu) Mediterranean In-situ Thematic Assembly Center (TAC). TAC collects data from the Operational Oceanography data providers along the Mediterranean Sea. Data used in this chapter to define natural variability are from MyOCEAN "Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006)" and from MyOcean (ESA-CCI) Mediterranean sea surface chlorophyll concentration from multi-satellite observation, reprocessed (1997-2012).

In Figure 32 the geographical localization of considered stations in the Mediterranean basin is visualized. Geographical coordinates of the middle of the square area here are considered as a representative of each station and are reported in Table 14**.** Obviously the data collection reported represent a simplification of the Mediterranean ecosystem but could be useful in this Guideline for a first definition of significant spatial and temporal trends of some variables and/or indicators useful to define some milestones of this Guideline concerning the Descriptor D5 – Eutrophication.

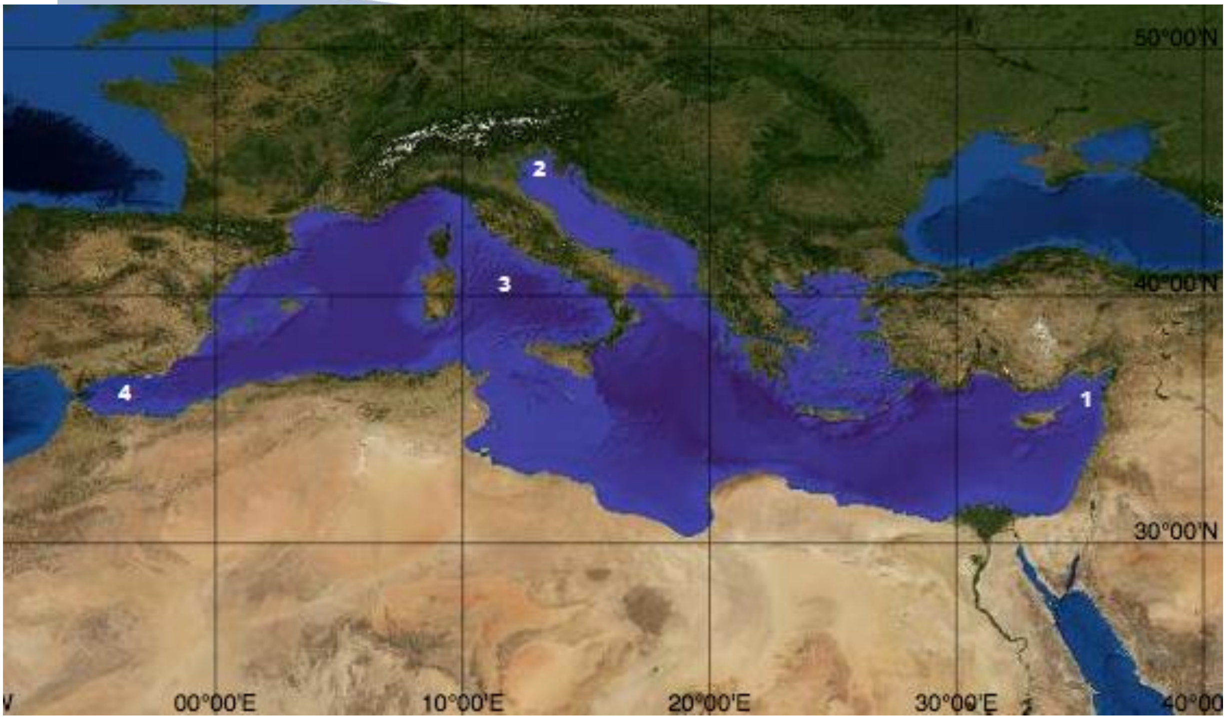

Figure 32. Localization of considered stations in the Mediterranean. Image from MyOCEAN Mediterranean Sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified.

Table 14. Geographical coordinates of the middle of the square area here considered as representative of each station.

Geographical location	Station number	Longitude	Latitude
Turkey	1	34.5	35.7
Northern-Adriatic sea	2	11.7	40.2
Central Thyrrenian Sea	3	12.9	44.8
Alboran Sea	4	-3.4	36.0

Concerning **nutrients**, the influence of the nutritional factor in the temperate zone is, generally based on the following facts: maximum nutrients concentrations are found at the end of the winter and early spring, shortly before phytoplankton blooms, followed by a sharp decrease of the nutrient concentrations after spring blooms, which persist often until autumn; changes into nutrients ratio are similar with those from phytoplankton populations. Thus, the biogenic elements reservoir controls directly the phytoplankton development and the Liebig Law (of the minimum) permits us to state that this development is directly controlled by that nutrient with minimum concentrations. A normal nutrition requires a stable ratio (Redfield ratio) within the main elements, C:N:P=106:16:1. If this ratio is deeply impaired (mainly due to the anthropogenic influences) the photosynthetic activity is altered. From the three elements only

Nitrogen and Phosphorus could play a limitative role (Carbon is found in sufficient quantities in the seawater due to the carbonate system).

The three inorganic nitrogen forms (nitrates, nitrites and ammonium) are equally utilized by the phytoplankton, but it seems that ammonium is preferred (due to the less energetic effort to reduce the nitrogen which has the NO=-3).

In this context the natural variability could be highly altered in the neighbourhood of the wastewater treatment plants or other ammonium sources. Still, due to its higher stability as final form of the oxidative processes, nitrates are an indicator of oxic water productivity. Phosphates concentration represents equally a must for the phytoplankon proliferation and represents an important variable as well for the natural fluctuations of the associated indicator (nutrient concentration).

Usually, the nutrients concentrations of the phytoplankton are higher than those of the seawater, thus it is outlined the role of the biological regeneration, nutrients input form the water masses circulation, resuspension from sediments.

As reported by Manca and colleagues (2004 and citations therein) "*nutrient enrichment is reported at the surface in the presence of convective chimneys in the Gulf of Lions, in the southern Adriatic gyre and during severe winters in the area of the northern Levantine occupied by the cyclonic Rhodes Gyre. On the contrary, low nutrient values have been detected in the neighbouring anticyclones. In the upper layer, nitrate concentrations are higher in winter than in summer, when oxygen rich and very low nutrient surface waters are rapidly capped creating conditions of high oligotrophy in the subsurface layer. Differences in nutrient concentration and changes in biodiversity between the Eastern and Western Mediterranean, due to the different physiography of the two interconnected basins, have been verified from a fully coupled physical and biochemical cycling model. The inverse estuarine circulations cause a net loss of nutrients in the eastern and western basins through the Sicily and the Gibraltar Straits, respectively*".

Nitrates - Spatial variability

In Table 15, levels of nitrates in surface waters are reported as range of variability (minimum

– maximum value) for each station in the Mediterranean Sea.

Table 15. Nitrates, spatial variability. Data were collected from MyOCEAN Mediterranean Sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006) and are referred to the period within 2013-July 2014 included.

Geographical location	Number of considered station	$mmol/m^3$ surface water
Turkey	1	<0.01 – 2.10
Northern-Adriatic sea	2	2.80 – 3.60
Central Thyrrenian Sea	3	<0.01 – 0.50
Alboran Sea	4	0.01 – 0.75

Nitrates – Temporal variability

In Table 16 levels of nitrates in surface waters are reported as a week average (standard deviation) for each station in the Mediterranean Sea both in July 2013 and 2014.

Table 16. Nitrates, temporal variability (surfaceperficial water, week average, standard deviation). Data were collected from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006) and are referred to the same week

Geographical location	Number of considered station	$mmol/m^3$ July 2013	$mmol/m^3$ July 2014
Turkey	1	2.13 (0.25)	0.34 (0.09)
Northern-Adriatic sea	2	3.45 (0.32)	2.80 (0.26)
Central Thyrrenian Sea	3	0.57 (0.09)	0.16 (0.05)
Alboran Sea	4	0.15 (0.04)	0.11 (0.03)

Nitrates –Spatial (Horizontal and Vertical) *versus* Temporal variability

Vertical profiles of levels of nitrates are reported for the same day in July 2013 (Figure 33) and July 2014 (Figure 34) for each station in the Mediterranean Sea.

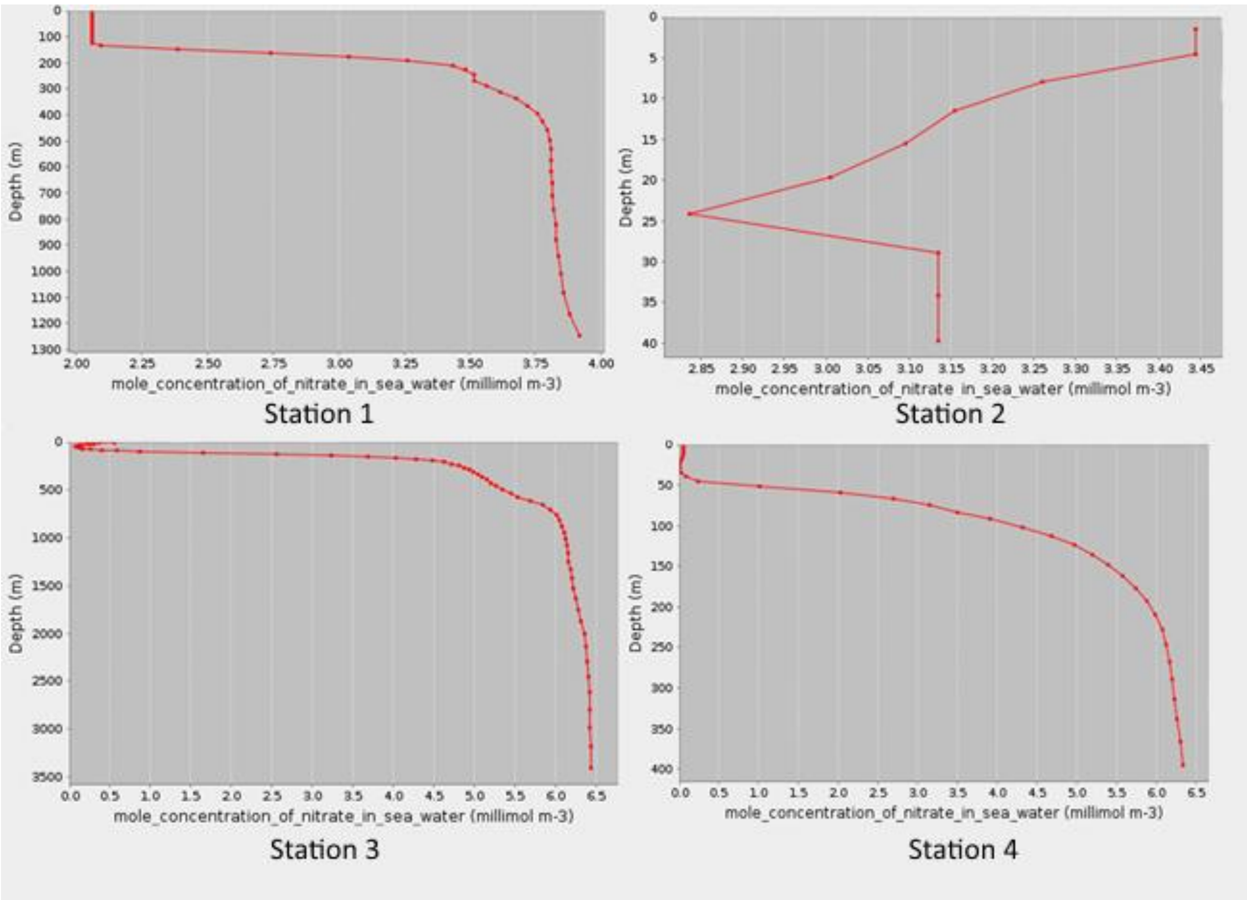

Figure 33. July 2013. Images are from MyOCEAN Mediterranean Sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified.

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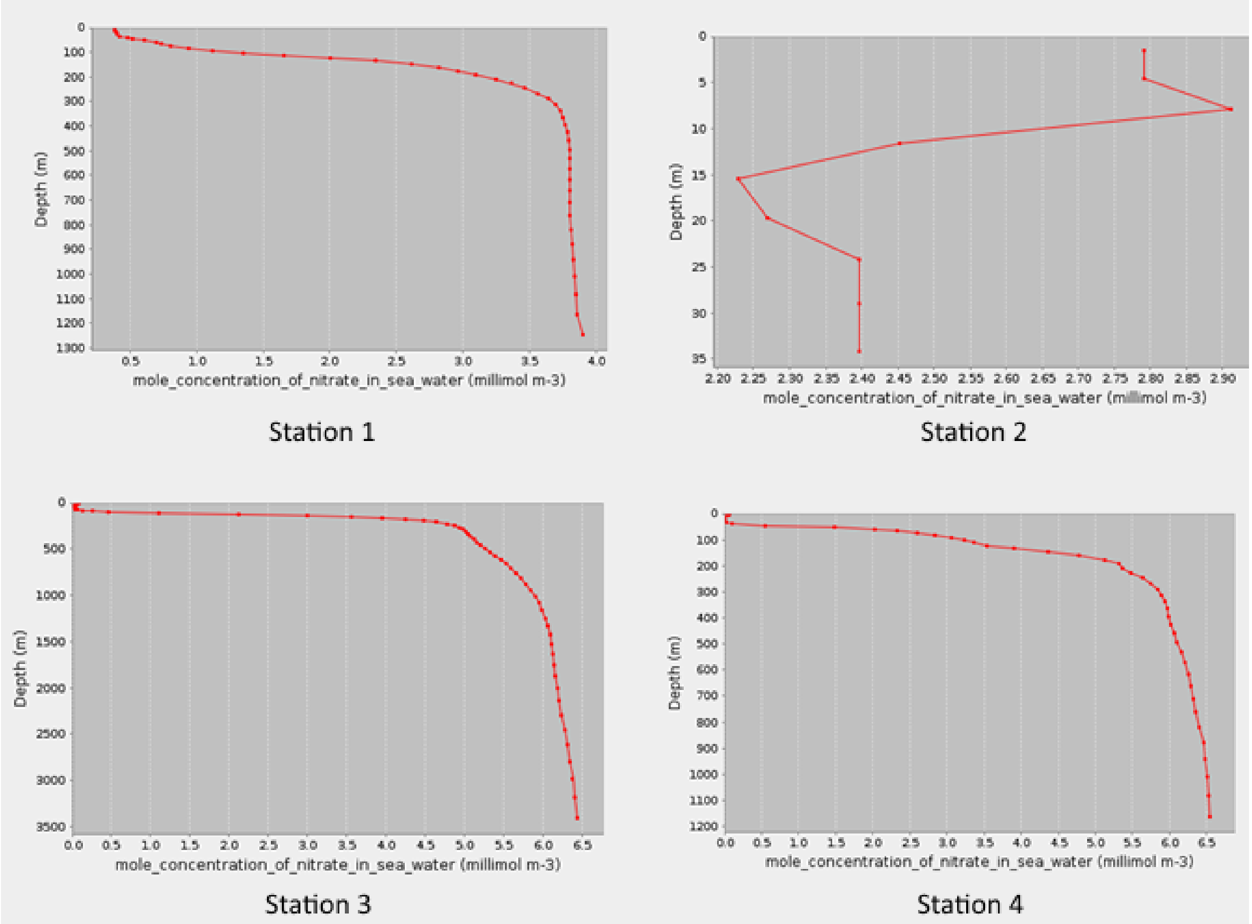


Figure 34. July 2014. Images are from MyOCEAN Mediterranean Sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified.

<u>Nitrates –Seasonality</u>

Vertical profiles of levels of nitrates are reported for different months during the years 2013-2014 for the Station 2 (Northern Adriatic, **Figure 35**) and Station 3 (Central Thyrrenian, Figure 36). Reported images are from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified.

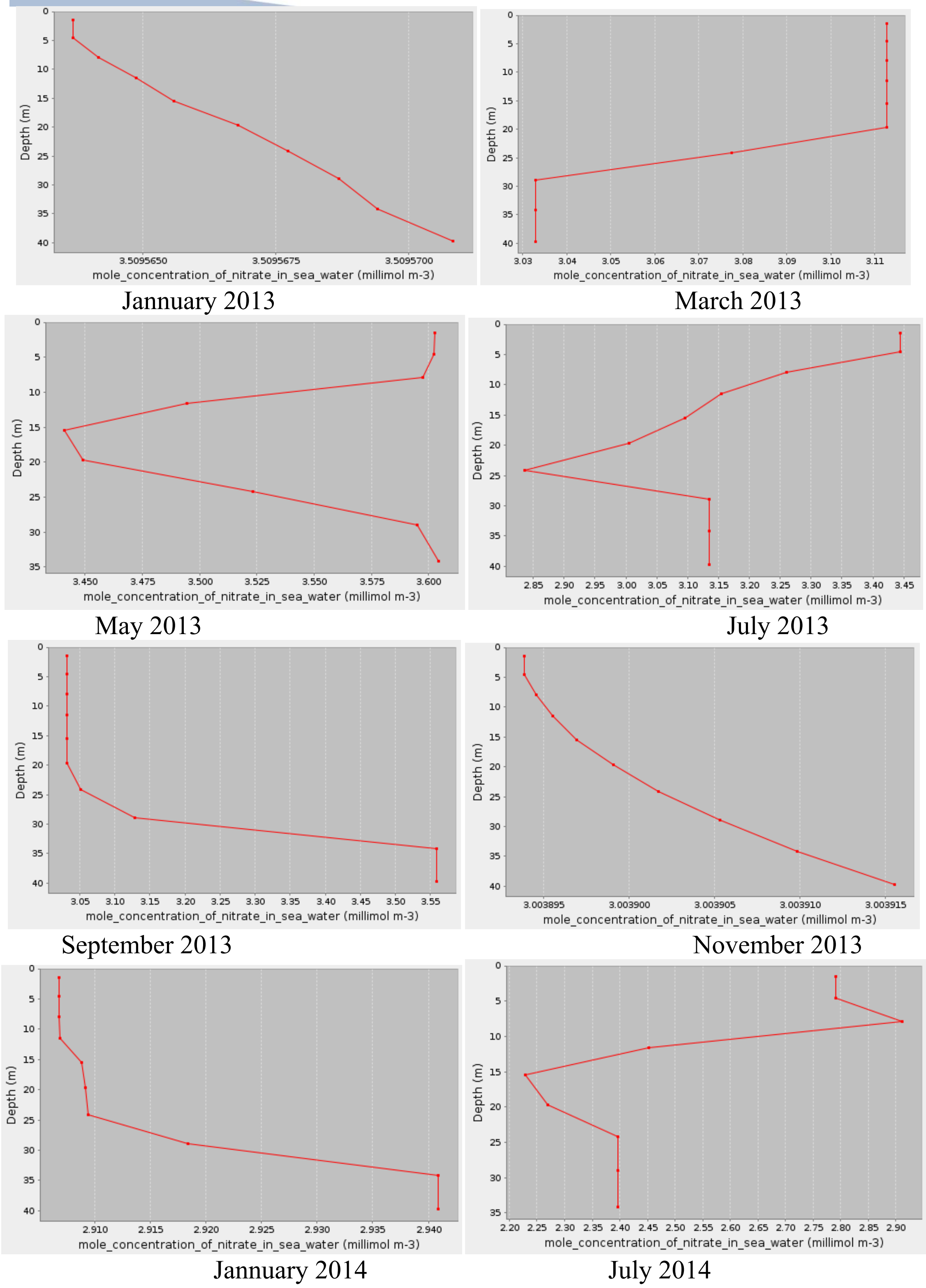


Figure 35. Vertical profiles of the levels of nitrates representative of different months during the years 2013-2014 for the Station 2 (Northern Adriatic).

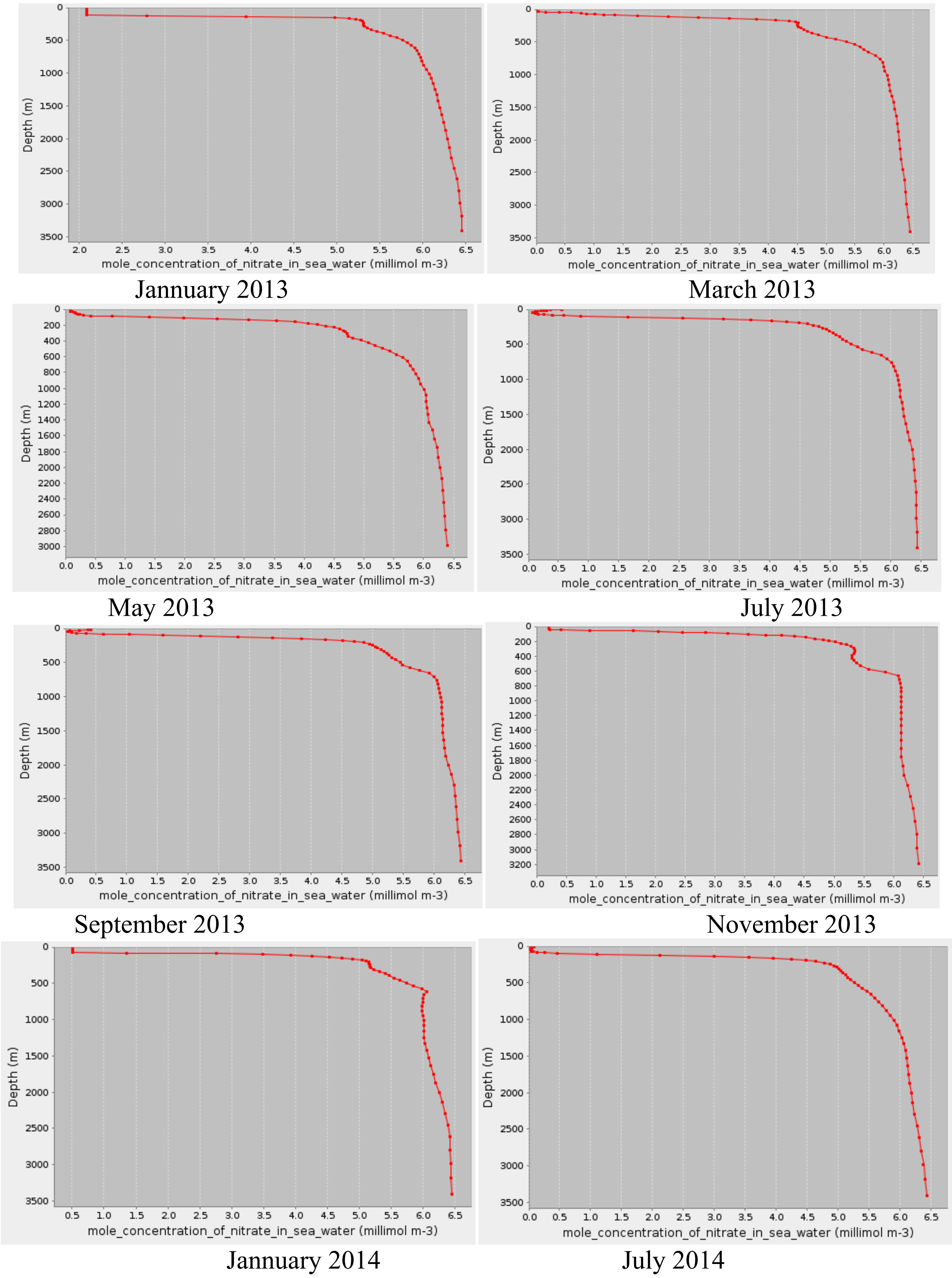


Figure 36. Vertical profiles of the levels of nitrates representative of different months during the years 2013-2014 for the Station 3 (Northern Adriatic).

Phosphates - Spatial variability

In Table 17 levels of phosphates in surface waters are reported as a range of variability (minimum – maximum value) for each station in the Mediterranean Sea.

Table 17. Phosphates, spatial variability. Data were collected from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006) and are referred to the period within 2013-July 2014 included.

Geographical location	Number of considered station	$mmol/m^3$ surface water
Turkey	1	0.006-0.040
Northern-Adriatic sea	2	0.004 – 0.018
Central Thyrrenian Sea	3	0.003 – 0.065
Alboran Sea	4	0.007 – 0.110

Phosphates – Temporal variability

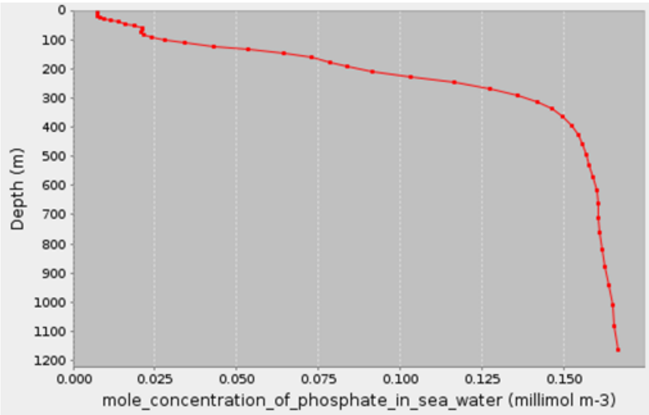
In Table 18 levels of phosphates in surface waters are reported as a week average (standard deviation) for each station in the Mediterranean Sea both in July 2013 and 2014.

Table 18. Phosphates, temporal variability (surface-superficial water, week average, standard deviation). Data were collected from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006) and are referred to the same period within July 2013 – 2014.

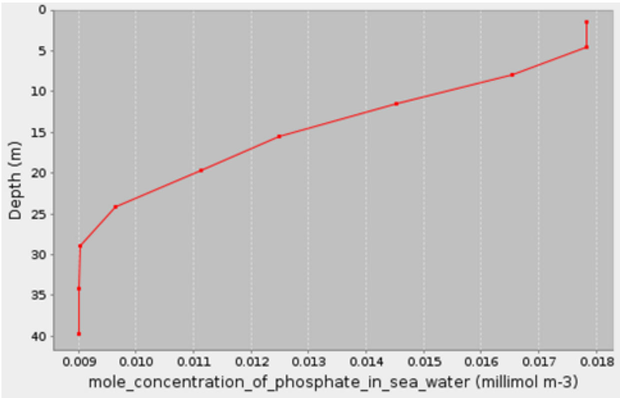
Geographical location	Number of considered station	$mmol/m^3$ July 2013	$mmol/m^3$ July 2014
Turkey	1	0.006	0.008
Northern-Adriatic sea	2	0.017	0.010
Central Thyrrenian Sea	3	0.013	0.008
Alboran Sea	4	0.025	0.014

Phosphates –Spatial (Horizontal and Vertical) *versus* temporal variability

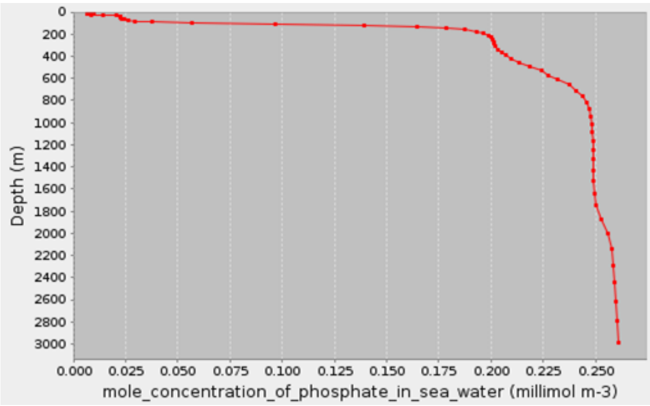
Vertical profiles of the levels of phosphates are reported for the same day in July 2013 (Figure 37) and July 2014 (Figure 38) for each station in the Mediterranean Sea.



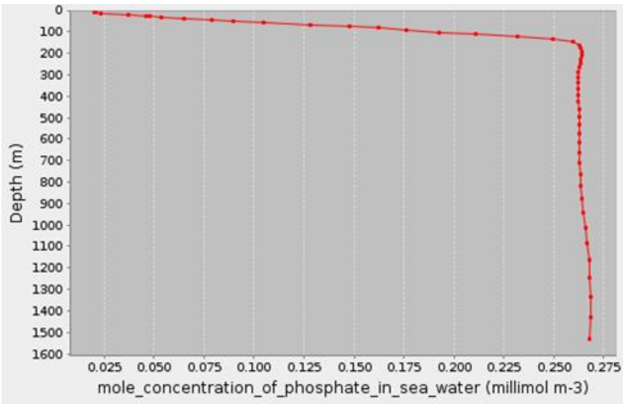
Station 1



Station 2

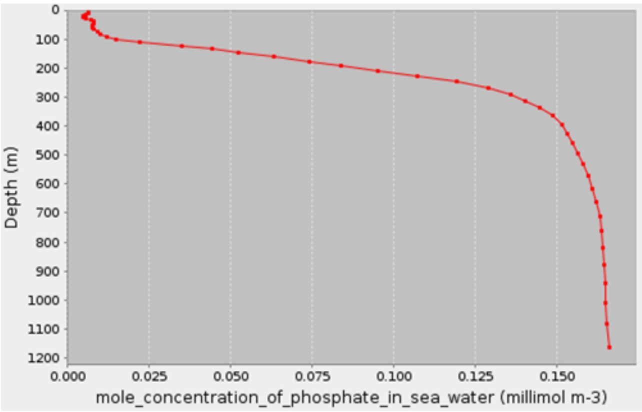


Station 3

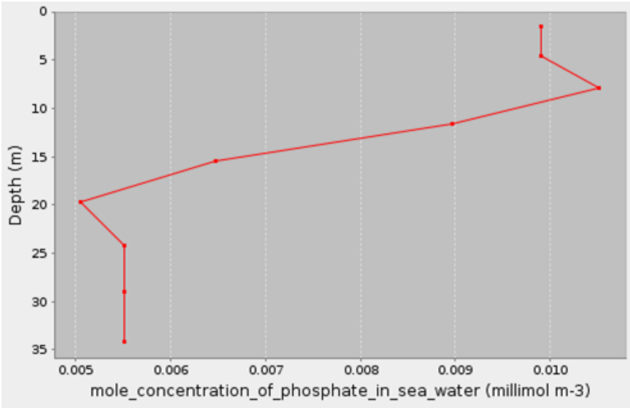


Station 4

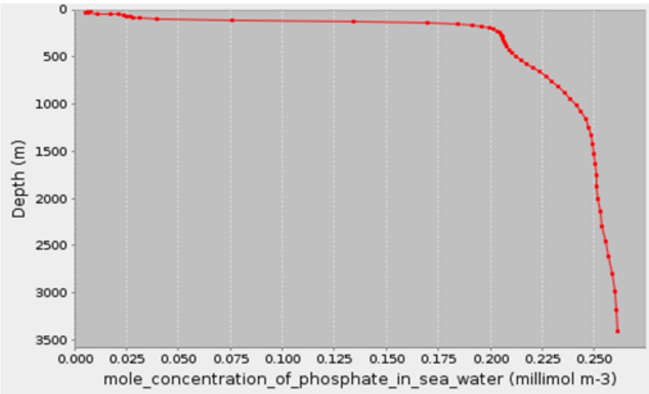
Figure 37. Phosphates –Spatial (Horizontal and Vertical) versus Temporal variability, July 2013. Images are from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified.



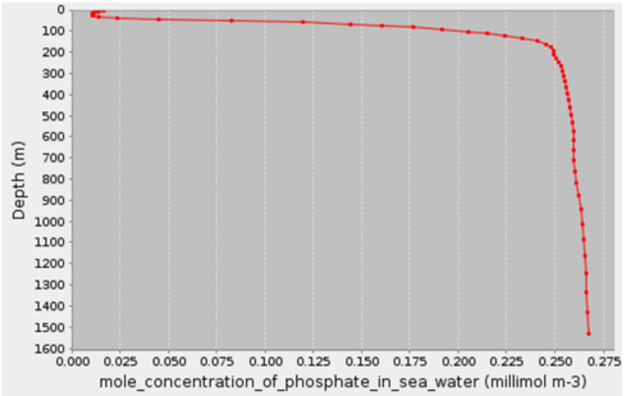
Station 1



Station 2



Station 3

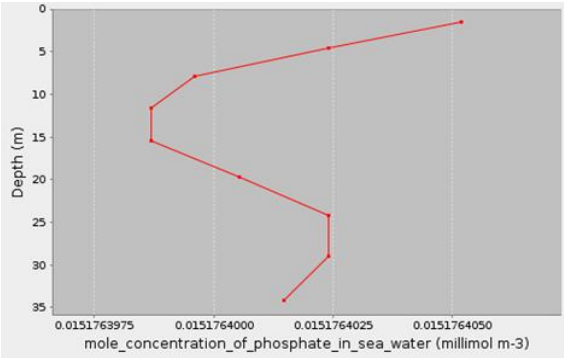


Station 4

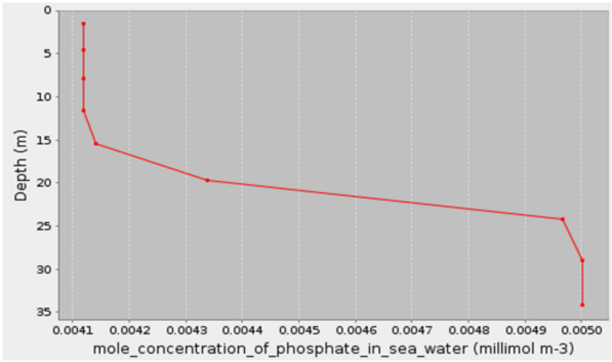
Figure 38. Phosphates –Spatial (Horizontal and Vertical) versus Temporal variability, July 2014. Images are from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified.

Phosphates –Seasonality

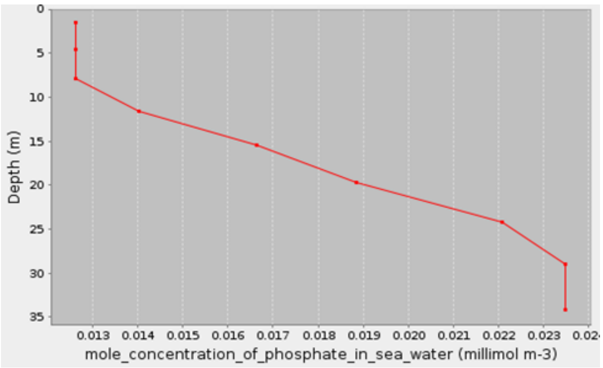
Vertical profiles of the levels of phosphates are reported for different months during the years 2013-2014 for the Station 2 (Northern Adriatic, Figure 39) and Station 3 (Central Thyrrenian, Figure 40).



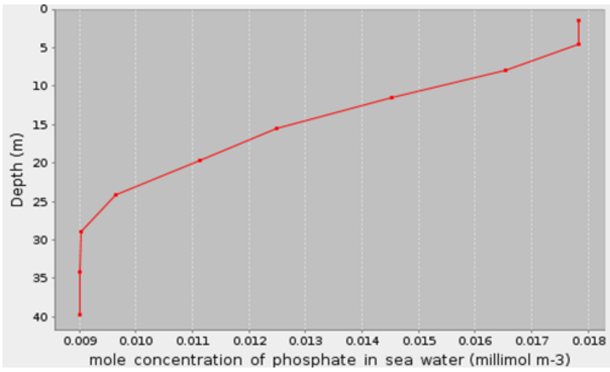
January 2013



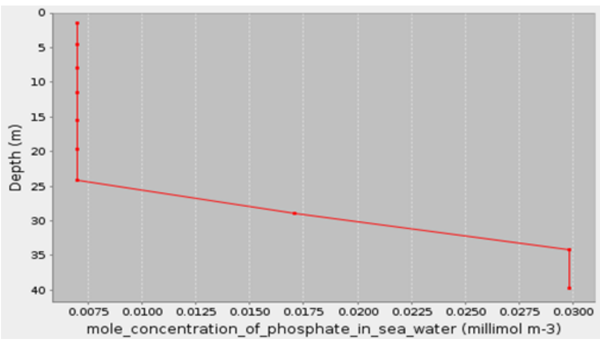
March 2013



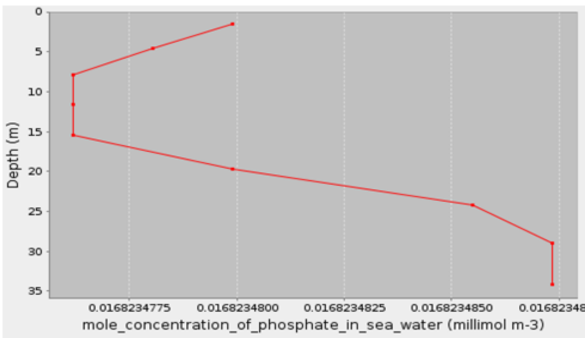
May 2013



July 2013

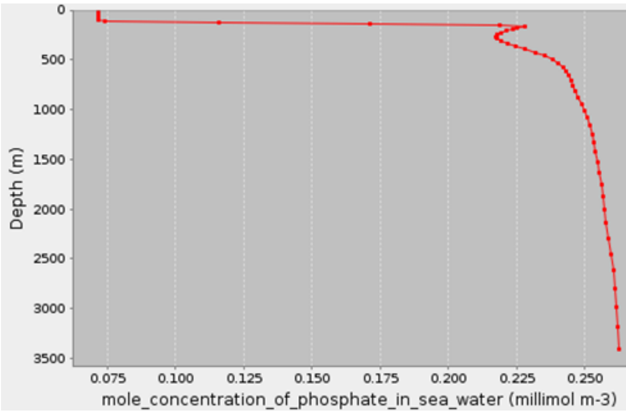


September 2013

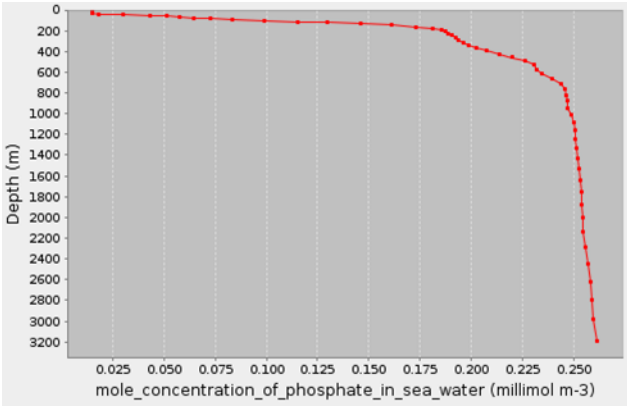


November 2013

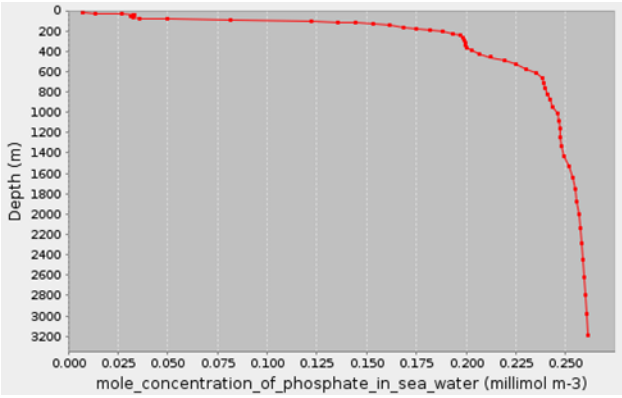
Figure 39. Vertical profiles of the levels of phosphates representative of different months during the year 2013 for the Station 2 (Northern Adriatic).



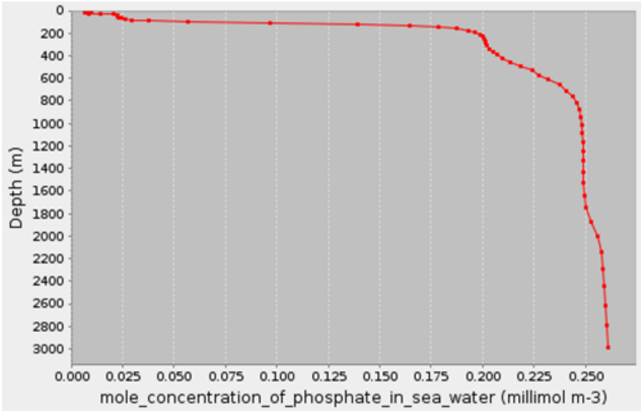
January 2013



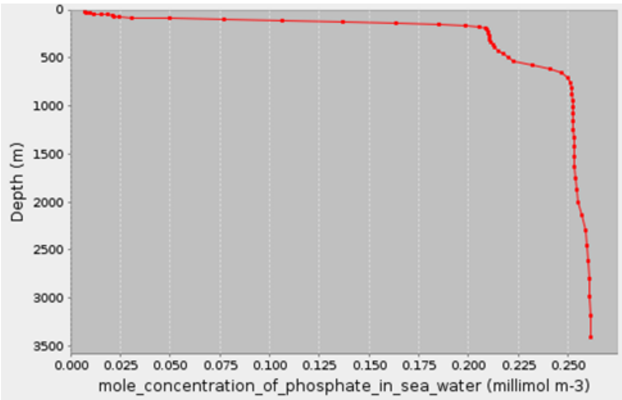
March 2013



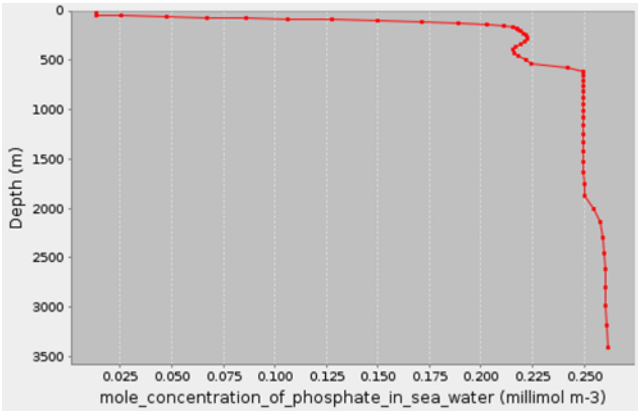
May 2013



July 2013



September 2013



November 2013

Figure 40. Vertical profiles of the levels of phosphates representative of different months during the year 2013 for the Station 3 (Northern Adriatic).

Concerning **water masses**, the Mediterranean Sea is a semi-enclosed basin characterised by rough bottom topography with a narrow continental shelf (<200 m) and a steep continental slope (Manca et al., 2004).

The Mediterranean Sea occupies an elongated area of about 2.5 million km^2 between Europe and Africa, and has only a restricted communication with the world ocean, through the narrow and shallow Strait of Gibraltar. It is further subdivided into two main basins, the Eastern Mediterranean (EMED) and the Western Mediterranean (WMED), communicating through the Sicily Channel. Due to its relatively small size, its geographical location, and its semi-land locked nature, the Mediterranean Sea is very sensitive and responds quickly to atmospheric forcings and/or anthropogenic influences.

The thermohaline circulation of the Mediterranean Sea exhibits strong seasonal and interannual variability, and is extremely complex, consisting of numerous eddies and current meanders. It is occupied at different levels by a number of water masses, either formed inside the sea or imported from the Atlantic Ocean. The upper branch of the Mediterranean Thermohaline Current (MTHC) that carries the relatively fresh AW towards the interior of the sea extends over the WMED and EMED and displays a rather complex surface circulation that could be considered as a superposition of interacting large-scale and mesoscale patterns, each of them showing their own variability. A debate on the surface circulation in the EMED still remains. The lower branch of the MTHC is differently affected by the sea topography. While Levantine Intermediate Water (LIW), the most important intermediate water, resides in the EMED at depths from which it can flow without major topographic constrictions through the Sicily Channel into the WMED, depicting a rather continuous return flow, the deep MWs circulation cells are separated by the topography of the channel and driven by specific DWF processes in the Adriatic/Aegean (for EMED) and the Provençal (for WMED) subbasins. Even if intermediate and deep circulations forming the lower branch of the MTHC are partially coupled to each other, they also have their own scales of variability that do not necessarily coincide.

The expected quick response, of a water body of reduced dimensions such as the Mediterranean Sea, to the atmospheric forcing variability through its surface has propitiated a considerable amount of literature regarding changes in MWs properties inside the sea. Recently a new possibility that changes that are being observed in the MWs properties have originated by changes in the properties of the inflowing AW or on the fresh water budgets modified by massive river damming (continental freshwater inputs) has opened new perspectives to the

analysis of the observed variability of MWs (Schroeder et al., 2012).

Among all dissolved gases in the seawater, oxygen is the most important and representative one for the ecosystem assessment, particularly due to the ease of its measurement. Its concentrations and influencing factors have a significant importance in the assessment of the eutrophication impact on the marine ecosystems.

The natural variability of dissolved oxygen depends on many factors sometimes acting antagonistically. The factors contributing to the seawater oxygen enrichment are: currents and winds regime, contact with atmosphere (in the surface layer), photosynthetic processes (both from phytoplankton and macroalgae). At the same time, factors are contributing to the seawater oxygen depletion as: increase of the temperature and salinity, respiration, organic matter decomposition, water masses stratification (Riley, 1971, Horne, 1969, Peres, 1961, Best, 2007).

Dissolved oxygen concentrations in the seawater are generally distributed in the water column in 4 layers, as follows (Horne, 1969):

- the surface layer (mixing layer) – dissolved oxygen is in equilibrium with the atmosphere content - high concentrations, homogenous.
- the photosynthetic layer–dissolved oxygen has maximum concentrations due to the photosynthetic production.
- the depth layer – the dissolved concentrations are decreased due to the organic matter composition.

As described by Manca and colleagues (2004 and references therein) "*Seasonal characteristics and interannual variations of the circulation elements affect the distribution of the biochemical species such as dissolved oxygen and nutrients, as well as the magnitude and the composition of phytoplankton biomass. It has been demonstrated that the Mediterranean Sea is in a non-steady-state situation. A marked long-term warming trend and salinity increase in the deep water of the Western Mediterranean has been detected since 1960, i.e. from the period when the accuracy of the observations have revealed differences significantly greater than possible instrumental errors. These variations have been mostly attributed to changes in climate. These trends have been estimated to be about 0.027 jC and 0.019 units per decade in temperature and salinity, respectivelyin the Aegean Sea. More saline, warmer and denser waters (Si38.85, hi13.80 jC, r_hi29.22 kg m^{-3}) than the EMDW of Adriatic origin (Si38.66, hi13.30 jC, r_hi29.18 kg m^{-3}), flowing out through the Cretan Arc Straits, sank to the bottom layer of the central*

Mediterranean regions, replacing almost 20% of the dense water below 1200 m. This event, named as Eastern Mediterranean Transient, has been attributed to an important meteorological anomaly that occurred in the Eastern Mediterranean at the beginning of the 1990s. Changes in the distributions of salt and of the biogeochemical materials have also been observed in the intermediate and deep layers of the Eastern Mediterranean".

Dissolved oxygen - Spatial variability

In Table 19, levels of dissolved oxygen in surface waters are reported as range of variability (minimum – maximum value) for each station in the Mediterranean Sea.

Table 19. Dissolved oxygen, spatial variability. Data were collected from MyOCEAN Mediterranean Sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006) and are referred to the period of July 2013-2014

Geographical location	Number of considered station	mmol/m^3 surface water
Turkey	1	177-191
Northern-Adriatic sea	2	175-203
Central Thyrrenian Sea	3	185-199
Alboran Sea	4	169-199

Dissolved oxygen – Temporal variability

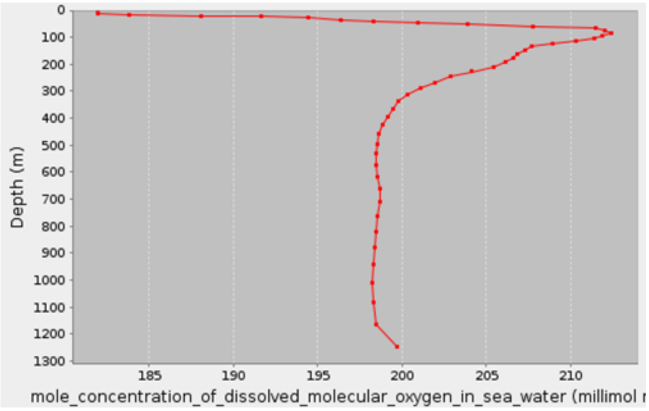
In Table 20, levels of dissolved oxygen in surface waters are reported as week average (standard deviation) for each station in the Mediterranean Sea both in July 2013 and 2014.

Table 20. Dissolved oxygen – Temporal variability (surfaceSuperficial water, week average, standard deviation). Data were collected from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006) and are referred to the period July 2013 – 2014.

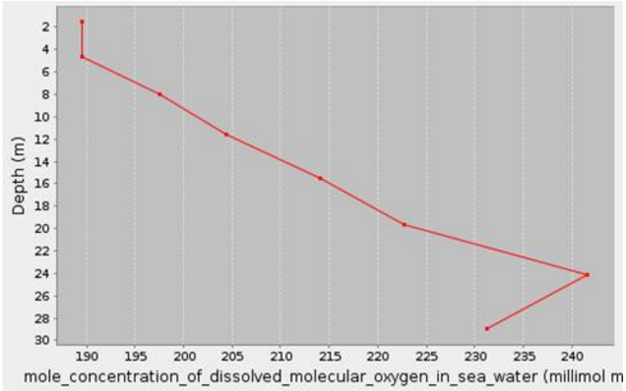
Geographical location	Number of Considered station	mmol/m^3 July 2013	mmol/m^3 July 2014
Turkey	1	182 (5)	184 (7)
Northern-Adriatic sea	2	190 (12)	195 (12)
Central Thyrrenian Sea	3	187 (3)	196 (4)
Alboran Sea	4	197 (5)	173 (9)

Dissolved oxygen –Spatial (Horizontal and Vertical) *versus* Temporal variability

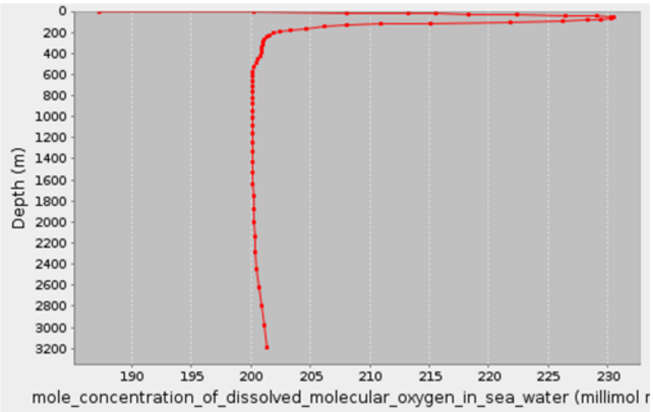
Vertical profiles of the levels of phosphates are reported for the same day in July 2013 (Figure 41) and July 2014 (Figure 42) for each station in the Mediterranean Sea.



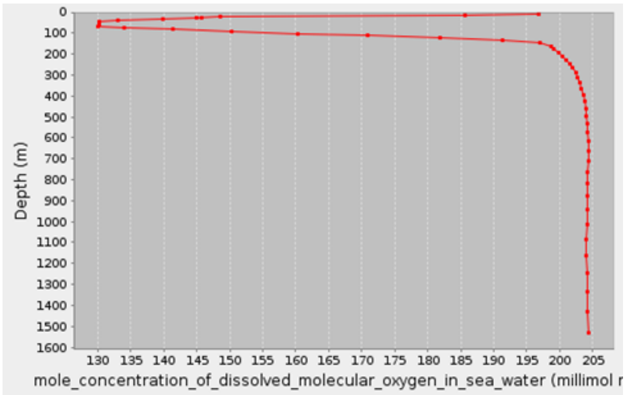
Station 1



Station 2

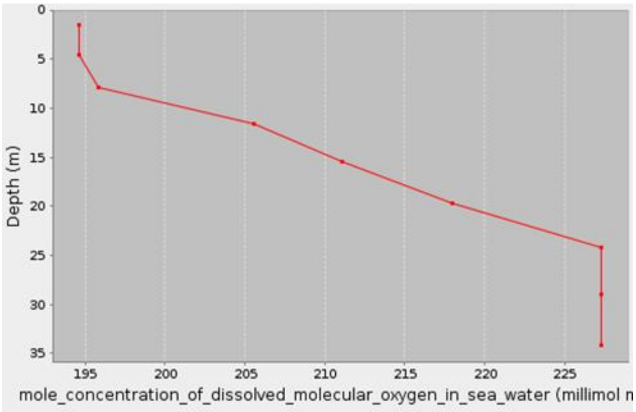


Station 3

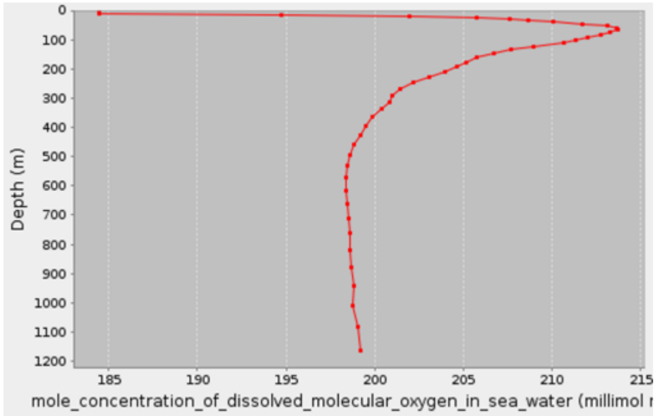


Station 4

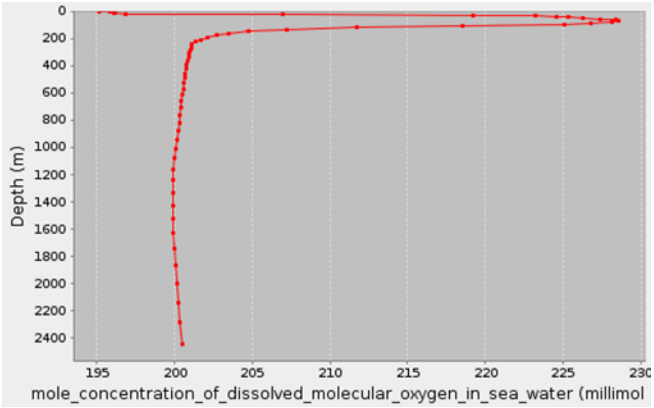
Figure 41. Dissolved oxygen, July 2013. Images are from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified.



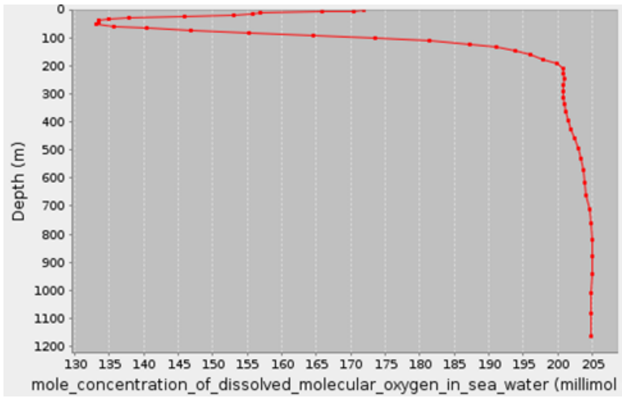
Station 1



Station 2



Station 3



Station 4

Figure 42. Dissolved oxygen, July 2014. Images are from MyOCEAN Mediterranean sea biogeochemical analysis and Forecast (MEDSEA-Analysis-Forecast-BIO-006-006), modified

A summary concerning nutrients and water masses descriptors for different areas of the Mediterranean Sea extracted from literature data is, also, reported (Manca et al., 2004). Average ± standard deviation (number of samples) values are reported for different geographical areas and depth for temperature (°C), salinity (PSU), Oxygen (mL/L), Nitrates (mmol/m^3), Phosphates (mmol/m^3) and Silicates (mmol/m^3). In particular are reported: i) the layer-averaged hydrographic properties and nutrient concentrations calculated for four regions in the Western Mediterranean (Table 21), ii) a summary of the most important water mass properties and their vertical and spatial differences in three regions of the Eastern Mediterranean (Table 22), iii) the hydrochemical properties in the marginal basins of the Eastern Mediterranean (Table 23).

Table 21. Hydrochemical properties of the water masses in four regions in the Western Mediterranean (by Manca et al., 2004).

Water mass	Temperature (°C)	Salinity (psu)	Oxygen (ml l^{-1})	Nitrate (mmol m^{-3})	Phosphate (mmol m^{-3})	Silicate (mmol m^{-3})
Gulf of Lions—DF2						
Surface water (0−5 m)	17.61±2.30 (14,218)	37.88±0.45 (9472)	5.44±0.24 (2182)	1.45±2.10 (352)	0.13±0.12 (757)	1.37±1.01 (820)
LIW (400 m)	13.17±0.11 (3101)	38.48±0.03 (2306)	4.48±0.17 (610)	6.31±0.58 (55)	0.34±0.08 (208)	6.26±1.02 (87)
WMDW (≥1500 m)	13.04±0.02 (3218)	38.42±0.01 (3473)	4.60±0.07 (1214)	7.78±0.51 (17)	0.39±0.04 (184)	8.16±0.71 (128)
Tyrrhenian North—DT1						
Surface water (0−5 m)	18.90±2.54 (11,208)	37.91±0.25 (2867)	5.55±0.26 (576)	0.24±0.22 (110)	0.08±0.07 (282)	2.17±0.59 (168)
LIW (500 m)	13.88±0.13 (593)	38.65±0.03 (391)	4.29±0.13 (179)	5.91±0.56 (32)	0.31±0.07 (61)	6.85±0.79 (29)
TDW (≥1500 m)	13.21±0.04 (277)	38.47±0.02 (185)	4.32±0.13 (139)	7.32±0.74 (10)	0.42±0.08 (20)	7.73±0.66 (12)
Algerian East—DS4						
Surface water (0−5 m)	19.56±2.52 (7684)	37.12±0.14 (1112)	5.31±0.33 (340)	0.05±0.10 (207)	0.04±0.05 (307)	0.99±0.33 (90)
LIW (500 m)	13.30±0.09 (988)	38.52±0.02 (459)	4.14±0.12 (168)	8.90±0.40 (36)	0.40±0.07 (81)	7.58±0.89 (26)
WMDW (≥1500 m)	13.04±0.04 (778)	38.42±0.02 (548)	4.49±0.12 (287)	8.28±0.11 (39)	0.39±0.05 (73)	9.29±0.27 (29)
Alboran Sea—DS1						
Surface water (0−5 m)	17.85±0.616 (18,874)	36.57±0.28 (7122)	5.44±0.33 (1877)	0.44±0.49 (733)	0.11±0.11 (1046)	1.33±1.20 (642)
LIW (500 m)	13.07±0.08 (2014)	38.45±0.04 (1588)	4.21±0.17 (320)	8.49±0.89 (126)	0.37±0.07 (121)	8.36±1.22 (112)
WMDW (≥1500 m)	13.08±0.03 (176)	38.44±0.01 (170)	4.50±0.09 (21)	9.13±3.91 (6)*	0.41±0.06 (8)*	8.381±0.99 (7)

Table 22. Hydrochemical properties of water masses in three regions of the Eastern Mediterranean (by Manca et al., 2004).

Water mass	Temperature (°C)	Salinity (psu)	Oxygen (ml l^{-1})	Nitrate (mmol m^{-3})	Phosphate (mmol m^{-3})	Silicate (mmol m^{-3})
Ionian South—DJ5						
Surface water (0−5 m)	20.26±2.49 (6208)	38.40±0.19 (545)	4.85±0.20 (531)	0.23±0.29 (103)	0.04±0.04 (244)	1.22±1.01 (139)
LIW (250 m)	14.72±0.18 (2390)	38.85±0.06 (284)	4.67±0.13 (279)	3.24±0.73 (49)	0.10±0.04 (108)	3.22±0.97 (68)
EMDW (≥1500 m)	13.70±0.02 (878)	38.69±0.02 (410)	4.40±0.11 (418)	4.89±0.37 (103)	0.18±0.04 (146)	8.65±0.49 (95)
Levantine North—DL1						
Surface water (0−5 m)	21.72±2.20 (8816)	39.16±0.10 (1995)	5.06±0.26 (1395)	0.21±0.24 (450)	0.06±0.05 (513)	0.89±0.78 (465)
LIW (125 m)	15.69±0.44 (4479)	39.02±0.05 (1087)	5.05±0.20 (720)	0.62±0.54 (237)	0.08±0.07 (313)	1.36±0.99 (262)
EMDW (≥1500 m)	13.77±0.04 (1973)	38.73±0.05 (972)	4.10±0.17 (1191)	4.59±0.92 (351)	0.25±0.07 (383)	8.94±1.47 (396)
Levantine South—DL3						
Surface water (0−5 m)	23.05±2.26 (5890)	38.97±0.39 (1116)	5.00±0.40 (1240)	0.21±0.42 (157)	0.09±0.04 (444)	1.86±1.70 (329)
LIW (125 m)	15.60±0.29 (2461)	38.97±0.06 (311)	5.03±0.15 (296)	1.13±0.63 (54)	0.09±0.05 (152)	1.92±1.23 (127)
EMDW (≥1500 m)	13.70±0.03 (632)	38.72±0.04 (309)	4.22±0.11 (385)	4.12±1.20 (90)	0.28±0.09 (129)	8.55±1.65 (96)*

Table 23. Hydrochemical properties in the marginal basins of the Eastern Mediterranean

(by Manca et al., 2004).

Water mass	Temperature (°C)	Salinity (psu)	Oxygen (ml l^{-1})	Nitrate (mmol m^{-3})	Phosphate (mmol m^{-3})	Silicate (mmol m^{-3})
Adriatic South—DJ3						
Surface water (0–5 m)	16.58±2.53 (14,218)	37.93±0.69 (9739)	5.47±0.26 (3000)	0.57±0.47 (1665)	0.04±0.03 (2442)	2.86±1.43 (1201)
LIW (200 m)	13.63±0.18 (1351)	38.70±0.04 (1039)	4.97±0.22 (372)	4.98±0.70 (235)	0.18±0.05 (244)	5.38±1.32 (210)
ADW (≥1000 m)	12.88±0.05 (812)	38.59±0.01 (768)	4.89±0.18 (272)	5.25±0.65 (208)	0.19±0.05 (245)	9.67±2.61 (193)
Sicily Strait—DI3						
Surface water (0–5 m)	19.23±2.59 (11,709)	37.47±0.20 (3476)	5.25±0.37 (1468)	0.48±0.76 (455)	0.06±0.06 (526)	1.09±0.59 (189)
LIW (400 m)	14.02±0.13 (1823)	38.75±0.02 (678)	4.30±0.13 (215)	5.46±0.87 (54)	0.20±0.05 (64)	7.25±1.33 (47)
EMDW (≥1500 m)	13.83±0.04 (12)	38.73±0.01 (10)	4.21±0.01 (4)	5.00±0.31 (2)*	0.18±0.04 (4)*	5.89±1.29 (4)**

* The deepest value is at 1200 m.
** The deepest value is at 1000 m.

Chlorophyll

It is well-known that concentrations of chlorophyll in the water are directly associated to phytoplankton abundance and due to this direct relationship among chlorophyll levels measured in the water and phytoplankton cell numbers this variable could be used (with opportune precautions) as proxy (indicator) of primary productivity. Increases of chlorophyll are associated to phytoplancton proliferations and, indirectly, to water eutrophication. Unicellular algal species are able to use different chlorophylls. Among these photosynthetic pigments the Chlorophyll-a is universal, while Chlorophyll-b is principally present in terrestrial plants and Chlorophyll-c is widespread in algae. Chlorophyll-d and –f are photosynthetic pigments that are typical of Cyanobacteria. Different methods to quantify the autotrophic standing stock in marine water are available. Among them, the principally used are i) the spetrophotometric determination of the chlorophyll in collected water samples (direct method); ii) the chlorophyll quantification throughout the use of multiparameter probe sensor (direct method); iii) the quantification of light emission at specific wavelenght of surface water throughout satellite imaging analysis (indirect method). The first and second methods require field survey to be performed. In the first case, collection, pre-treatments, storage and laboratory analyses are required, while in the second case acquisitions are performed throughout a multiparameter field-probe within standard physico-chemical descriptors of water masses (e.g. temperature, salinity). Even if the first method is characterized by higher sensitivity and by the possibility to make distinctions between different chlorophyll pigments, the second allow acquiring chlorophyll data with the same sampling frequency of physico-chemical ones performing

complete vertical profiles of this variable at lower costs. Furthermore, errors associated to collected data are reduced due to the reduced number of steps of the data acquisition procedure. The chance to collect data on chlorophyll using the satellites technology is a recent powerful and interesting challenge. The great spatio-temporal coverage that this technique could provide for the detection of chlorophyll in the water allow to monitor the whole surface water mass of national competence at real time increasing data acquired (in theory each pixel of a satellite image contain information on concentration of chlorophyll) and allow to detect surfacesources of eutrophication. The principal limits of these methods are represented by cloud interferences, application related only to surface layers of water, low possibility to detect surface stratifications and costs for the satellite images acquisitions. Data obtained by different methods are difficult to compare, for this reason the source of data considered for the definition of spatial and temporal variability of chlorophyll should be indicated and accurately discussed.

Chlorophyll– Spatial variability

In Table 24 levels of chlorophyll in surface waters are reported as range of variability (minimum – maximum value) for each station in the Mediterranean Sea.

Table 24. Chlorophyll, spatial variability. Data were collected from MyOcean platform and are referred to the same stations or when impossible to stations closed to those reported in Tables. To determine spatial ranges, values are collected in the same day of the year.

Geographical location	Station number	Chlorophyll-a levels (mg/m^3) range 1998-2012
Turkey	1	0.018-0.106 (0.024)
Northern-Adriatic sea	2	0.089-4.570 (1.078)
Central Thyrrenian Sea	3	0.023-0.180 (0.035)
Alboran Sea	4	0.066-7.354 (2.173)

Chlorophyll – Temporal variability

In Figure 43 levels of chlorophyll in surface waters are reported for each station in the Mediterranean Sea during the period 1998-2012.

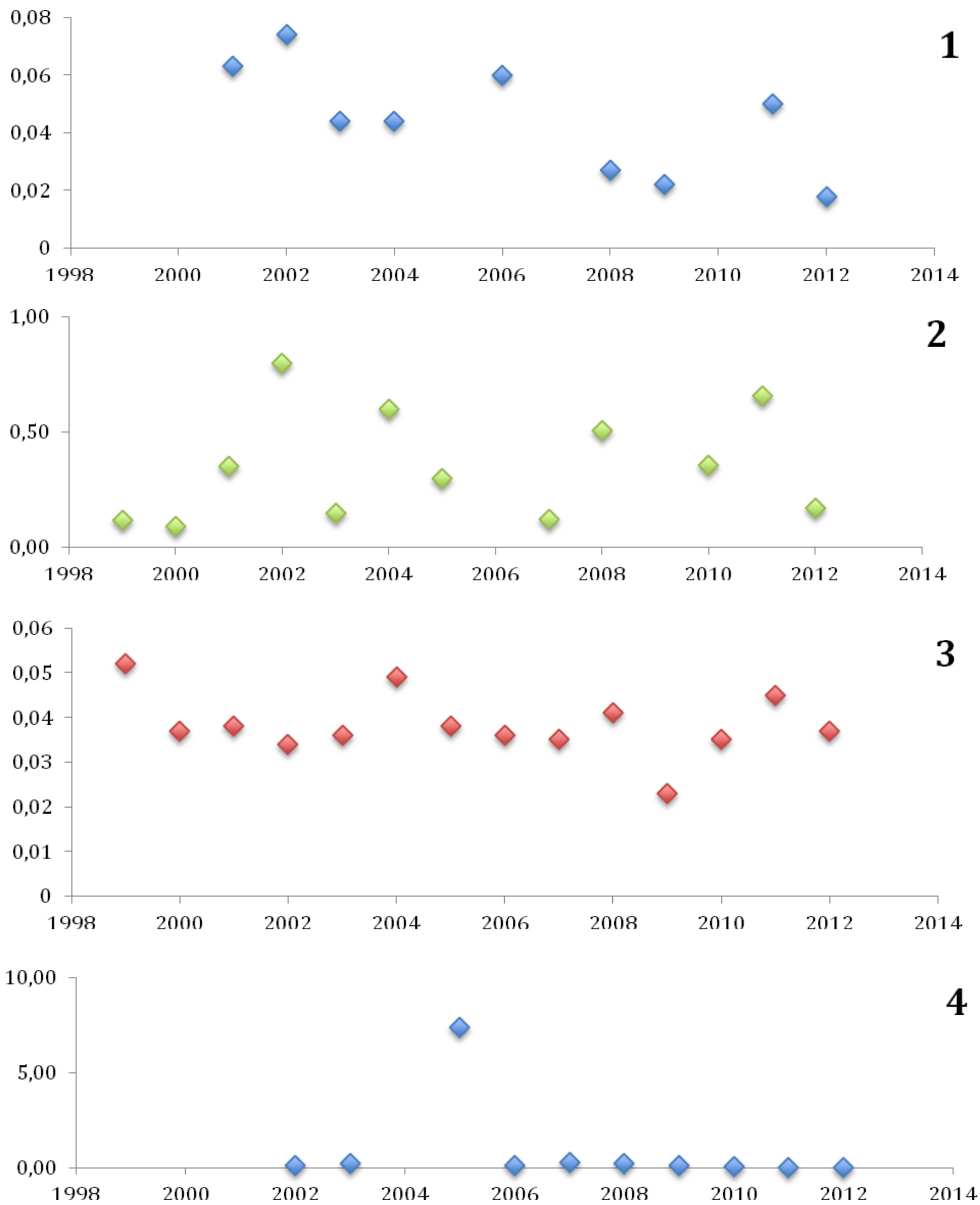


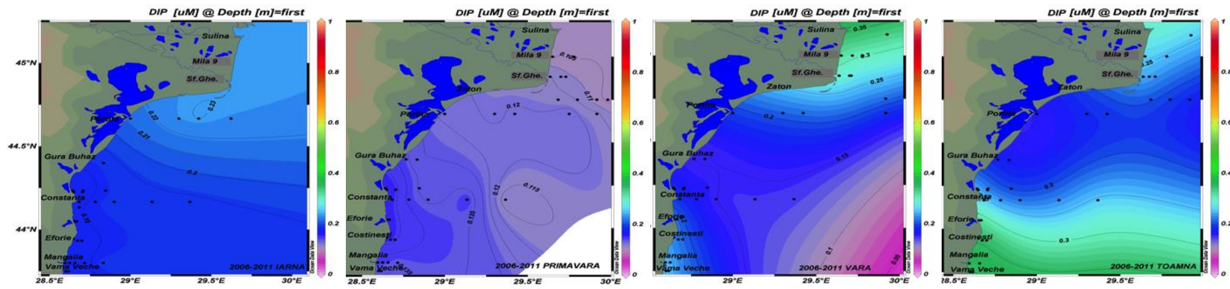
Figure 43. **Chlorophyll in surface waters at the relevant stations (see Table 24). Data used for these elaborations are taken fromMyOcean platform. Concentrations are expressed as mg/m^3.**

III.3.2. Black Sea

The spatial and temporal variability of nutrients in the Black Sea is reported and discussed in this section. The data were acquired during 2006-2011 within the Romanian national monitoring program on a network consisting of 36 stations along the entire Romanian coast, between the Danube's mouth Sulina and Vama Veche, with 5-30m bottom depths. Additionally, historical data were used from the transect EST Constanţa, consisting of 5 sampled stationsin the water column at standard depths (0m, 10m, 20m, 30m, 50m), between 1964-2011 (phosphorus) and 1980-2011 (nitrogen).

<u>Dissolved phosphorus (DIP)</u>

The DIP concentrations (n=1529) ranged between 0.01-16.50µM (mean 0.31µM, median 0.15 µM, SD. 0.96µM), normal distributed, with 93% values in the interval "*undetectable*" –0.05 µM. The extreme values, higher than 4.00µM, were recorded seasonally, on restricted area, in the influence zone of the cities Constanţa and Mangalia. In winter, the 0.2µM isoline marksat the surface, showed a slight gradient between transitional and coastal waters. In any case, are enough homogenous and do not allow an evident distinction between fluvial and coastal sources input. In spring, the concentrations decrease, being the lowest throughout the year because of the specific phytoplanktonic consumption. In summer, the fluvial inputs become, on average, more significant, but with concentrations in the natural variability. At the end of the warm season the areas nearby Constanţa and Mangalia are delimited by the highest averages of the year. Thus, at Romanian Black Sea coasts are distinguished two inorganic phosphorus sources: the Danube and the WWTPs Constanţa Sud and Mangalia. Because of the very different flows/input of the sources we consider as more significant the fluvial input (Figure 44).



2006-2011 Winter | **2006-2011 Spring** | **2006-2011 Summer** | **2006-2011 Autumn**

Figure 44. The spatiotemporal distribution of DIP at the surface – 2006-2011, Romanian Black Sea waters.

The DIP vertical variation is influenced by the ecosystem's biological activity and physical phenomena. The water column has two distinct layers, delimited at any season by the 0.2 μM isoline. We find the maximum concentration in winter due to phosphate regeneration from phytoplankton, detritus and dissolved organic compounds. The uptakes of the phytoplankton during the specific spring bloom lead to minimum concentrations. The end of spring and early summer are characterized by the increasing gradient with depth, with maximum concentrations at 50m assuming that inorganic phosphorus is accumulated in the sediments. In autumn, these values are not found, even if the gradient is still evident but in a lower range. The DIP vertical distribution is generally characterized by two maxima: the smaller one in the 0-20m layers and the other at the interface water-sediment (Figure 45).

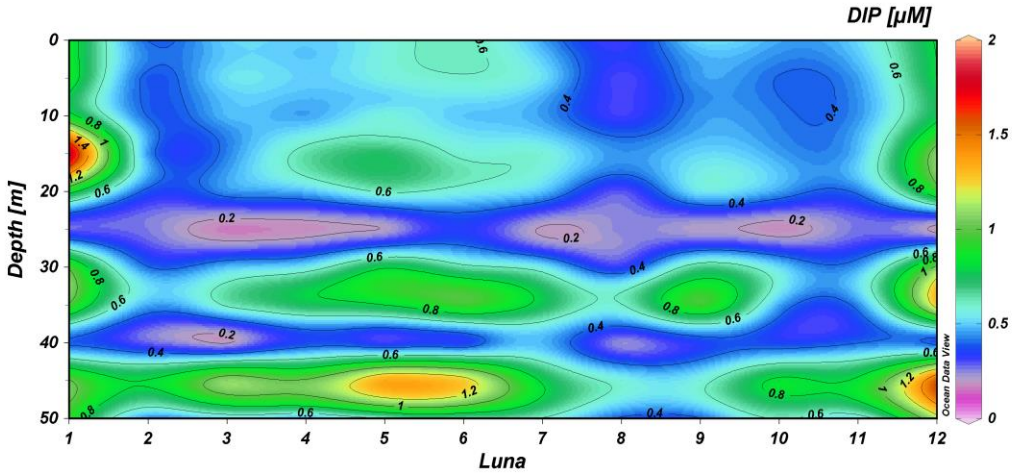


Figure 45. Water column DIP (µM). Multiannual monthly means 1964-2011, Est Constanţa

Long-term (1964-2011, n=6964) is observed the DIP concentrations decrease se up to comparable values with 60's, reference period for the good quality of the Romanian Black Sea waters (Figure 46).

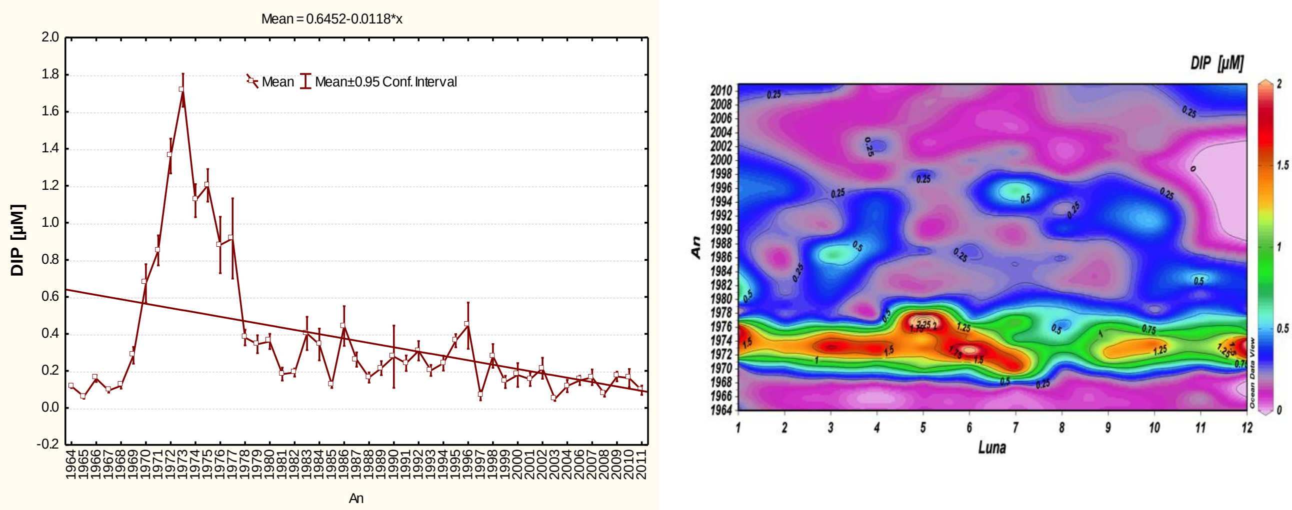


Figure 46. Annual and monthly DIP means concentrations in Est Constanţa.

The inorganic phosphorus content of the Romanian Black Sea waters is influenced by the Danube's and WWTPs input. Due to their different flow, the fluvial input is more significant. In the water column, the DIP seasonal variation is more influenced by the biological activity and it is characterized by two maxima: the smaller one in the 0-20m layer and the other at the interface water. A long-term (1964-2011, n=6964) decrease is observed in the DIP concentrations reaching comparable values with 60's, reference period for the good quality of the Romanian Black Sea waters. These low values give to phosphorus the feature of a limitative element for the phytoplankton's proliferation.

Dissolved nitrogen (DIN)

In the assessment DIN concentrations represent a sum of nitrate, nitrite and ammonium. The DIN concentrations (n=1536) range within 1.14 – 160.04 µM (mean 10.21 µM, median 6.70 µM, standard deviation 13.24 µM), normally distributed, with 90,8% values in the interval "*undetectable*" – 20.0 µM. The extremes are, as in the phosphorus case, seasonally and punctiform, in the WWTP Constanţa Sud neighborhood.

Generally, throughout the year, the highest mean concentrations are observed in the Northern part of the coast, under the Danube's direct influence. However, in the surface layer we observe seasonal variations as a result of the biological activity, more pronounced in the coastal waters. In spring, the 18.00 µM isoline marks, at the water surface, the front between transitional and coastal waters, approx. nearby Portiţa station. Unlike phosphate, the inorganic nitrogen input is more outlined in spring and autumn, with the increased precipitations. These input is reduced due to the biological consumption from spring, still limited by phosphorus, and, in summer, the

concentrations gradient become decreased from North to South. In the coastal zone, in summer ammonia become predominant due to higher WWTP discharges, phytoplankton decomposition, zooplankton and excretions of fishes, etc. In winter, the mean concentrations are homogenous and quite low along the entire coast (Figure 47).

The DIN vertical variation is influenced by ecosystem's biological activities, and the physical and chemical processes. The water column has two distinct layers delimited, at any season by the 2.0 μM isoline, at approximately 25 m depth. The highest values are found in spring, in the superior layer, due to fluvial and coastal inputs but also due to the seasonal thermocline delineation. The specific biological consumption during spring presents is led finding maximum of the superior layer, and at the early summer, at 10-30m instead of surface. Thus, the inorganic nitrogen regeneration is emphasized in the superior layer as well as the trend to sedimentation. The end of summer is characterized by the interruption of the inventory regeneration by a second bloom, which leads to minimum concentrations, in the whole of the water column.

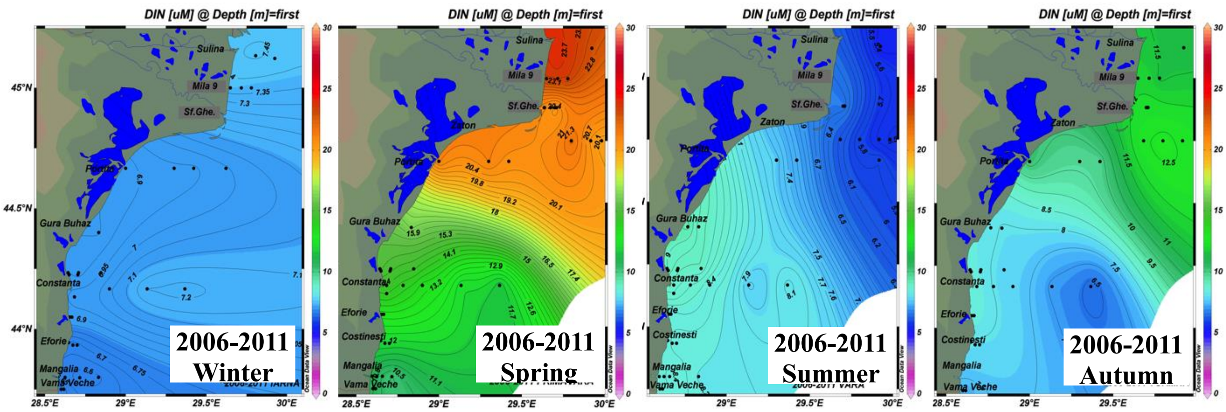


Figure 47. The spatiotemporal distribution of DIN at the surface – 2006-2011, Romanian Black Sea waters.

In November, due to the breaking thermocline and increase of the input from land the DIN regeneration starts and continues throughout the winter (Figure 48).

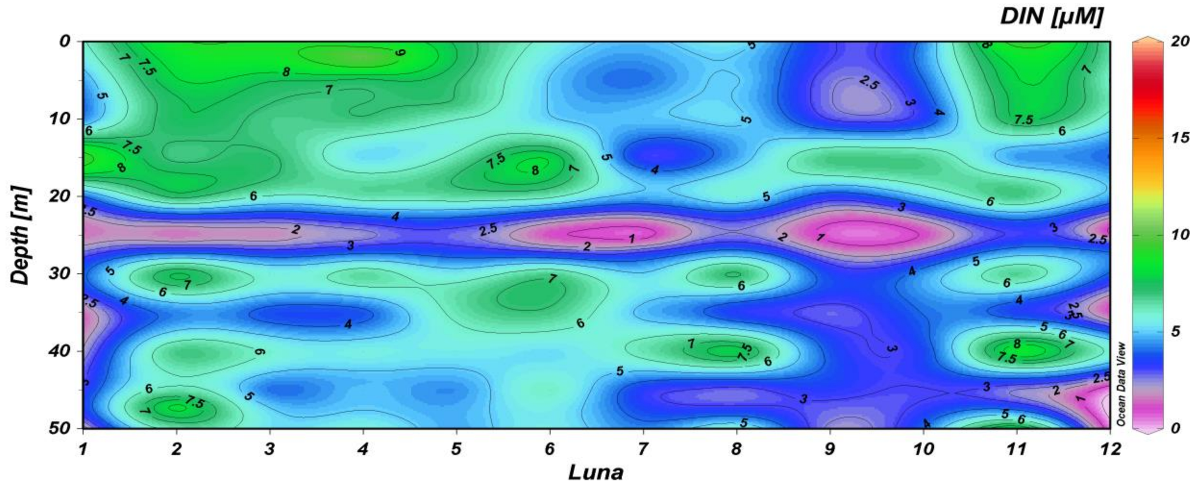
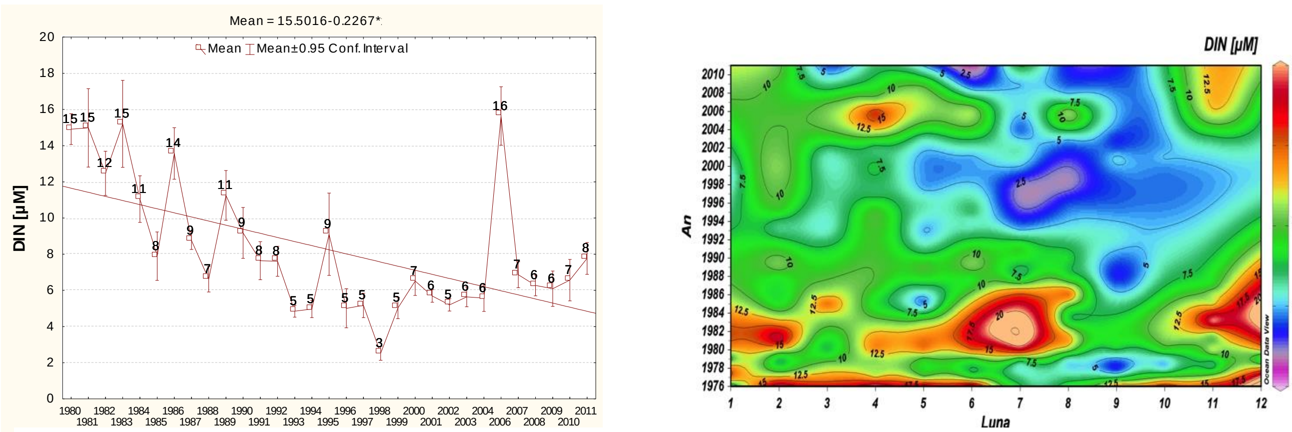


Figure 48. Water column DIN (µM). Multiannual monthly means 1964-2011, Est Constanţa.

In a long-term (1980-2011, n=3914), it is generally observed the decrease of DIN mean concentrations, up to the comparable values from 1991-1992, when the eutrophication intensity of the Romanian Black Sea waters starts to decrease. However, nowadays, DIN values are slightly increasing than at the end of 90’s, when minimum was observed (Figure 49a, b).



(a) (b)

Figure 49. Annual (a) and monthly (b) DIN means concentrations, Est Constanţa.

The dissolved inorganic nitrogen content of the Romanian Black Sea waters is mainly influenced, at the surface by the Danube’s input. Seasonally and on the restricted area, we found higher concentrations of ammonia in the neighborhood of WWTPs, ammonia higher concentrations. DIN seasonal variation in the water column is characterized by two maxima: the smaller one at approx. depth of 10m and the other at the interface water – sediment. In a long-term (1980-2011), even if actually a slightly increasing trend is observed, the decreasing concentrations up to the level of 1991-1992 are noticed, when the intensity of the eutrophication

started to decrease.

Dissolved oxygen (DO)

Concerning DO, the Black Sea shows some particularities. The Black Sea is a strong stratified system. The biogeochemistry of the superior layer located above permanent anoxic waters, lifeless (except the anaerobic bacteria) implies four distinct layers (BSC, 2008, Sorokin, 2002, Konovalov, 2000):

The oxic layer. The approximative thickness of the oxic layer is 0-50 m (up to approximatively 1% light) – characterized by active biological processes (e.g. nutrients uptake, phytoplanktonic blooms, respiration, mortality, etc.), and high DO concentrations (approx. 300 μM). In this euphotic layer the DO concentrations variability range usually within 250-450 μM.

The oxycline. The superior limit of the oxycline, where the DO levels starts to decrease corresponds to 35-40 m depth in the cyclonic areas and 70-100 m depth in the coastal zones. The inferior limit is defined by concentrations of approximately 10 μM and is located at approx. 50-100 m of depth.

The suboxic layer. The oxygen deficient layer (with concentrations less than 10 μM) is generally located at 100-130 m depths and has 20-40 m thickness, at the inferior limit of the nitracline. In this layer, the DO concentrations decrease while the sulphide hydrogen ones increase, the two compounds coexisting (BSC, 2008).

The anoxic layer. The oxygen disappears above the anoxic interface at depths over 150-200 m. Due to the presence of hydrogen sulphide and absence of the DO it is lifeless. The layer is defined through a particular chemistry with three main characteristics: nitrate and nitrite low concentrations due to the denitrification consumption (anaerobic phenomena), sulphate reduction and forming of the hydrogen sulphide, reducing of the redox potential and organic matter oxidation (Horne, 1969). The assessment of the Romanian Black Sea waters DO content is done only for stations with maximum bottom depth up to 50 m, in the oxic layer.

The spatio-temporal distribution of dissolved oxygen

The surface seawater has DO concentrations (n =725) within 152.3 – 732.9 μM, normal distributed, with 90% in the range 250.0 – 450.0 μM, indicating good oxygenation, any season (Figure 50).

In winter, concentrations are homogenous along the entire coast, within 318.0 – 456.9 μM, due

to lower air and seawater temperatures as well as water masses vertical mixing. During the warm season the DO variability increases, outlining the decreased gradient from the Danube's mouths to the South due to higher seawater temperatures.

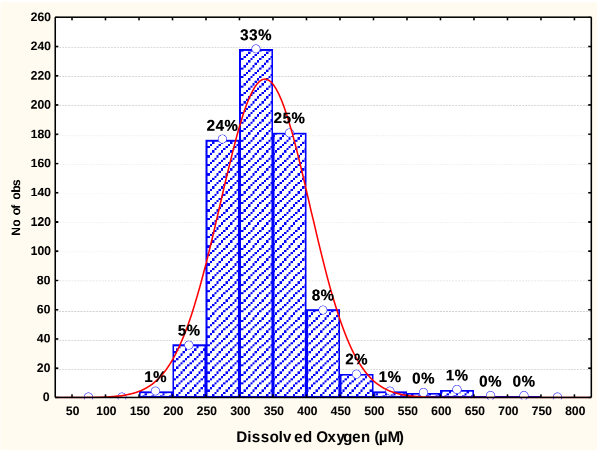


Figure 50. Histogram of DO, Romanian Black Sea waters, surface.

The phytoplanktonic blooms, producing oxygen in spring, are more intense in the Northern part due to the fluvial input, leading to an extreme value of 732.9 µM (April, Sulina). On the other hand, in the areas with anthropogenic impact (neighboring of the WWTP Constanta Sud) the DO starts to decrease up to 152.3 – 162.6 µM. In summer and autumn the values ranged in the same intervals (150.0 –500.0 µM), being higher in the transitional waters. The lower values from the warm season are due to both higher temperatures and to the specific consumption of the organic matter oxidation (Figure 51).

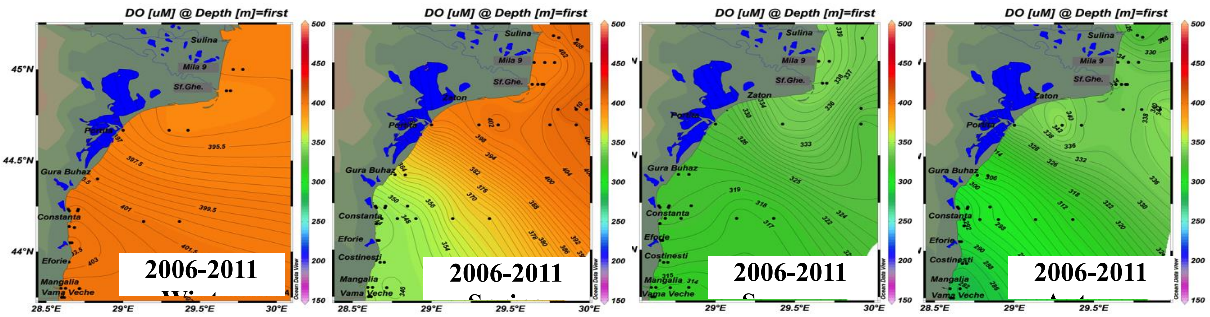


Figure 51. The spatio - temporal distribution of DO at the surface – 2006-2011, Romanian Black Sea waters.

In the water column, at 0-50m, the DO mean concentrations suffer pronounced seasonal variations in the range of 200.0 – 405.0 µM, additional changes due to the water masses stratification. In winter, the gradient is less outlined, with mean concentrations being within 320.0 – 405.0 µM, due to the vertical mixing. The maximum concentrations are achieved in February-March, at the air-water interface in a layer with approximate thickness of 0-5 m,

which coincides with the coldest mixing layer of the whole year. Starting with the warm season, in April, the stratification becomes more pronounced, with the isoline of 320.0 µM rising to 40 m depths. As the heating continues, the mixing layer becomes thinner (0-10 m) and the average decrease from 360.0 µM in spring to 280.0 µM in September. In autumn, until November, the minimum concentrations, 205.0 µM, are found at depths over 40 m and the maximum value is not exceeding 280.0 µM. The lower temperatures from October – November gradually cools the layer 0-10 m where concentrations over 300.0 µM are found in December at about 20 m (Figure 52)

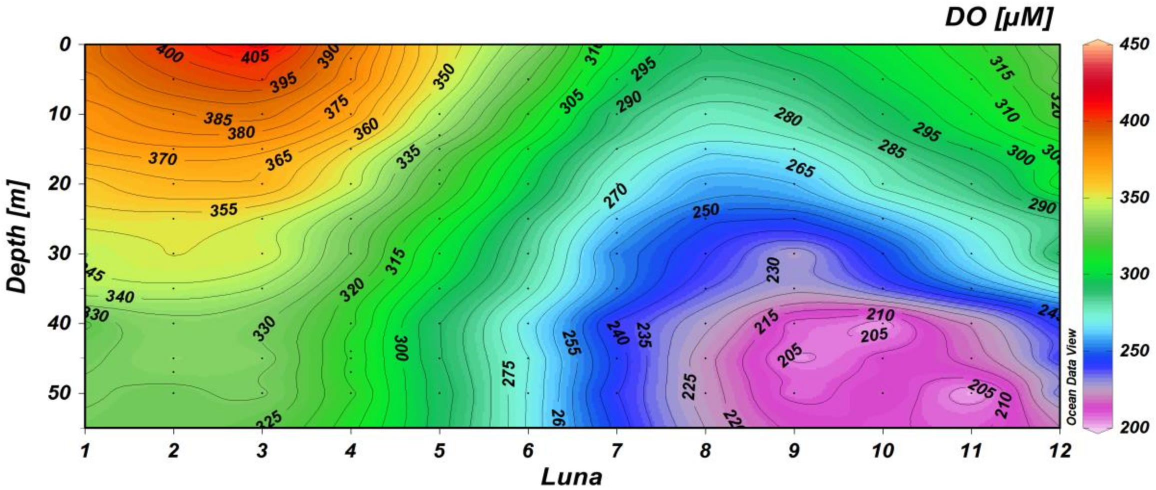


Figure 52. Water column DO (µM). Multiannual monthly means 1964-2011 - Est Constanţa.

Concerning DO, as for the Mediterranean Sea, numerous factors could affect its levels. The interface seawater-bottom sediments show lower concentrations due to the biological processes, which are correlated with the organic carbon content in the sediments (Peres, 1961).

IRIS-SES

Integrated regional monitoring implementation strategy in the South European Seas

www.iris-ses.eu

PART 2

Gap analysis and relative Annexes

Project coordinator: Dr. Kalliopi Pagou
Grant Agreement:
07.0335/ 659540 / SUB / C

1. INTRODUCTION

The Marine Strategy Framework Directive (2008/56/EC) of the European Parliament and of the Council (17th June 2008) establishes a framework for community action in the field of marine environmental policy. It comes into force on the 15th of July 2008. It determines that EU Member States have to define Good Environmental Status (Article 9), to set environmental target (Article 10), to develop operative monitoring programmes (Article 11) and to assess every six years the environmental status of their marine water (Article 8; Article 17(2)). On an operative point of view, monitoring programmes have to be sized and set by 2016 following a six year cycle. Regarding MSFD, GES is described by eleven different descriptors numbered from D1 to D11. A crucial issue will be to determine the spatial and temporal resolution needed for the monitoring programs, improving the existing ones in order to cover the MSFD requirements. The scale of the assessment should be aligned with the ecosystem temporal and spatial natural variability and prioritising areas where pressures and impacts are important. This Report addresses the comparison and identification of gaps in the existent national monitoring programmes. Important recommendations of the Diagnostic Report will be taken into consideration, such as: spatial expanding the monitoring programmes towards the open sea; revision of the existing monitoring programmes to include new parameters and frequencies of observation according to all relevant Directives and especially to MSFD. In this report we perform a gap-analysis on the existing institutional data on marine ecosystem monitoring, considering the existing gaps along the spatial and temporal scales between available data and MSFD requirements and discussing potential gaps related to the natural variability of descriptors and parameters included in the MSFD (Fig. 53). This report in general represents a case study of running MSFD related national monitoring programmes in year 2012, selected as a representative year for the monitoring plans that have already been planned from the member states for the near future.

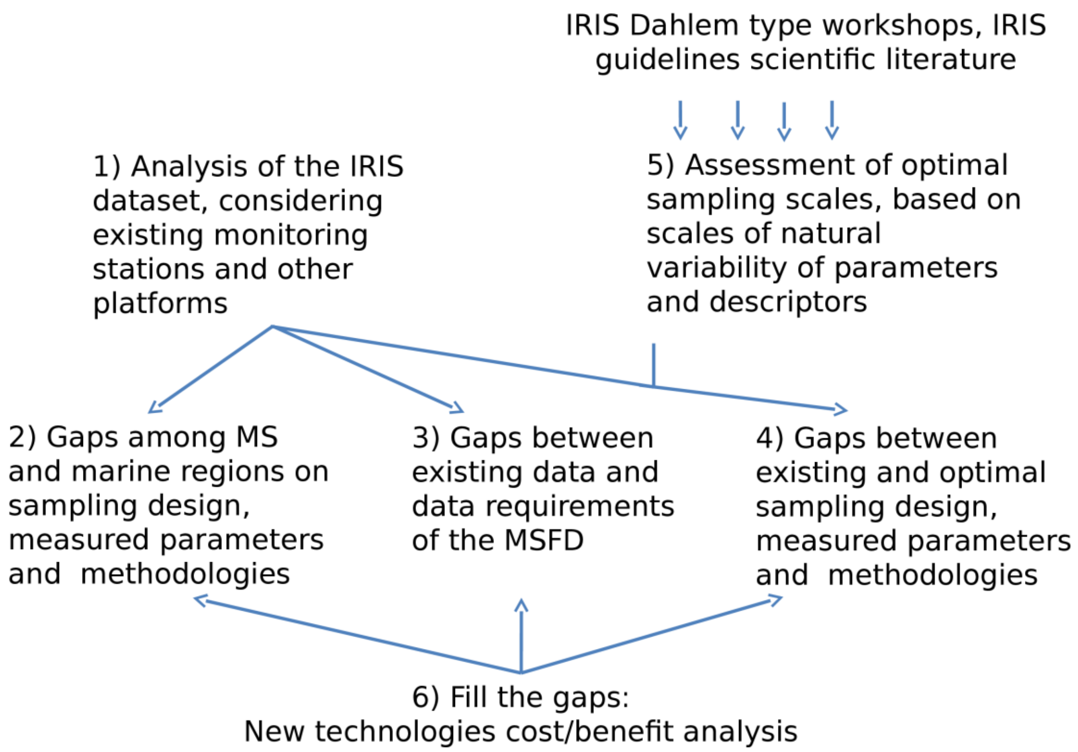


Figure 53. Conceptual scheme

2. MATERIAL & METHODS

Data sources

Data on monitoring stations have been provided for all member states of IRIS-SES and Croatia, due to personal contributions of Croatian scientists to the development of ISIS-SES activity 2 actions.

The IRIS Dataset

Data on monitoring stations are the result of a joined effort of several institutions. They are reported by different organizations (Table 25):

Table 25. Collected data.

Country	Descriptor	Collected from	Reported to
Bulgaria	D1 D4 D6 Fish	IO-BAS	MOEW, WISE
Bulgaria	D1 D4 D6 Seabed habitats PhyB	IO-BAS	MOEW, Bucharest Convention, EEA, WISE
Bulgaria	D1 D4 D6 Seabed habitats ZooB	IO-BAS	MOEW, BSBD, Bucharest Convention, EEA, WISE
Bulgaria	D1 D4 D6 Water column habitats FPK	IO-BAS	MOEW, Bucharest Convention, EEA, WISE
Bulgaria	D1 D4 D6 Water column habitats ZPK	IO-BAS	MOEW, WISE
Bulgaria	D2 Non indigenous species	IO-BAS	MOEW, BSBD, Bucharest Convention, EEA, WISE
Bulgaria	D2 Non indigenous species	IO-BAS	regional, national standards
Bulgaria	D3 Commercial fish shellfish	IO-BAS	NAFA (National Agency for Fisheries and Aquaculture), WISE
Bulgaria	D3 Commercial fish shellfish	IO-BAS	MOEW, WISE
Bulgaria	D5 Eutrophication	IO-BAS	MOEW, Bucharest Convention, EEA, WISE
Bulgaria	D7 Hydrographical changes	IO-BAS	MOEW, Bucharest Convention, EEA, WISE
Bulgaria	D8 Contaminants in sediment	IO-BAS	Not available
Bulgaria	D8 Contaminants in sediment	Medical University	Not available
Croatia	D1 D4 D6 Seabed habitats PhyB	IOF	Not available
Croatia	D1 D4 D6 Seabed habitats ZooB	IOF	Not available
Croatia	D1 D4 D6 Water column habitats FPK	IOF	Not available
Croatia	D1 D4 D6 Water column habitats ZPK	IOF	Not available
Croatia	D2 Non indigenous species	IOF	Not available
Croatia	D2 Non indigenous species	IOR	MEDITS, BY-CATCH
Croatia	D2 Non indigenous species	IOR	Not available
Croatia	D3 Commercial fish shellfish	IOR	Not available
Croatia	D5 Eutrophication	IOF	Not available
Croatia	D7 Hydrographical changes	IOF	Not available
Croatia	D8 Contaminants in biota	IOF	Not available
Croatia	D8 Contaminants in sediment	IOF	Not available
Cyprus	D1 D4 D6 Water column habitats FPK	Dept of Fisheries and Marine Research	Not available
Cyprus	D3 Commercial fish shellfish	Dept of Fisheries and Marine Research	MEDITS
Cyprus	D5 Eutrophication	Dept of Fisheries and Marine Research	EIONET

Cyprus	D8 Contaminants in water	Dept of Fisheries and Marine Research	EIONET
Greece	D1 D4 D6 Seabed habitats PhyB	HCMR	EIONET
Greece	D1 D4 D6 Seabed habitats PhyB	HCMR	WISE (EU)-MINENV (national)
Greece	D1 D4 D6 Seabed habitats PhyB	HCMR	Not available
Greece	D1 D4 D6 Seabed habitats PhyB	HCMR	EU-minenv (NATURA)
Greece	D1 D4 D6 Seabed habitats PhyB	Archipelagos IMC AKOA	Not available
Greece	D1 D4 D6 Seabed habitats ZooB	HCMR	EIONET
Greece	D1 D4 D6 Seabed habitats ZooB	HCMR	WISE (EU)-MINENV (national)
Greece	D1 D4 D6 Seabed habitats ZooB	HCMR	EYDAP SA Company, EU
Greece	D1 D4 D6 Seabed habitats ZooB	HCMR	Aluminium Company
Greece	D1 D4 D6 Water column habitats FPK	HCMR	Not available
Greece	D1 D4 D6 Water column habitats ZPK	HCMR	Not available
Greece	D2 Non indigenous species	HCMR	EIONET
Greece	D2 Non indigenous species	HCMR	http:/perseus-net.eu
Greece	D2 Non indigenous species	HCMR	EYDAP SA Company, EU
Greece	D3 Commercial fish shellfish	HCMR	EU, JRC
Greece	D3 Commercial fish shellfish	HCMR	EU, ICCAT, JRC
Greece	D5 Eutrophication	HCMR	Not available
Greece	D7 Hydrographical changes	HCMR	Not available
Greece	D7 Hydrographical changes	University of Athens	Not available
Greece	D8 Contaminants in biota	Ministry of Rural Development and Food	Ministry of Rural Development and Food, Veterinary Services
Greece	D8 Contaminants in sediment	HCMR	Not available
Greece	D8 Contaminants in sediment	HCMR	Water agency, Greek Government
Greece	D8 Contaminants in sediment	University of Athens	Not available
Greece	D8 Contaminants in water	HCMR	WISE (EU)-MINENV (national)
Greece	D8 Contaminants in water	HCMR	EIONET
Greece	D8 Contaminants in water	HCMR	Not available
Greece	D8 Contaminants in water	University of Athens	Not available
Italy	D1 D4 D6 Seabed habitats PhyB	ARPA, ISPRA, CNR	SIDIMAR
Italy	D1 D4 D6 Seabed habitats ZooB	ARPA, ISPRA, CNR	EIONET
Italy	D1 D4 D6 Water column habitats FPK	ARPA, ISPRA, CNR	EIONET
Italy	D1 D4 D6 Water column habitats ZPK	ARPA, ISPRA, CNR	EIONET
Italy	D2 Non indigenous species	ARPA, ISPRA, CNR	EIONET
Italy	D5 Eutrophication	ARPA, ISPRA, CNR	EIONET
Italy	D7 Hydrographical changes	ARPA, ISPRA, CNR	EIONET
Italy	D8 Contaminants in sediment	ARPA, ISPRA, CNR	EIONET
Italy	D8 Contaminants in water	ARPA, ISPRA, CNR	EIONET
Romania	D1 D4 D6 Fish	NIMRD	MOEW, BSC, GFCM
Romania	D1 D4 D6 Fish	NIMRD	Not available
Romania	D1 D4 D6 Seabed habitats PhyB	NIMRD	Ministry of Environment and Climate Changes
Romania	D1 D4 D6 Seabed habitats ZooB	NIMRD	Ministry of Environment and Climate Changes
Romania	D1 D4 D6 Seabed habitats ZooB	GeoEcoMar	Not reported
Romania	D1 D4 D6 Water column habitats FPK	NIMRD	Ministry of Environment and Climate Changes
Romania	D1 D4 D6 Water column habitats FPK	WBADL	AN "Romanian Waters"
Romania	D1 D4 D6 Water column habitats ZPK	NIMRD	Not available
Romania	D2 Non indigenous species	NIMRD	Ministry of Environment and Climate Changes
Romania	D2 Non indigenous species	GeoEcoMar	Not reported

Romania	D2 Non indigenous species	NIMRD	regional, national standards
Romania	D2 Non indigenous species	NIMRD	Not available
Romania	D3 Commercial fish shellfish	NIMRD	MOEW, BSC, GFCM
Romania	D3 Commercial fish shellfish	NIMRD	BSC, GFCM, JRC, EC
Romania	D3 Commercial fish shellfish	NIMRD	Not available
Romania	D3 Commercial fish shellfish	NAFA	GFCM, JRC, EC
Romania	D5 Eutrophication	NIMRD	Ministry of Environment and Climate Changes
Romania	D5 Eutrophication	GeoEcoMar	Not reported
Romania	D5 Eutrophication	Tulcea County, Department of Public Health	Ministry of Health, Department for Public Health and Public Health control
Romania	D5 Eutrophication	Constanta County, Department of Public Health	Ministry of Health, Department for Public Health and Public Health control
Romania	D7 Hydrographical changes	NIMRD	Ministry of Environment and Climate Changes
Romania	D7 Hydrographical changes	GeoEcoMar	Not reported
Romania	D8 Contaminants in biota	NIMRD	EIONET,BSC
Romania	D8 Contaminants in sediment	NIMRD	EIONET,BSC
Romania	D8 Contaminants in sediment	WBADL	AN "Romanian Waters"
Romania	D8 Contaminants in sediment	GeoEcoMar	Not reported
Romania	D8 Contaminants in water	NIMRD	EIONET,BSC
Romania	D8 Contaminants in water	WBADL	AN "Romanian Waters"
Romania	D9 Contaminants in seafood	NIMRD	EIONET,BSC
Spain	D1 D4 D6 Fish	MEDITS	MEDITS
Spain	D1 D4 D6 Fish	MEDIAS	MEDIAS
Spain	D1 D4 D6 Seabed habitats PhyB	ACA	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	Direccion General de Pesca, Consejeria de Agricultura, Medio Ambiente y Territorio de las Islas Baleares	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	Servicio de Estudios y Planificacion, Direccion General de Recursos Hidricos, Consejeria de Agricultura, Medio Ambiente y Territorio	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	Servei de Planificacio, Direccion General de Medio Natural, Educacion Ambiental y Cambio ClimAtico, Consejeria de Agricultura, Medio Ambiente y Territorio. Illes Balears	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	Conselleria de Infraestructuras, Territorio y Medio Ambiente	Not reported

Spain	D1 D4 D6 Seabed habitats PhyB	Conselleria de Presidencia y Agricultura, Pesca, Alimentacion y Agua, Comunidad Valenciana	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	CITMA	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	Servicio de Pesca y Acuicultura. Consejeria de Agricultura, Agua y Medio Ambiente. Comunidad Autonoma Region de Murcia.	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	Consejeria de Medio Ambiente y Ordenacion del Territorio, Junta de Andalucia	Not reported
Spain	D1 D4 D6 Seabed habitats PhyB	Consejeria de Agricultura, Pesca y Medio Ambiente, Junta de Andalucia,	Not reported
Spain	D1 D4 D6 Seabed habitats ZooB	Consejeria de Agricultura, Medio Ambiente y Territorio. Govern Balear.	MAGRAMA, SIA, WISE, FIC
Spain	D1 D4 D6 Seabed habitats ZooB	Consejeria de Agricultura, Medio Ambiente y Territorio. Govern Balear.	MAGRAMA
Spain	D1 D4 D6 Seabed habitats ZooB	Centro de Investigacion Marina. Universidad de Alicante	MAGRAMA
Spain	D1 D4 D6 Seabed habitats ZooB	Junta de Andalucia	MAGRAMA
Spain	D1 D4 D6 Seabed habitats ZooB	Ministerio de agricultura, alimentacion y medio ambiente. Medio Marino	Secretara General de Pesca.
Spain	D1 D4 D6 Seabed habitats ZooB	Consejeria de Agricultura, Medio Ambiente y Territorio. Govern Balear.	-
Spain	D1 D4 D6 Seabed habitats ZooB	Universidad Catolica de Valencia.	MAGRAMA
Spain	D1 D4 D6 Seabed habitats ZooB	Departamento de Agricultura, Ganadera, Pesca, Alimentacion y Medio Natural. Generalitat de Catalunya.	MEDPAN
Spain	D1 D4 D6 Seabed habitats ZooB	CITMA	Regional government

Spain	D1 D4 D6 Water column habitats FPK	IEO	Not available
Spain	D1 D4 D6 Water column habitats FPK	IEO	X
Spain	D1 D4 D6 Water column habitats ZPK	COB-IEO	under request
Spain	D1 D4 D6 Water column habitats ZPK	IEO	Available for the responsible national agency of the Barcelona Convention
Spain	D2 Non indigenous species	Not available	Secretaria General de Pesca
Spain	D2 Non indigenous species	Not available	Generalitat de Catalunya. Part reported to MEDPAN
Spain	D2 Non indigenous species	Not available	Murcia Regional Government
Spain	D2 Non indigenous species	Departamento de Agricultura, Ganadera, Pesca, Alimentacion y Medio Natural. Generalitat de Catalunya.	MEDPAN
Spain	D2 Non indigenous species	Consejeria de Agricultura, Medio Ambiente y Territorio. Govern Balear.	MAGRAMA, SIA, WISE, FIC
Spain	D2 Non indigenous species	MEDITS	Not available
Spain	D2 Non indigenous species	MEDIAS	Not available
Spain	D3 Commercial fish shellfish	MEDITS	MEDITS
Spain	D3 Commercial fish shellfish	MEDIAS	MEDIAS
Spain	D5 Eutrophication	IEO	Not available
Spain	D5 Eutrophication	IEO	Spanish Misnistry of Environment
Spain	D7 Hydrographical changes	COB-IEO	Seadatanet
Spain	D7 Hydrographical changes	COB-IEO	Not available
Spain	D8 Contaminants in biota	D.G. de Medio Rural y Marino. Islas Baleares	MAGRAMA
Spain	D8 Contaminants in biota	D.G. Planificacion y Gestion del D.P.H./Sv. Calidad y D.P.H. Regional. Andalucïa	MAGRAMA
Spain	D8 Contaminants in biota	IEO	MAGRAMA
Spain	D8 Contaminants in sediment	IEO	MAGRAMA
Spain	D9 Contaminants in seafood	D.G. de Medio Rural y Marino. Islas Baleares	MAGRAMA
Spain	D9 Contaminants in seafood	Not available	MAGRAMA, Jacumar, ICES, ACSA, D.G. de Pesca de Cataluna
Spain	D9 Contaminants in seafood	IRTA y Direccion General de Pesca de Cataluna	MAGRAMA, Jacumar, ICES, ACSA, D.G. de Pesca de Cataluna
Turkey	D1 D4 D6 Seabed habitats PhyB	Min. of Environment and Urbanization	Not available
Turkey	D1 D4 D6 Seabed habitats ZooB	Min. of Environment and Urbanization	BSC
Turkey	D1 D4 D6 Seabed habitats ZooB	Min. of Environment and Urbanization	Not available
Turkey	D1 D4 D6 Water column habitats FPK	Min. of Environment and Urbanization	BSC

Turkey	D1 D4 D6 Water column habitats FPK	Min. of Environment and Urbanization	Not reported
Turkey	D1 D4 D6 Water column habitats FPK	Min. of Environment and Urbanization	Data collected according to protocols and submitted to the Ministry (data owner), the Min. submit the data to the RSC. Ministry has to submit the same data to the NODC. Data is available to the users (experts, other project owners) upon signed project agreements or official request.
Turkey	D2 Non indigenous species	Min. of Environment and Urbanization	BSC
Turkey	D2 Non indigenous species	Min. of Environment and Urbanization	Not available
Turkey	D5 Eutrophication	Min. of Environment and Urbanization	Black sea Commission
Turkey	D5 Eutrophication	Min. of Environment and Urbanization	UNEP/MAP

Methodology

Stations reported in institutional, public available dataset and for which it is planned a future, regular monitoring are reported in Figs 54-55. Processable metadata were joint in a unique georeferenced dataset. For any accounted Descriptors, we used the cross-classifying factors ’State’, ’Descriptors’ and ’Measured parameter for descriptor’, to build contingency tables of the counts at each combination of factor levels (Figure 59A & B as example for descriptor D5). The integration between Descriptors was calculated as percentage of point for different descriptors reporting the same coordinates (Figure 59C); it does not account for synchrony in sampling. Distances from coastline (Figure 59 D as example, for descriptor D5) and densities of sampling stations (Figure 60) and frequency of monitoring (Figure 61) were calculated on the base of http://www.marineregions.org shapefiles. For an easier visualization, sampling points were also plotted as point and raster maps (Figure 62). All analysis was performed with R statistical package.

3. RESULTS

Gaps in actual monitoring:

Gaps in information occur on a spatial scale, both along ideal transects from the coast to the open ocean and along the coastline, crossing administrative boundaries between regions and member states. Considering the former spatial dimension a) marine monitoring is intensively implemented in the coastal zone (< 1 NM from the coastline), while it is scarce and not regularly scheduled in the open sea and b) member states largely differ each other in the spatial and

temporal sampling scale, and the number and typology of parameters measured for each descriptors.

In the majority of the reported stations (with the exception of Romania) only one descriptor is measured. Generally, the Descriptors Fish (D1 D4 D6), Commercial Fish & Shellfish (D3) and Seabed habitat Phytobenthos (D1 D4 D6) have scarce or no spatial overlap at all with other descriptors (Figure 56). Oppositely, Non indigenous species (D2), Contaminants in biota (D8) and Water column habitats Zooplankton (D1 D4 D6) are highly integrated with other Descriptors.

Descriptors

- **D1 D4 D6 Fish** (Figure 59, Figure 60, Figure 61 & Figure 62): A total of 407 (404 of which complete of coordinates and sampling frequency) stations were reported from Bulgaria, Croatia, Romania and Spain. The parameters 'Relative abundance', 'Relative biomass' and 'Length' are measured from all countries. The parameters 'Species level taxonomy' and 'Age' are measured from Bulgaria and Romania only (Figure 59). The 75 % of sampling stations is located between 1 and 12 NM from the coastline (Figure 59).

Additional comments:

- Spain: Descriptors related to fish are almost fully covered by large annual surveys cofunded by the European Union within the framework of Common Fisheries Policy, the Demersal trawl surveys Medits and the acoustic surveys MEDIAS, which in addition are carried out in a coordinated and standardized way with many other Mediterranean countries, and for sure will constitute the basis for some of the future joint monitoring programs under MSFD, not only for the D3 to which were originally directed, but many others, as these of 1, 4, 6 and are also already used as platforms for carrying out hydrographic sampling, ampling for pollution in biota and sediments and even for plankton tows.

  Medits survey covers all the shelf and slope areas in the Spanish western Mediterranean. It follows an stratified sampling design, and several stations by depth strata and area are covered every year, with a total of around 200 stations by year. However, since the exact position of the stations change from year to year,

only the locations of the hauls carried out during the 2013 survey were reported, as an example of their spatial coverage.

Medias acoustic surveys not only estimate the biomass of a group of target small pelagic, but thanks to the hauls carried out to define the proportion of different species in the echoes, it is also useful to address these descriptors. In this case the sampling only covers the shelf of the mainland Spanish Mediterranean coasts.

- **D1 D4 D6 Seabed habitats Phytobenthos** (Figure 63, Figure 64, Figure 65 & Figure 66): A total of 3312 stations (3297 of which complete of coordinates and sampling frequency) were reported from Bulgaria, Croatia, Greece, Italy, Romania, Spain and Turkey (Figure 63). Generally, there is scarce overlapping between D1 D4 D6 Seabed habitats Phytobenthos and other descriptors (Figure 64). The parameters 'Species coverage', and 'Species taxonomy' are measured from the majority of the reporting countries (Figure 66). The 50 % of sampling stations is located in less than 0.1 NM from the coastline and the 25% between 10 and 100 NM from the coastline (Fig. 65).

Additional comments:

  - Greece: Most stations are part of WFD monitoring, therefore mostly refer to coastal waters. Due to the high indentation of Greece coastlines, the baseline bays and gulfs with islands is drawn as a straight line thus enclosing in the coastal water bodies of entire gulfs thus extending offshore within the MSFD coverage.
  - Turkey: Implemented only in 2011 and 2013. New NMP (2014-2016) has this module too.

- **D1 D4 D6 Seabed habitats Zoobenthos** (Figure 67, Figure 68, Figure 69 & Figure 70): A total of 622 (422 of which complete of coordinates and sampling frequency) stations were reported from Bulgaria, Croatia, Greece, Italy, Romania, Spain and Turkey (Figure 70). This descriptor has an high overlap (> 60%) with D1 D4 D6 Seabed habitats Phytobenthos and D1 D4 D6 Water column habitats Phytoplankton (Figure 67). The parameters 'Relative abundance', 'Relative biomass', and 'Species level taxonomy' are measured from the majority of the reporting countries. The 50 % of sampling stations is located more than 1 NM from the coastline and the 25% between 3 and 100 NM from the coastline (Figure 67).

Additional comments:

- Greece: Most stations are part of WFD monitoring, therefore mostly refer to coastal waters. Due to the high indentation of Greece coastlines, the baseline bays and gulfs with islands is drawn as a straight line thus enclosing in the coastal water bodies entire gulfs thus extending offshore within the MSFD coverage.

- Spain: Exact positions of permanent sampling sites were not provided, but it was indicated the number of regularly sampled stations focusing on zoobenthos in at least some ofl the Mediterranean Spanish regions, in coastal or territorial (<12 miles) waters: 56 in Andalusia, 87 along the coasts of Balearic islands, and also an undetermined n of stations covering diverse marine reserves in Catalonia and Valencia regions. Moreover, within the framework of Medits annual surveys, not only the usual bottom trawl hauls are useful for monitoring certain benthic species, but complementary hauls by means of epibenthic devices also are useful for zoobenthos sampling.

- Turkey: Implemented only in 2011 and 2013. New NMP (2014-2016) has this module too.

- **D1 D4 D6 Water column habitats Phytoplankton** (Figure 71, Figure 72, Figure 73 & Figure 74): A total of 514 stations (510 of which complete of coordinates and sampling frequency) were reported from Bulgaria, Croatia, Greece, Italy, Romania and Turkey (Figure 74). This descriptor has an high overlap (> 60%) with D5 Eutrophication (> 80%) D1 D4 D6 Seabed habitats Zoobenthos (> 60%) (Figure 71). The parameters 'Relative biomass' is accounted from Bulgaria, Croatia and Italy only, while the parameter 'Species level taxonomy' is the only one reported for Cyprus. The 50 % of sampling stations is located more than 1 NM from the coastline and the 25% between 10 and 100 NM from the coastline (Figure 71).

Additional comments:

- Bulgaria: monitoring starts in May and is monthly until September / October

- Turkey: New NMP (2014-2016) has this module too, with some modifications.

- **D1 D4 D6 Water column habitats Zooplankton** (Figure 75, Figure 76, Figure 77 & Figure 78): A total of 339 stations (336 of which complete of coordinates and sampling frequency) were reported from Bulgaria, Croatia, Greece, Italy, Romania, Spain and Turkey (Figure 78). This descriptor has an high overlap with D1 D4 D6 Water column habitats Phytoplankton (> 80%), D5 Eutrophication (> 70%) and D1 D4 D6 Seabed habitat Zoobenthos (60%) (Figure 75). The parameters 'Relative abundance' and 'Species level taxonomy' are measured from the majority of the reporting countries (Figure 75). The 'Relative biomass' is reported from Bulgaria, Greece and Romania. Bulgaria is the only country reporting 'Developmental stage' and 'Sex' (Figure 75). The 50 % of sampling stations is located more than 1 NM from the coastline and the 25% between 3 and 50 NM from the coastline (Figure 75).

Additional comments:

  - Spain: Regular samples are only taken from 100 m depth to surface, and hence deeper zooplankton is only occasionally sampled within occasional research projects.
  - Turkey: Not done at routine basis. Monitored only in 2011 at the MEDiterranean sts, but not in 2013 and not included in the new NMP (2014-2016). It will be considered only in 2016 at the pilot level at above mentioned AUs.

- **D2 Non indigenous species** (Figure 79, Figure 80, Figure 81 & Figure 82): All stations for the descriptors D1 D4 D6 Fish and D1 D4 D6 Seabed habitat zoobenthos for which was measured the parameter Species level taxonomy were included in D2 Non indigenous species. A total of 1112 (918 of which complete of coordinates and sampling frequency) stations were reported from Bulgaria, Croatia, Italy, Greece, Romania, Spain and Turkey (Figure 82). The parameters 'Relative abundance' and 'Species level taxonomy' are measured from the majority of the reporting countries (Figure 79). The 50 % of sampling stations is located more than 3 NM from the coastline and the 25% between 10 and 100 NM from the coastline (Figure 79). Very important gaps affect this descriptor, since planktonic and micro or meiobenthonic organisms are not monitored

regularly, and in general only coastal areas, specially the circalitoral level, are properly sampled for some target groups, as macroalgae.

Additional comments:

- Romania: The most important in species for the Black Sea (*Mnemiopsis leidy* and *Beroe ovata*) have been monitored in addition to the ZPK monitoring programe (biannually in the last years).
- Turkey: No specific programmes for D2 at ports or risk areas.

- **D3 Commercial fish and shellfish** (Figure 83, Figure 84, Figure 85 & Figure 86): All stations for the descriptors D1 D4 D6 Fish were included in D3 Commercial fish and shellfish. A total of 533 stations (424 of which complete of coordinates and sampling frequency) stations were reported from from Bulgaria, Croatia, Cyprus, Greece, Romania, Spain and Turkey (Figure 86). The 50 % of sampling stations is located more than 7 NM from the coastline and the 25% between 10 and 32 NM from the coastline (Figure 83). Additional information can be get from landings statistics. Recreational fisheries and maybe some very small scale artisanal fisheries would be not completely monitored.

Additional comments:

- Turkey: Only activities on project basis under the responsibility of Ministry of Food, Agriculture.

- **D5 Eutrophication** (Figure 87, Figure 88, Figure 89 & Figure 90): A total of 720 stations (704 of which complete of coordinates and sampling frequency) were reported from Bulgaria, Croatia, Cyprus, Greece, Italy, Romania, Spain and Turkey (Figure 90). The Parameters ’DON’, ’DOC’ and ’POC’ are measured from Spain and Croatia only (Figure 87). This descriptor has an high overlap with D1 D4 D6 Water column habitats Phytoplankton (> 60%), and D7 Hydrographical change (ca. 50%) (Figure 87). The 50 % of sampling stations with know location is located in more than 1 NM from the coastline and the 25% between 10 and 100 NM from the coastline (Figure 87).

Additional comments:

- Turkey: While monitoring is performed, no measured parameters were reported on the dataset.

- **D7 Hydrographical changes** (Figure 91, Figure 92, Figure 93 & Figure 94): A total of 474 stations (467 of which complete of coordinates and sampling frequency) were reported from Bulgaria, Croatia, Greece, Italy, Romania and Spain (Figure 94). This descriptor has an high overlap with D5 Eutrophication (ca. 80%) and D1 D4 D6 Seabed habitat Zoobenthos (ca. 50%) (Figure 91). The 50 % of sampling stations with know location is located more than 2 NM from the coastline and the 25% between 10 and 100 NM from the coastline (Figure 91). In addition to the reported monitoring stations, a large numbers of alternative platforms exists for the monitoring of this descriptor (Appendix 1).

  Additional comments:

  - Turkey: There is no dedicated programme, but some of the used parameters (pH, Temperature and Salinity) are measured in D5, Eutrophication.

- **D8 Contaminants in biota** (Figure 95, Figure 96, Figure 97 & Figure 98): A total of 128 stations (117 of which complete of coordinates and sampling frequency) were reported from Croatia, Greece, Italy, Romania and Spain (Figure 98). This descriptor has an high overlap with D7 Hydrographical changes and D1 D4 D6 Water column habitat Phytoplankton (ca. 80%) (Figure 95). The 50 % of sampling stations with know location is located more than 0.6 NM from the coastline and the 25% between 1.5 and 32 NM from the coastline (Figure 95).

  Additional comments:

- Turkey: Implemented in 2011 and 2013. New NMP (2014-2016) has this module too with modifications at sampling stations. For Turkey there is no biota data for Black Sea.

- **D8 Contaminants in sediment** (Figure 99, Figure 100, Figure 101 & Figure 102): A total of 548 stations (535 of which complete of coordinates and sampling frequency) were reported from Bulgaria, Croatia, Greece, Italy, Romania, and Spain (Figure 102). This

descriptor has a high overlap with D1 D4 D6 Water column habitat Phytoplankton, D1 D4 D6 Seabed habitats Zooplankton and D1 D4 D6 Seabed habitats Zoobenthos (ca. 60%) (Figure 99). The 50 % of sampling stations with know location is located more than 2 NM from the coastline and the 25% between 8 and 100 NM from the coastline (Figure 99).

Additional comments:

- Bulgaria: there is no official monitoring for contaminants in the sediments, in the Monitoring catalogue we have provided available fragmented data for the period 2010-2011, the national monitoring started in May 2014.

- Spain: stations are located over the continental shelf, and hence do not consider the sediments in slope and deeper areas of the basins.

- **D8 Contaminants in water** (Figure 103, Figure 104, Figure 105 & Figure 106): A total of 203 stations were reported from Bulgaria, Croatia, Greece, Italy and Romania (Figure 106). This descriptor has a moderate overlap with D1 D4 D6 Seabed habitats Zoobenthos (ca. 35%) (Figure 103). The 50 % of sampling stations with know location is located less than 1 NM from the coastline and the 25% between 3 and 20 NM from the coastline (Figure 103).

- **D9 Contaminants in seafood** (Figure 107, Figure 108, Figure 109 & Figure 110): A total of 62 stations were reported from Greece, Italy, Romania and Spain (Figure 110). While Greece did not indicate the measured parameters, Spain did not report the coordinates of sampling sites. This descriptor has a complete overlap with D8 Contaminants in sediment (Figure 110). The 50 % of sampling stations with know location is located less than 0.5 NM from igs. the coastline and the 25% between 1 and 10 NM from the coastline (Figure 110).

4. CONCLUSIONS

This report is a first integrated assessment of the Mediterranean monitoring in a MFSD perspective. From the present analysis emerges clearly that there are bias related to data availability, especially for the descriptors D1, D4, D6 (Mammals, Birds, Fish, Reptiles), D3 Commercial Fish and Shellfish, D8 Contaminants in biota and D9 Contaminants in seafood. While monitoring on these descriptors is implemented in many of the MS, metadata are not available or not sufficiently organized to be reported in this analysis. Real gaps of knowledge exist for the new descriptors accounted in MSFD (D10, D11), for which national monitoring plans have still to be implemented. A large heterogeneity across member states has been observed in terms of density of sampling stations, sampling frequency and measured descriptors and parameters.

Overlap between descriptors: **In the majority of the reported stations (with the exception of**

Romania) only one descriptor is measured (

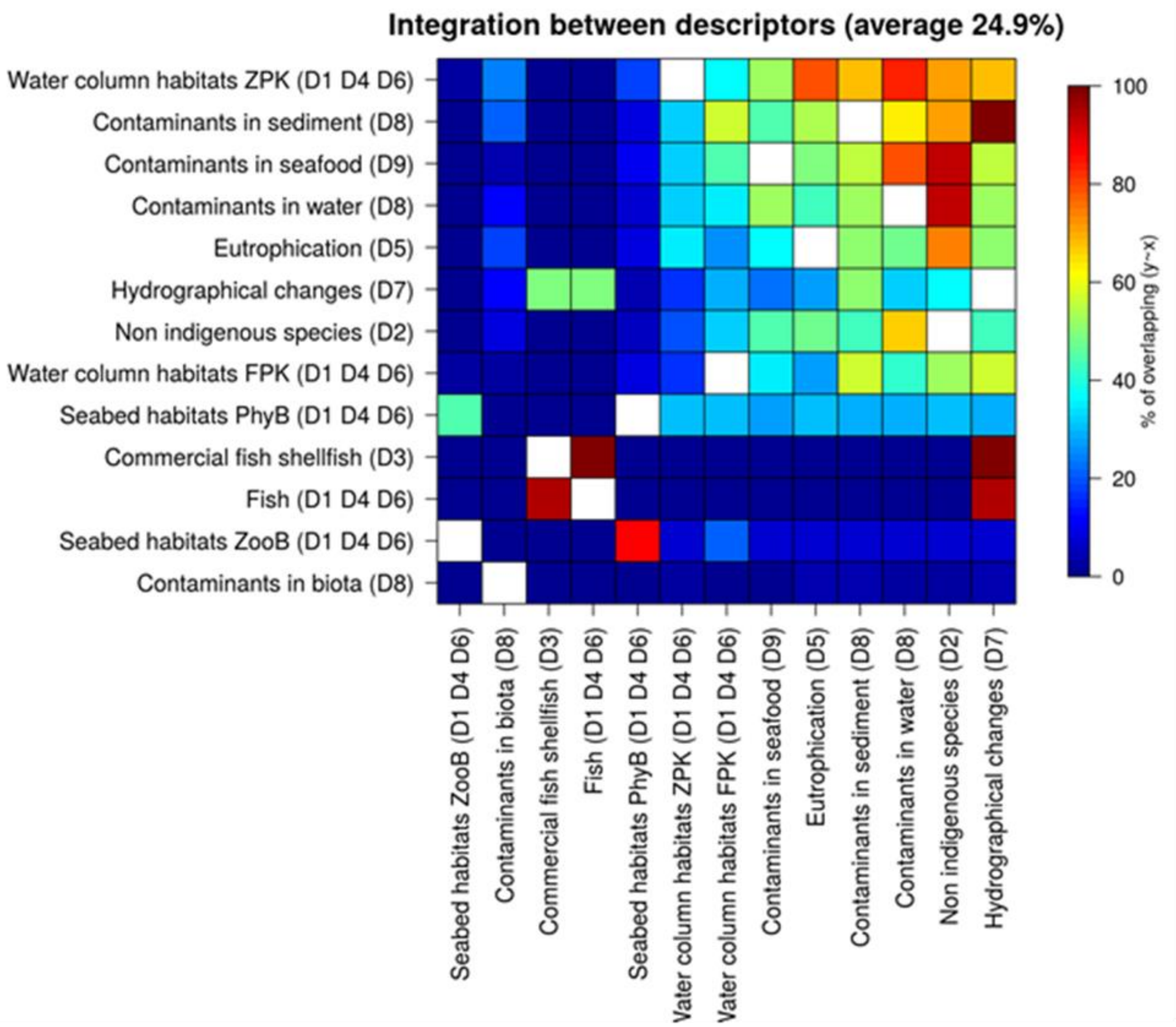


Figure 56). Several descriptors have a good percentage of spatial overlapping (

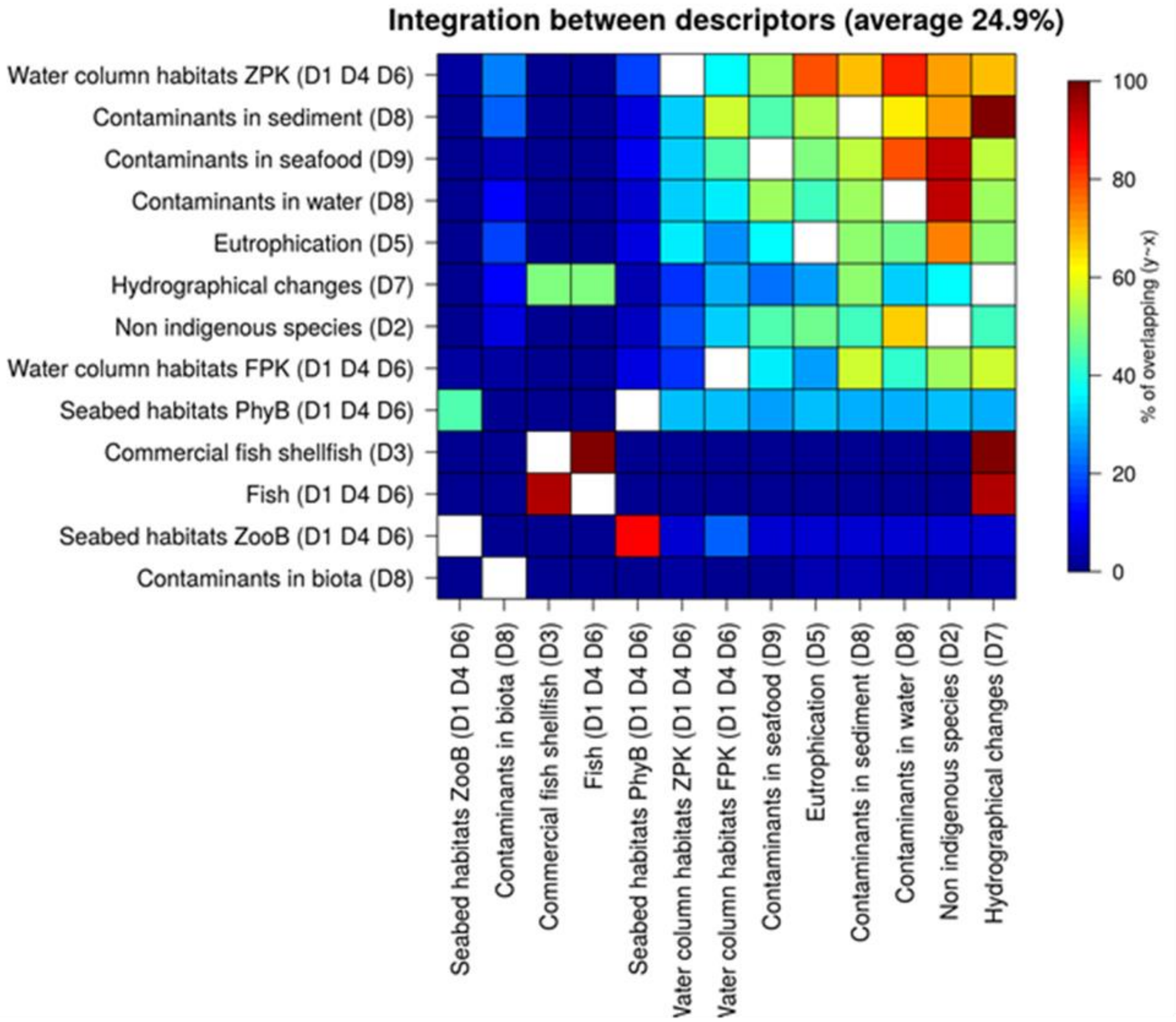


Figure 56,

Figure 57 & Figure 58). In particular Romania and Turkey have highly integrated monitoring plans (

Figure 57 & Figure 58). Generally, D1 D4 D6 Fish, D3 Commercial Fish & Shellfish and D1 D4 D6 Seabed habitat Phytobenthos have scarce or no spatial overlap at all with other

descriptors (

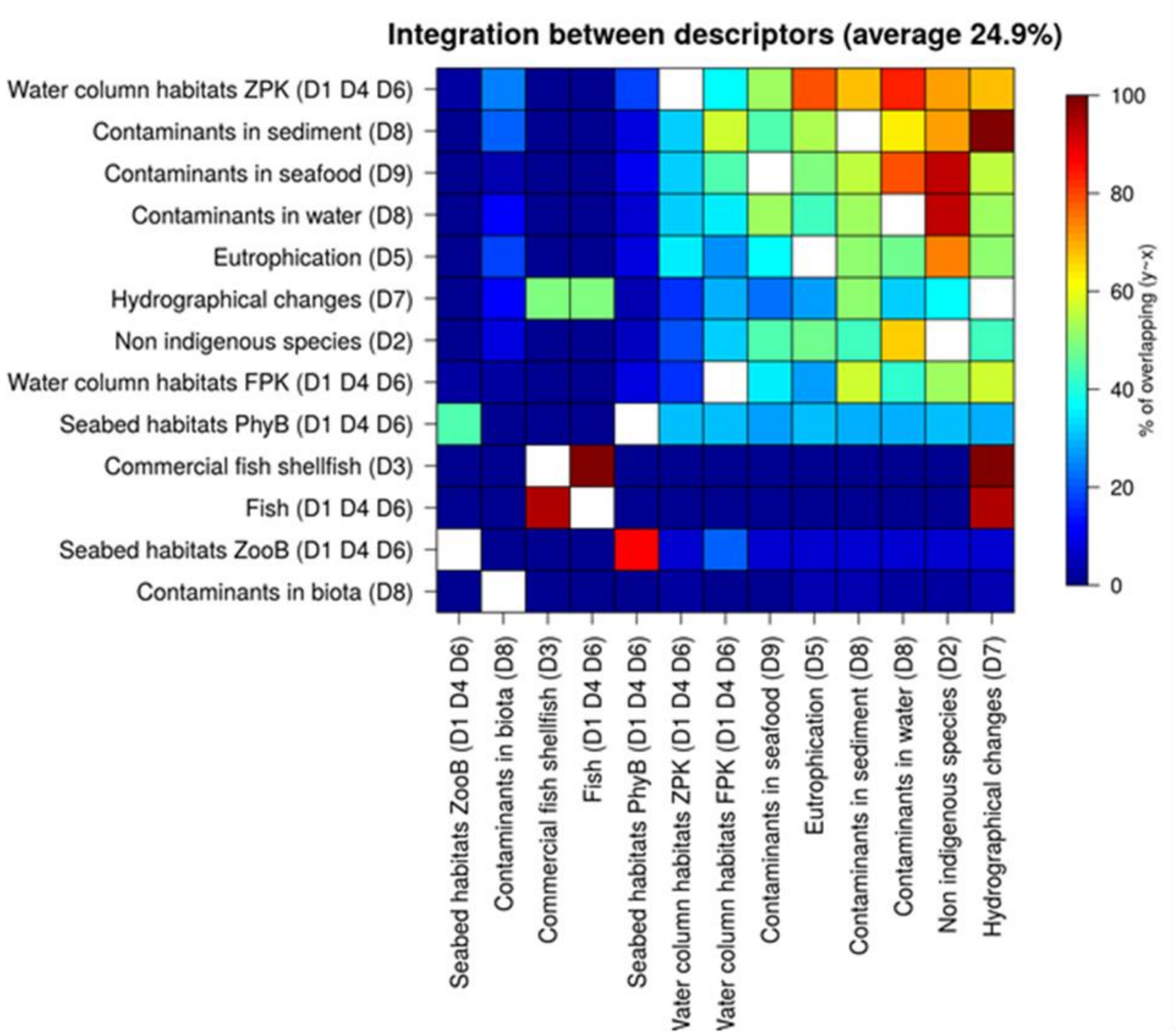


Figure 56). D2 Non indigenous species, D8 Contaminats in biota and D1 D4 D6 Water

column habitats Zooplankton have an high overlap with other descriptors (

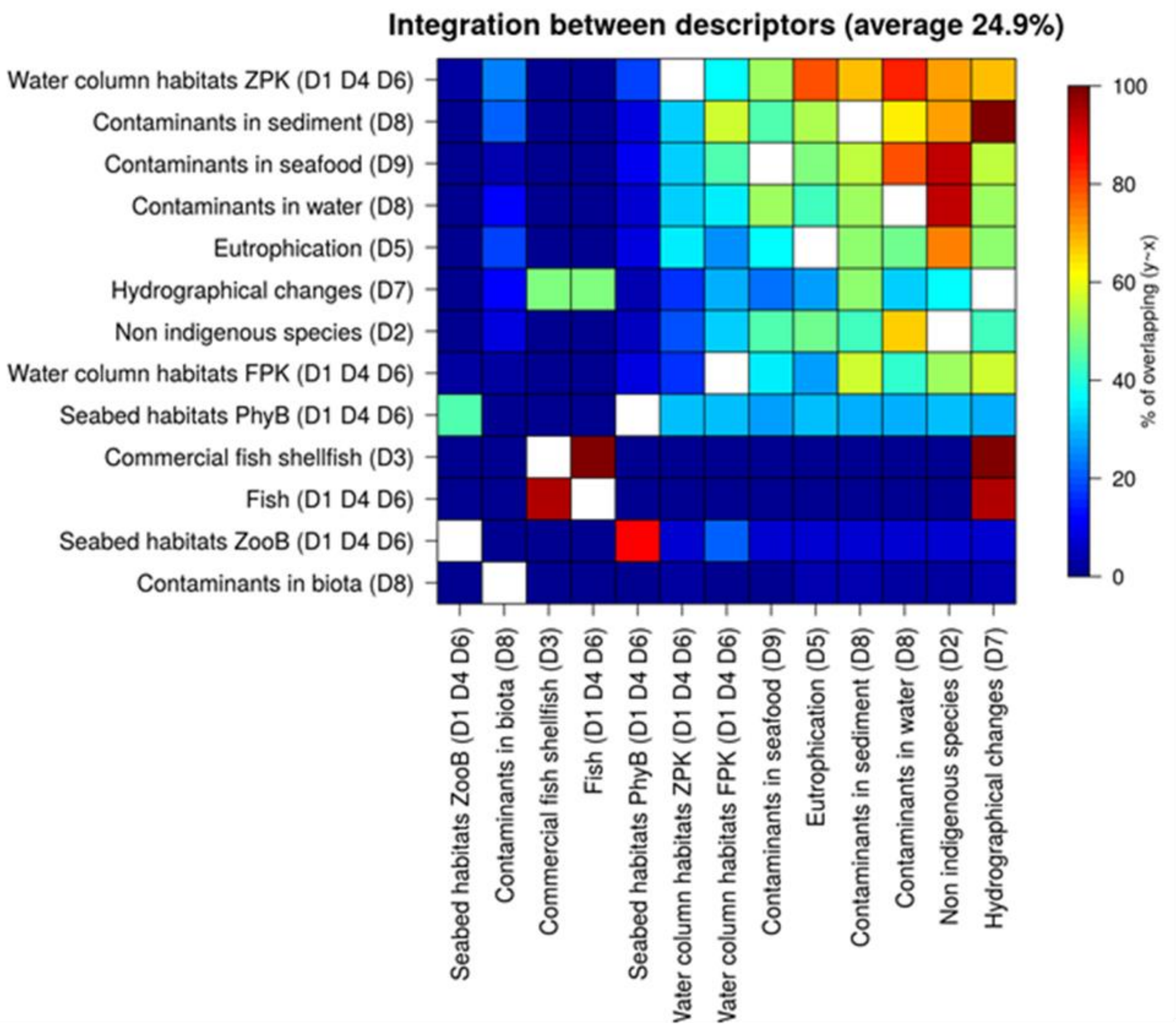


Figure 56).

The Task 2.1 of IRIS-SES has focused on the compilation of information on the existing monitoring in the Mediterranean and Black Sea. The present document provides a helpful synthesis of the main findings. Activity 3 will integrate the outcomes of Activity 1 and Activity 2 into a GIS planning tool including many scales and levels on which the MSFD Directive has been built on, such as the characteristics level (e.g. biological features, physicochemical features), pressure and impact, indicator/threshold, spatial (location of monitoring stations) and

temporal (frequency-periodicity) across regions-subregions-countries. Moreover, we have not included in the report a comparison of the gap existing among current monitoring programmes and optimal programmes accounting for the natural variability of descriptors and parameters, which is part of the Guidance (Part I) on monitoring accounting for natural variability reported as D2 in the IRIS-SES activity 2 products. Raw data are included as Appendix II in a cd format incorporating the excel files behind this gap analysis part.

DETAILED RESULTS

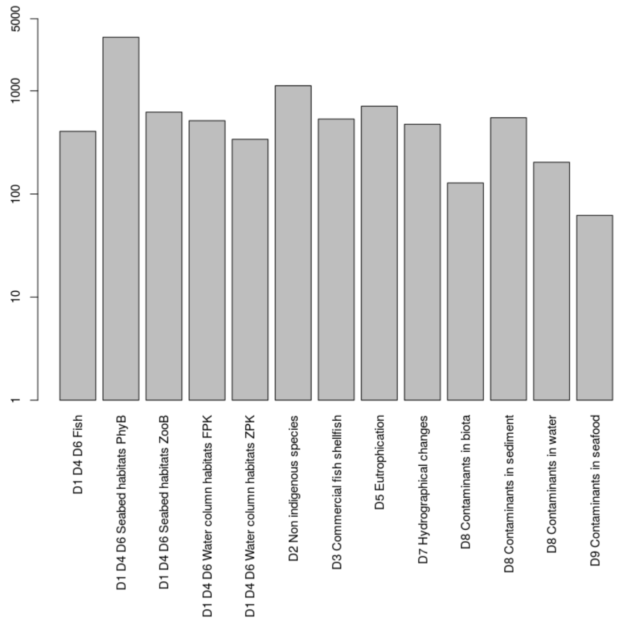


Figure 54. N. of reported stations for descriptors.

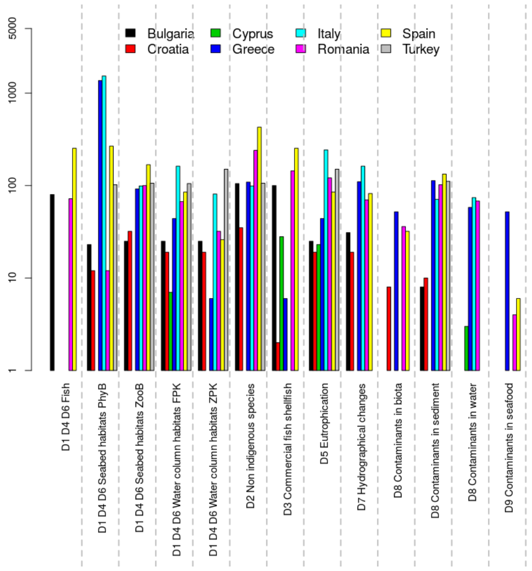


Figure 55. N. of reported stations for descriptors, divided by MS.

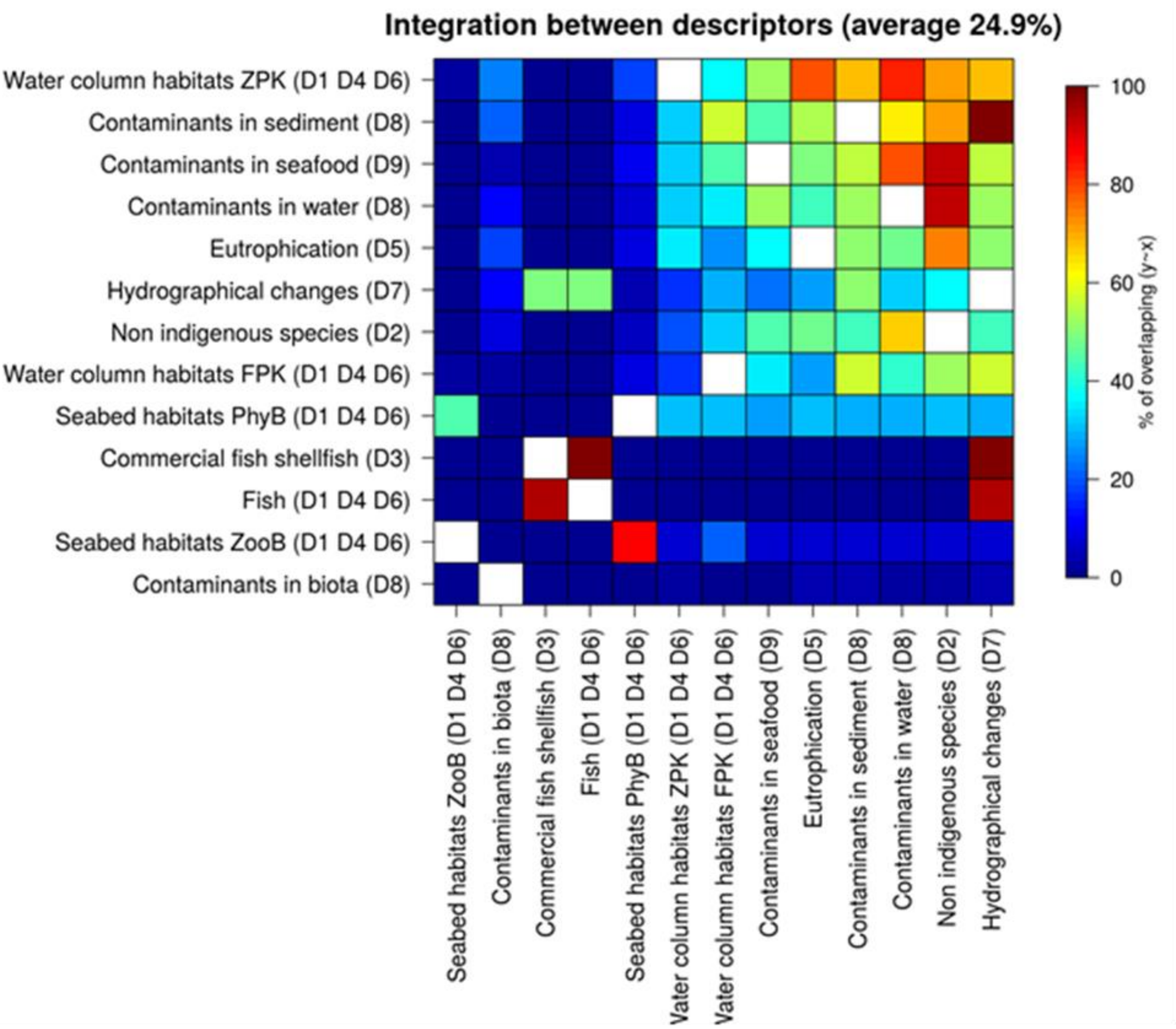


Figure 56. **Spatial overlaps between descriptors, expressed as % of stations for the descriptor on the y-axis for which the descriptor on the x axis is measured. Numbers between parentheses on the y axis indicates the total number of stations for descriptor (only stations with known location).**

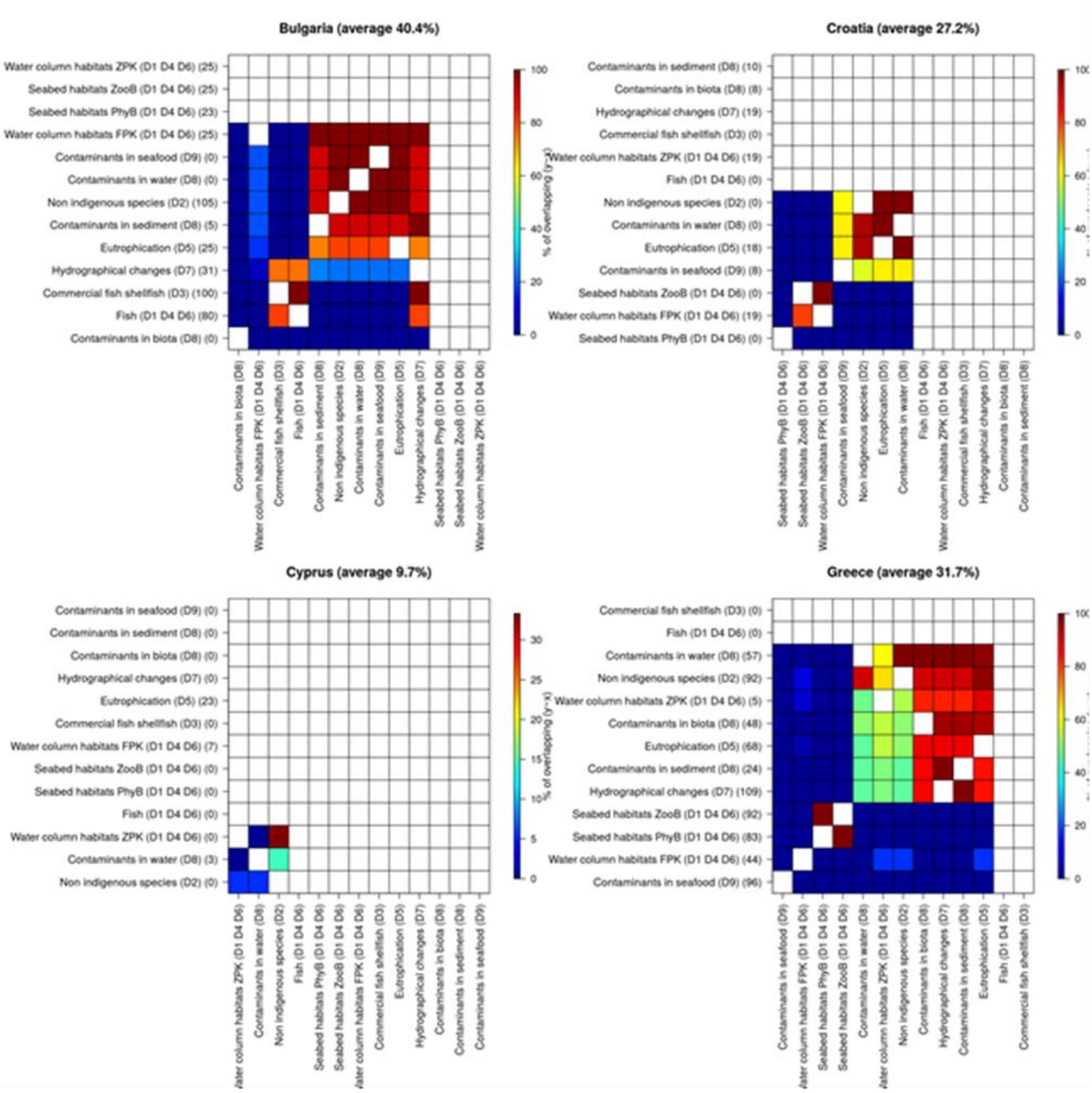


Figure 57. Spatial overlaps between descriptors divided by MS, expressed as percentage of stations for the descriptor on the y-axis for which the descriptor on the x axis is measured. Numbers between parenthesis on the y axis indicates the total number of stations for descriptor (only stations with known location).

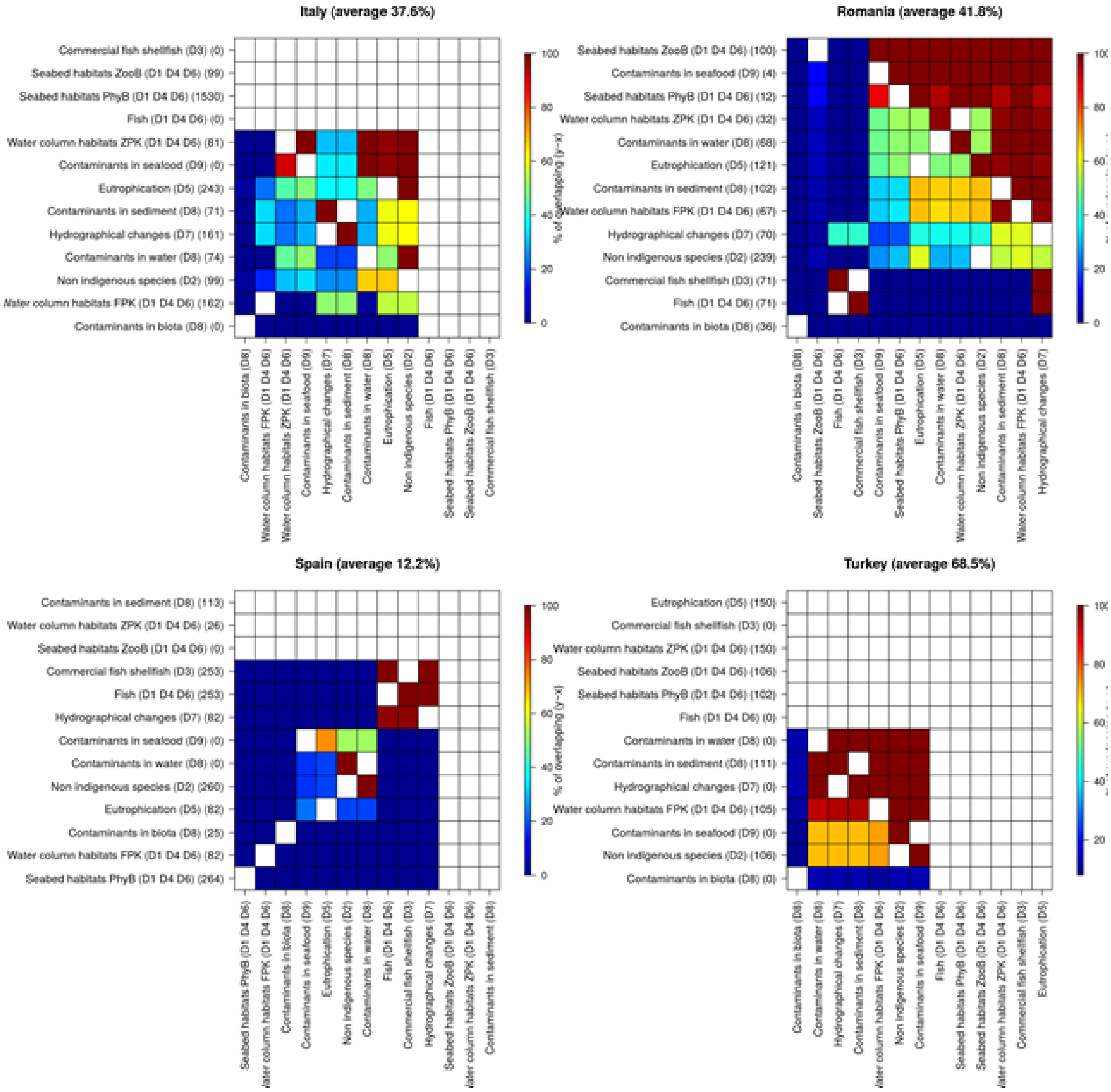


Figure 58. Spatial overlaps between descriptors divided by MS, expressed as percentage of stations for the descriptor on the y-axis for which the descriptor on the x axis is measured. Numbers between parentheses on the y-axis indicates the total number of stations for descriptor (only stations with known location).

D1 D4 D6 Fish

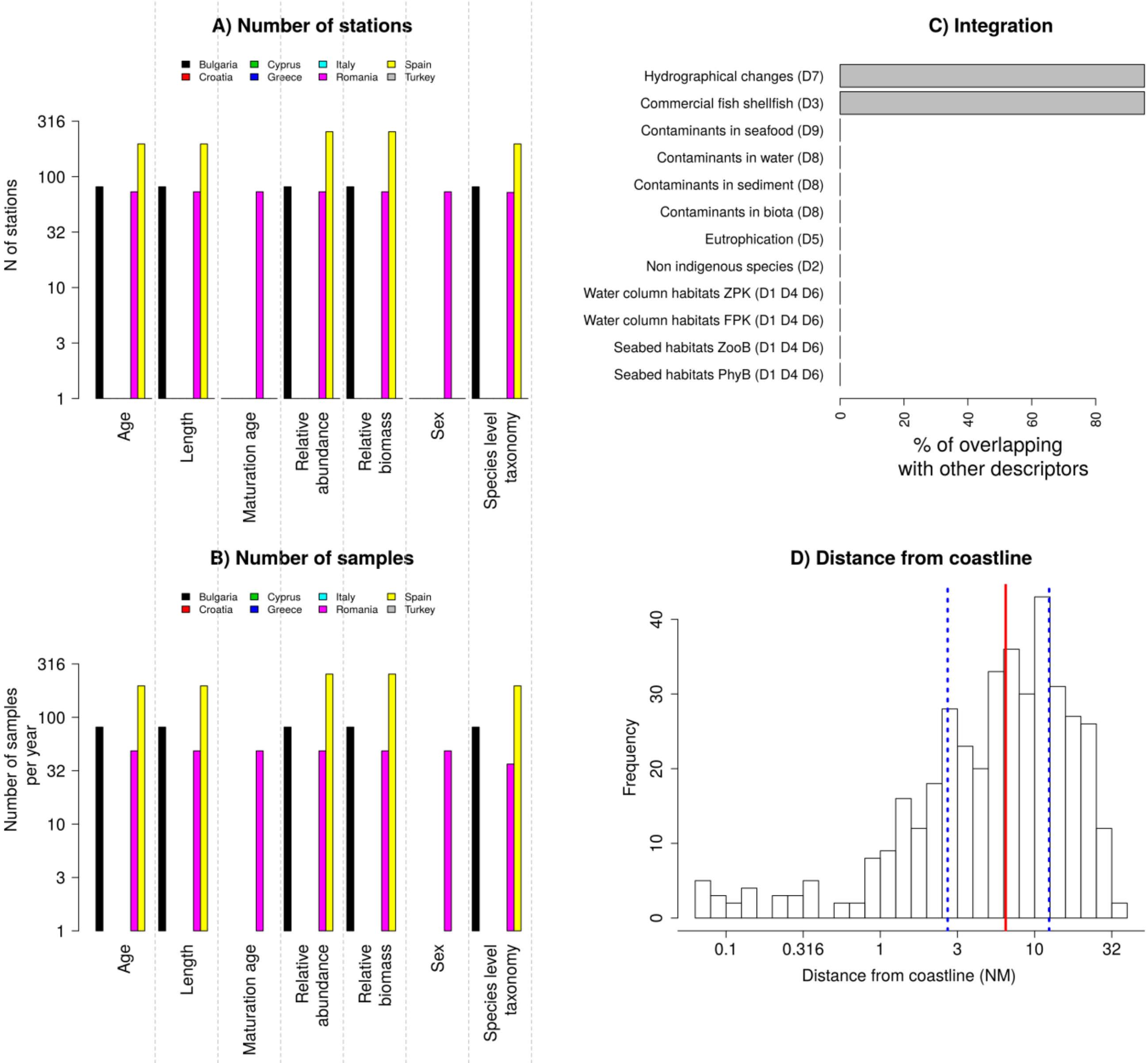


Figure 59. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25th and 75th quantile (stations with known location).

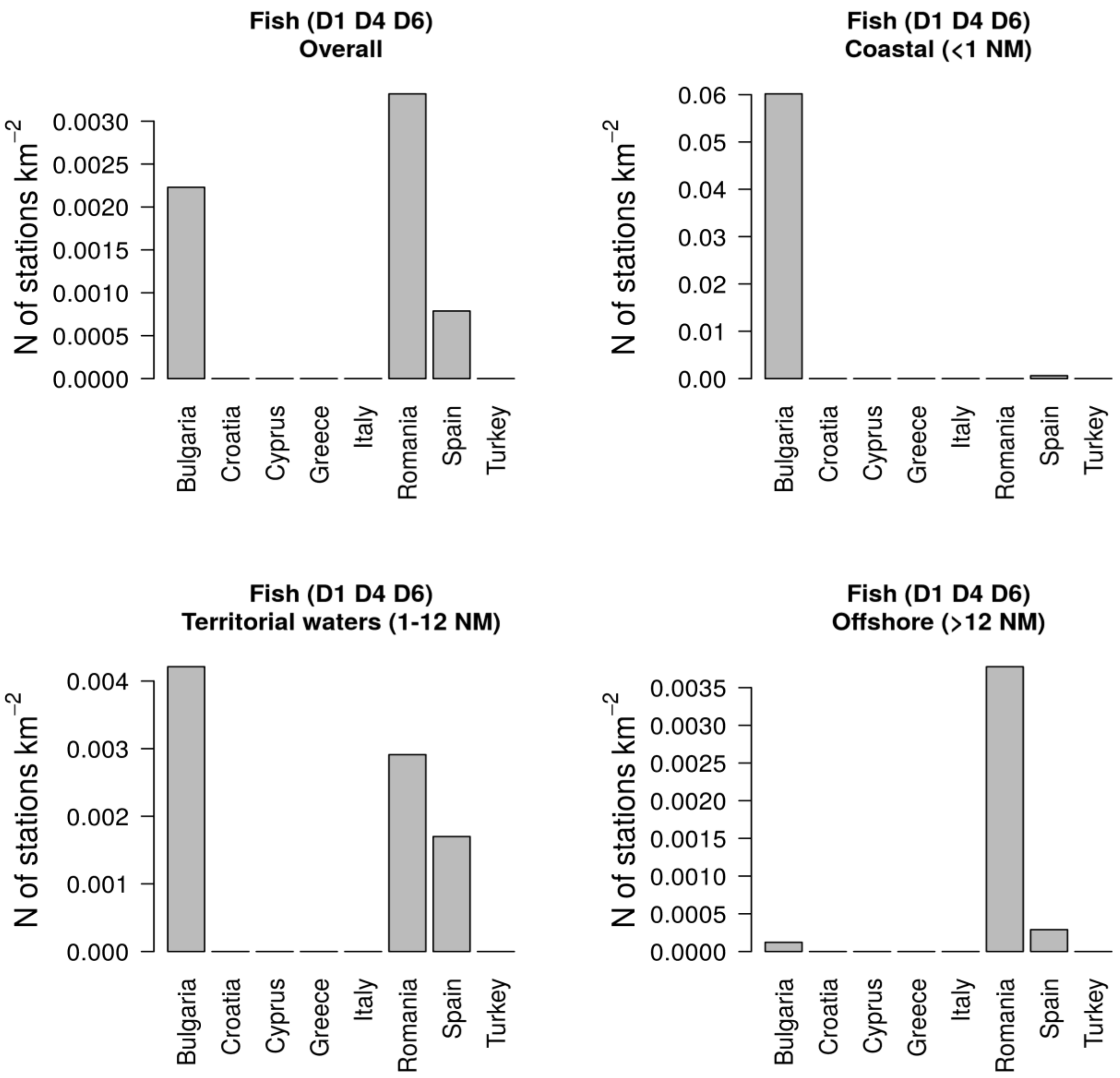


Figure 60. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known

location).

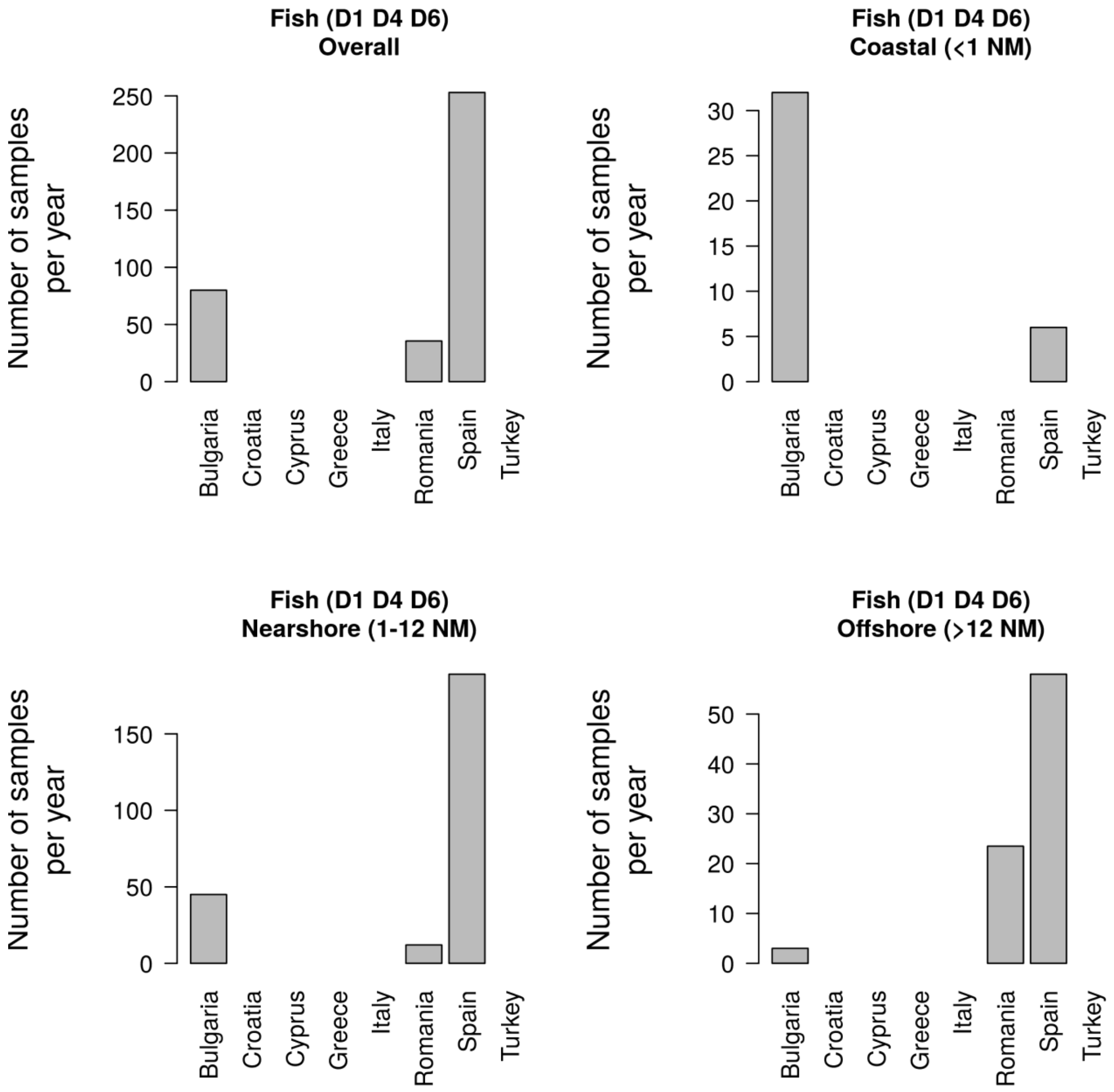


Figure 61. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

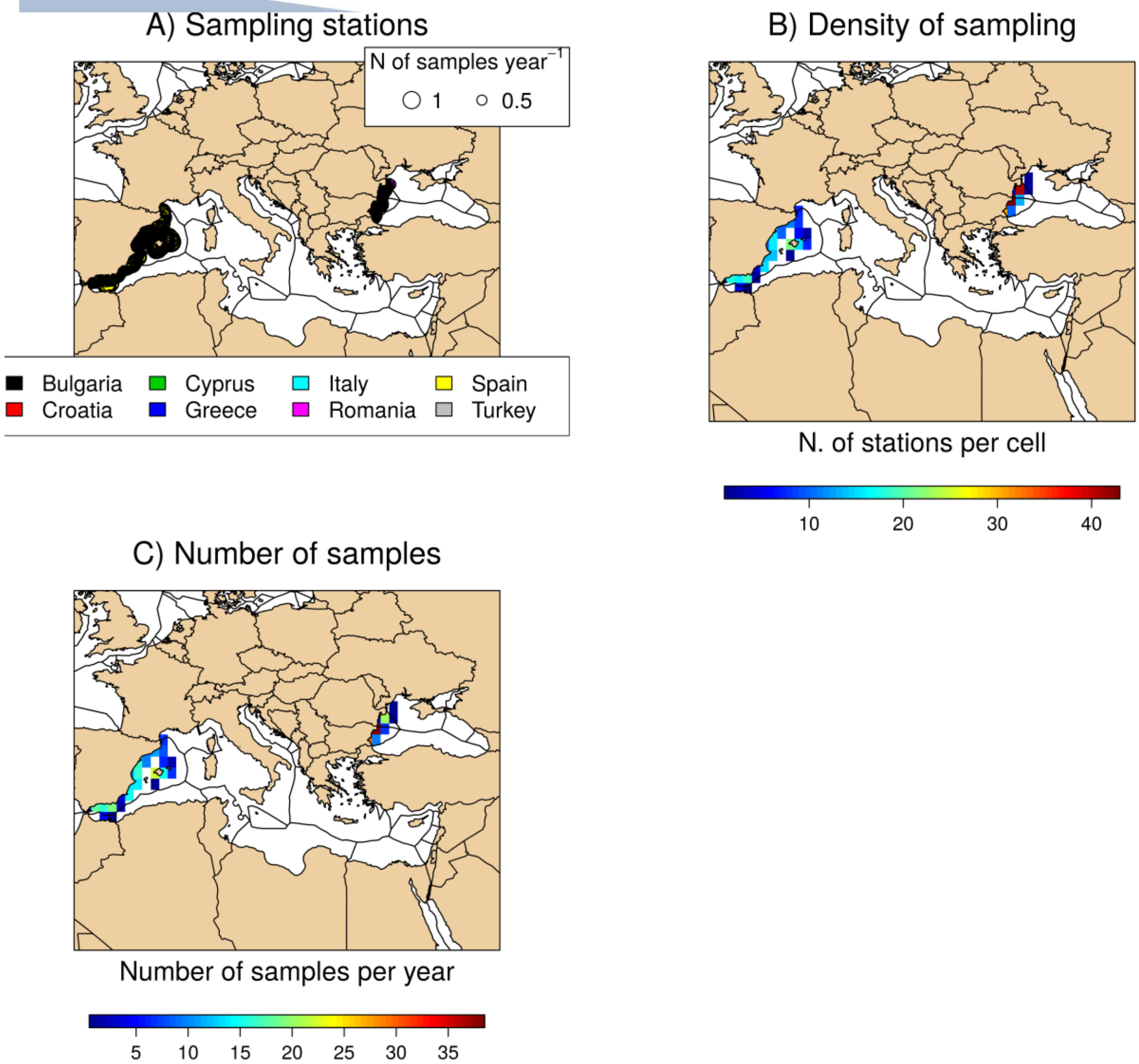


Figure 62. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D1 D4 D6 Seabed habitats Phytobenthos

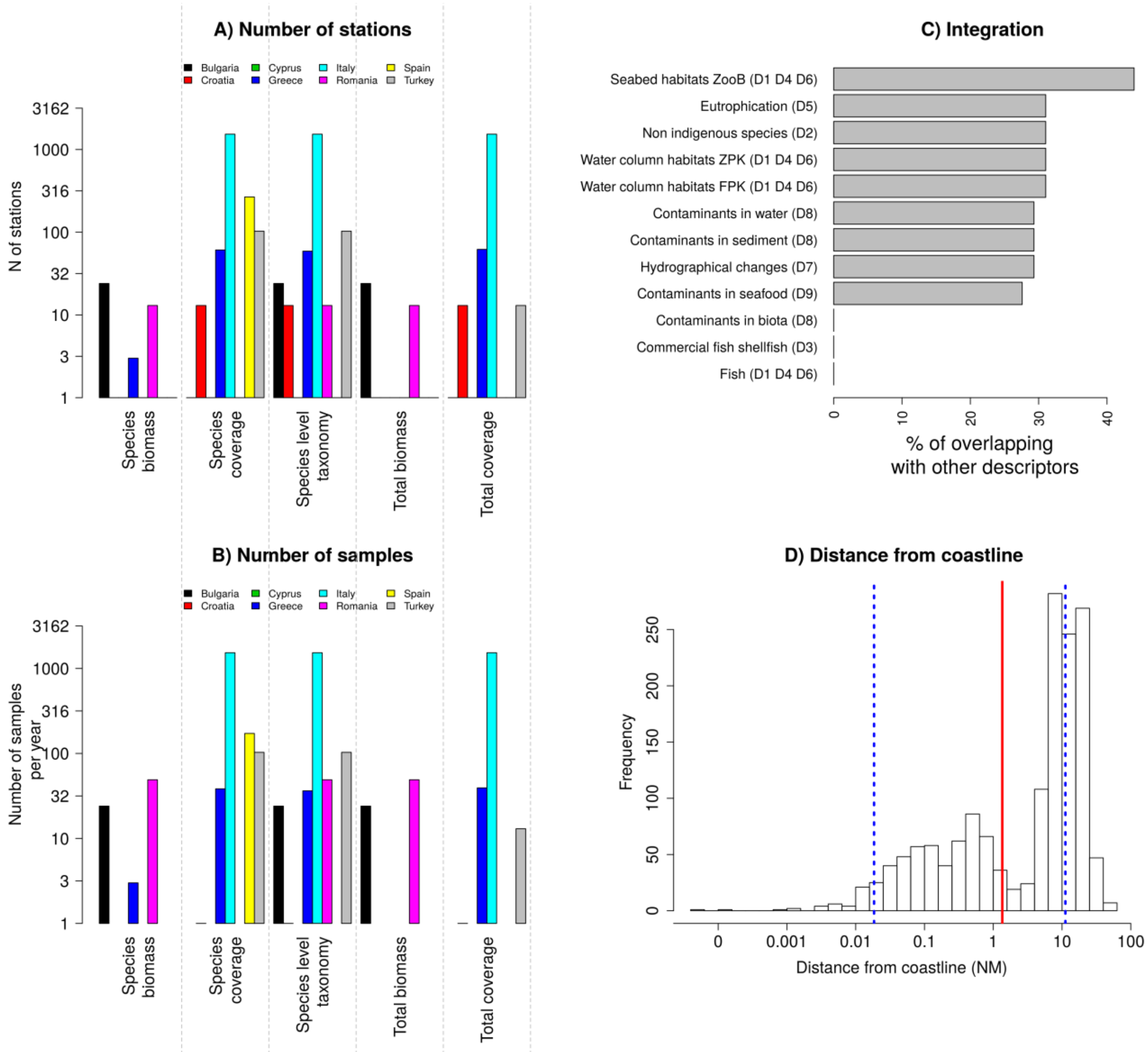


Figure 63. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value; the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

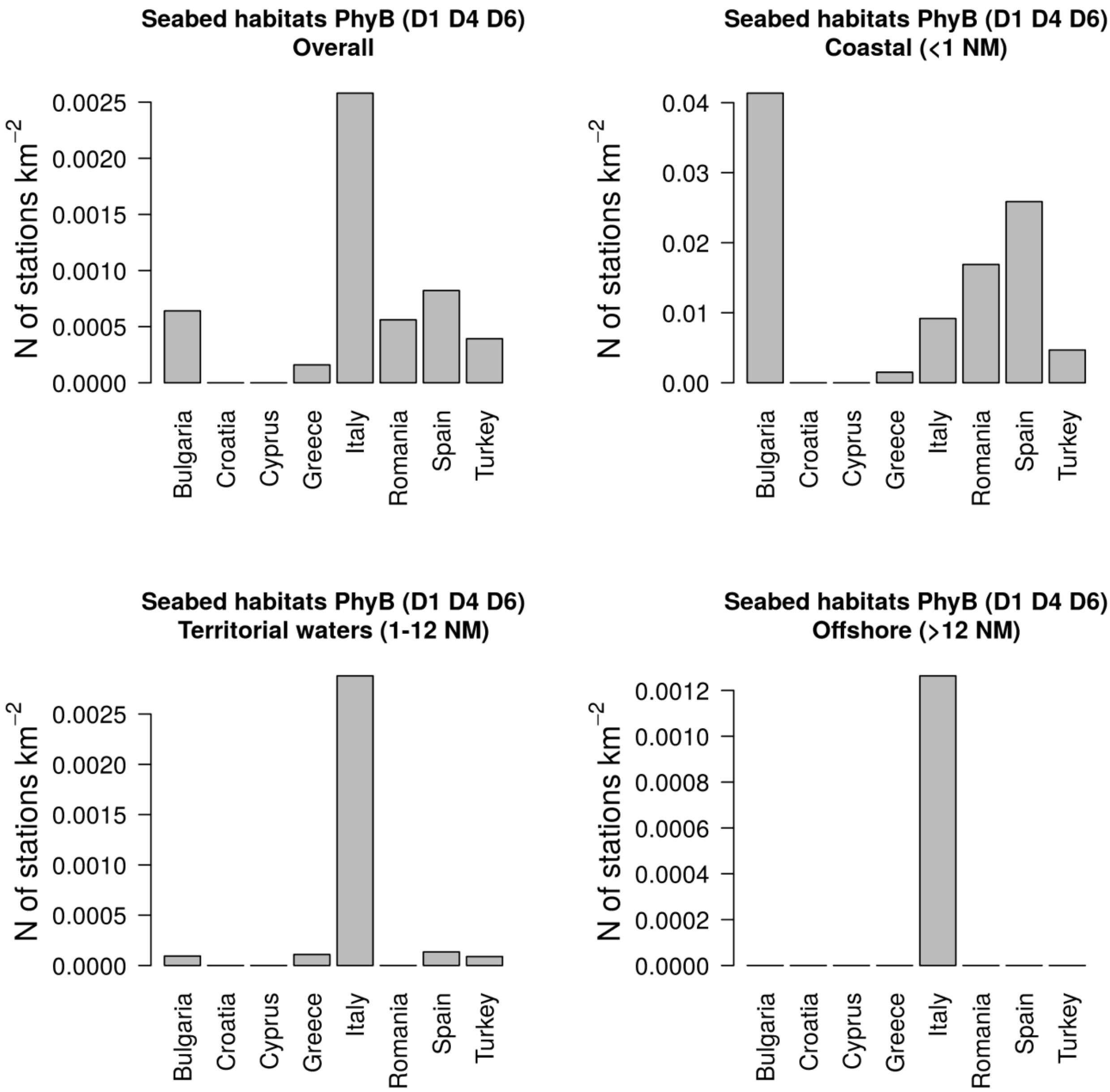


Figure 64. Density of sampling stations (N of station km^{-2}) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

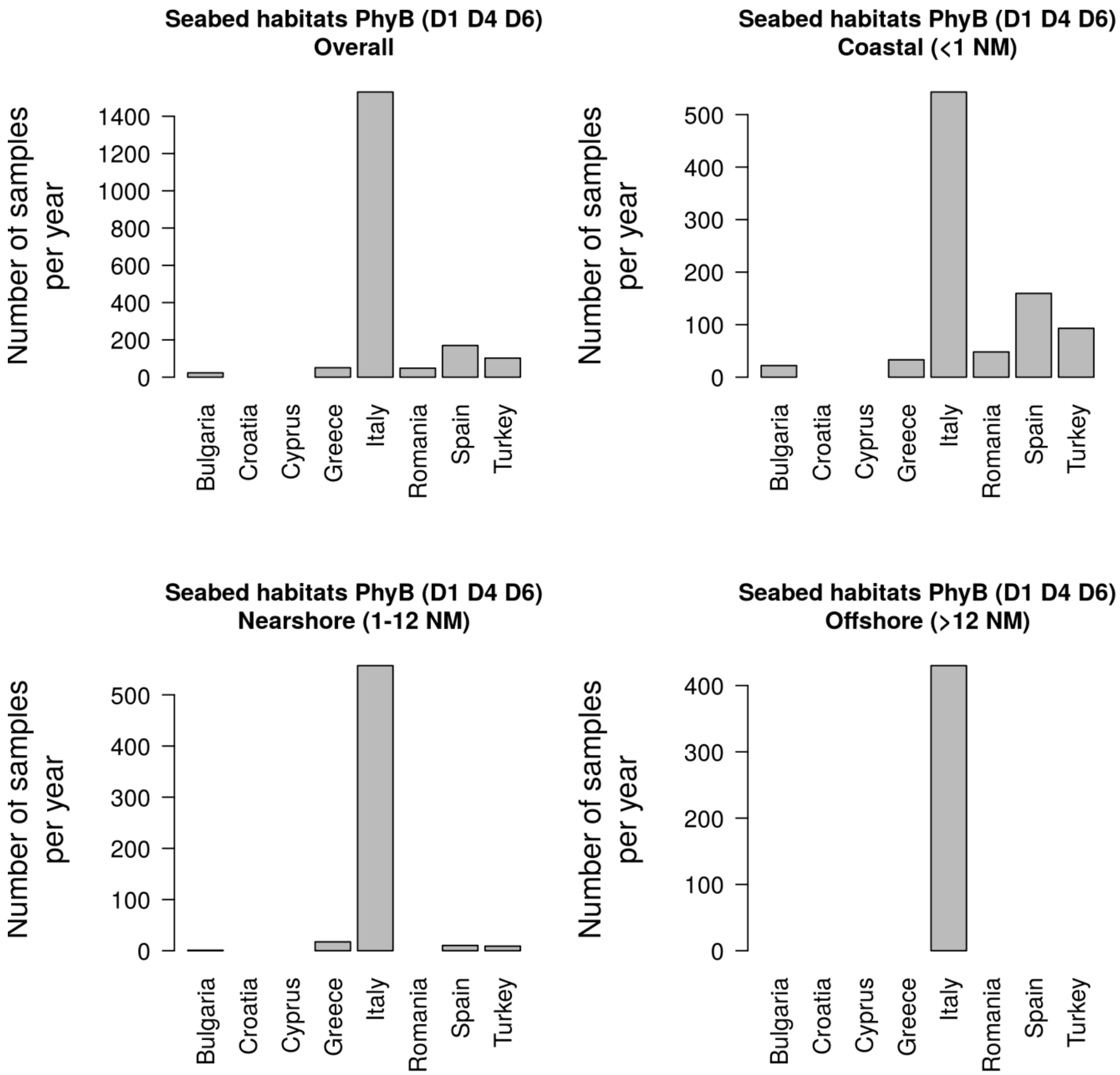


Figure 65. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

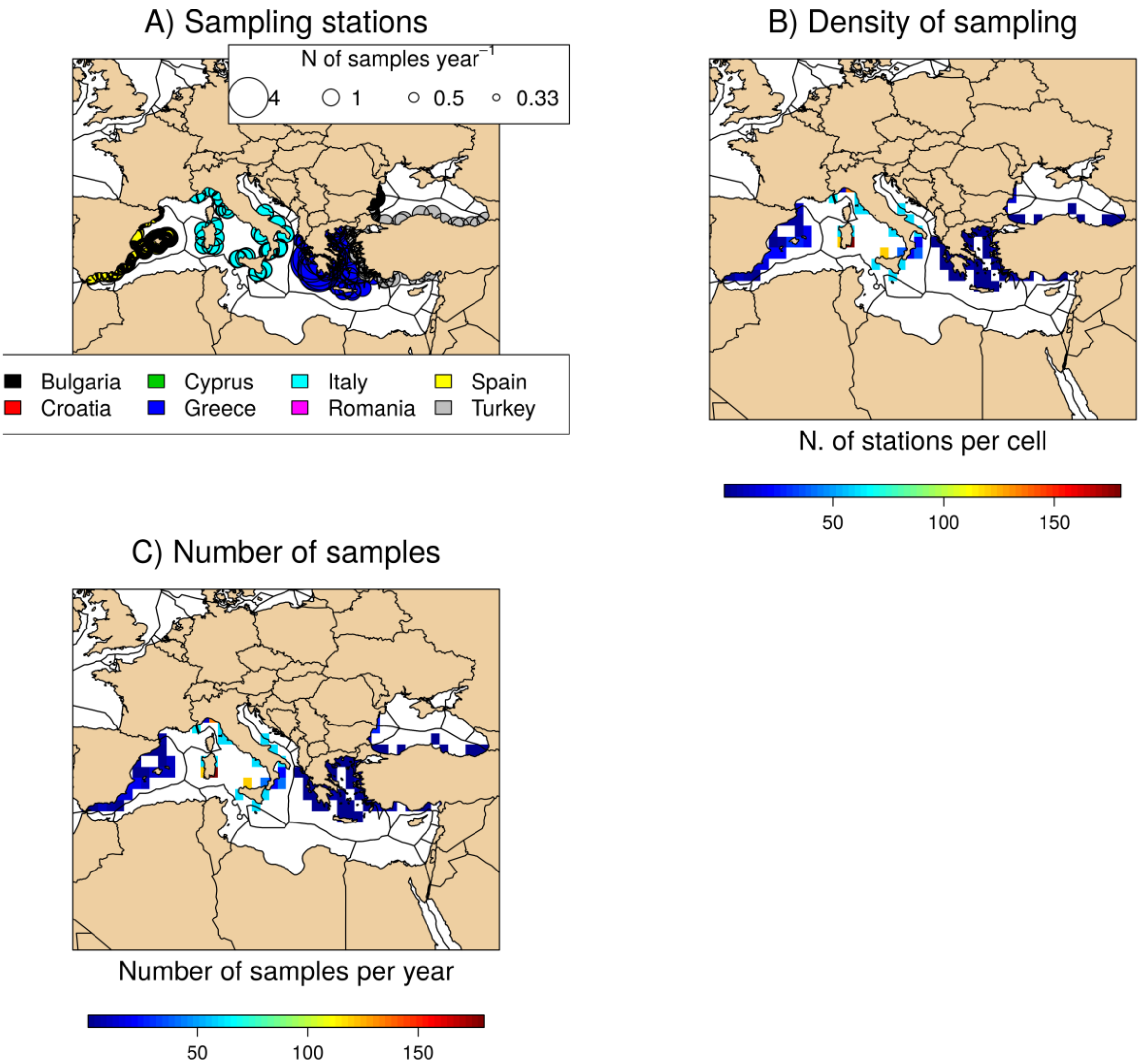


Figure 66. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D1 D4 D6 Seabed habitats Zoobenthos

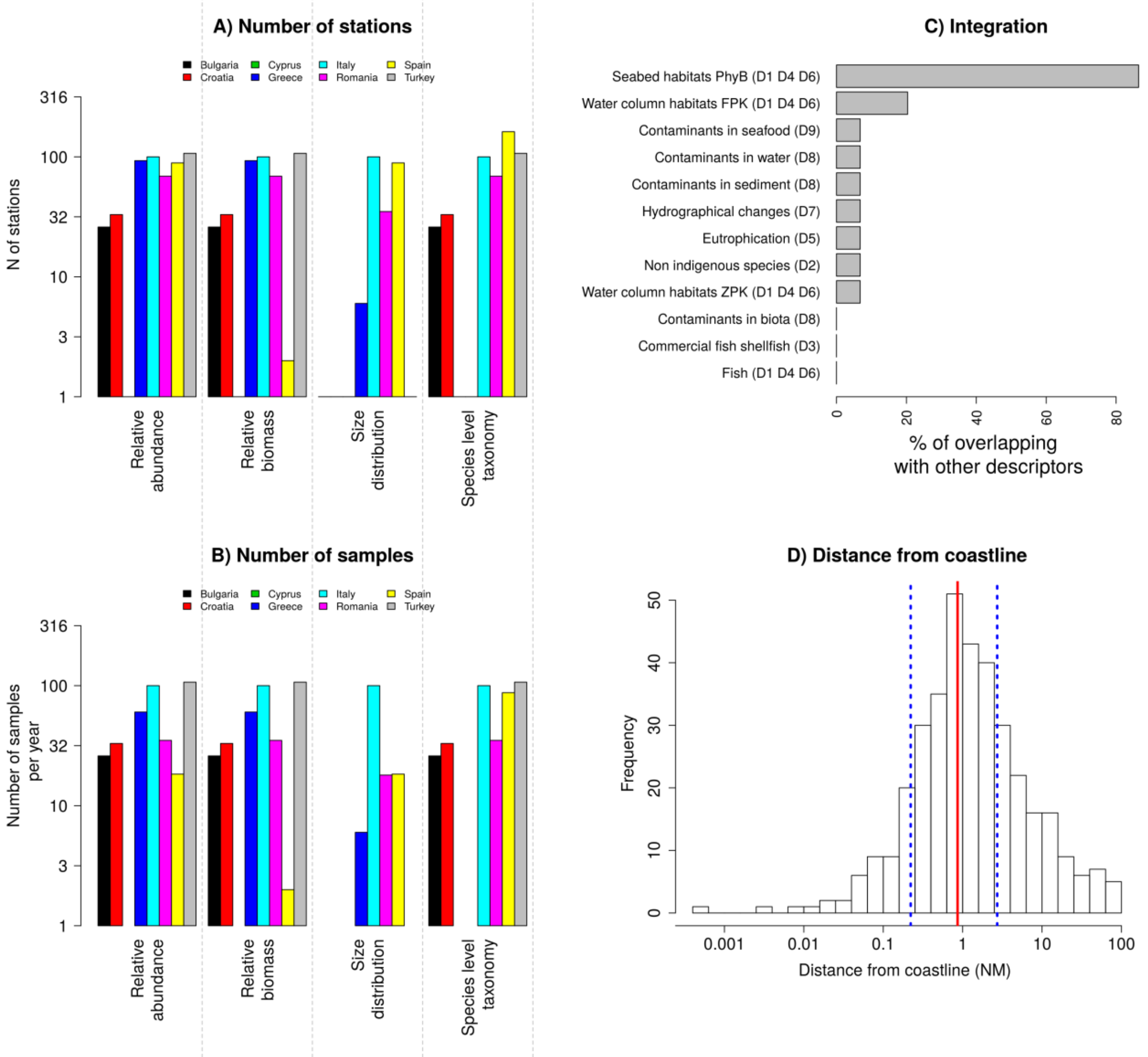


Figure 67. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

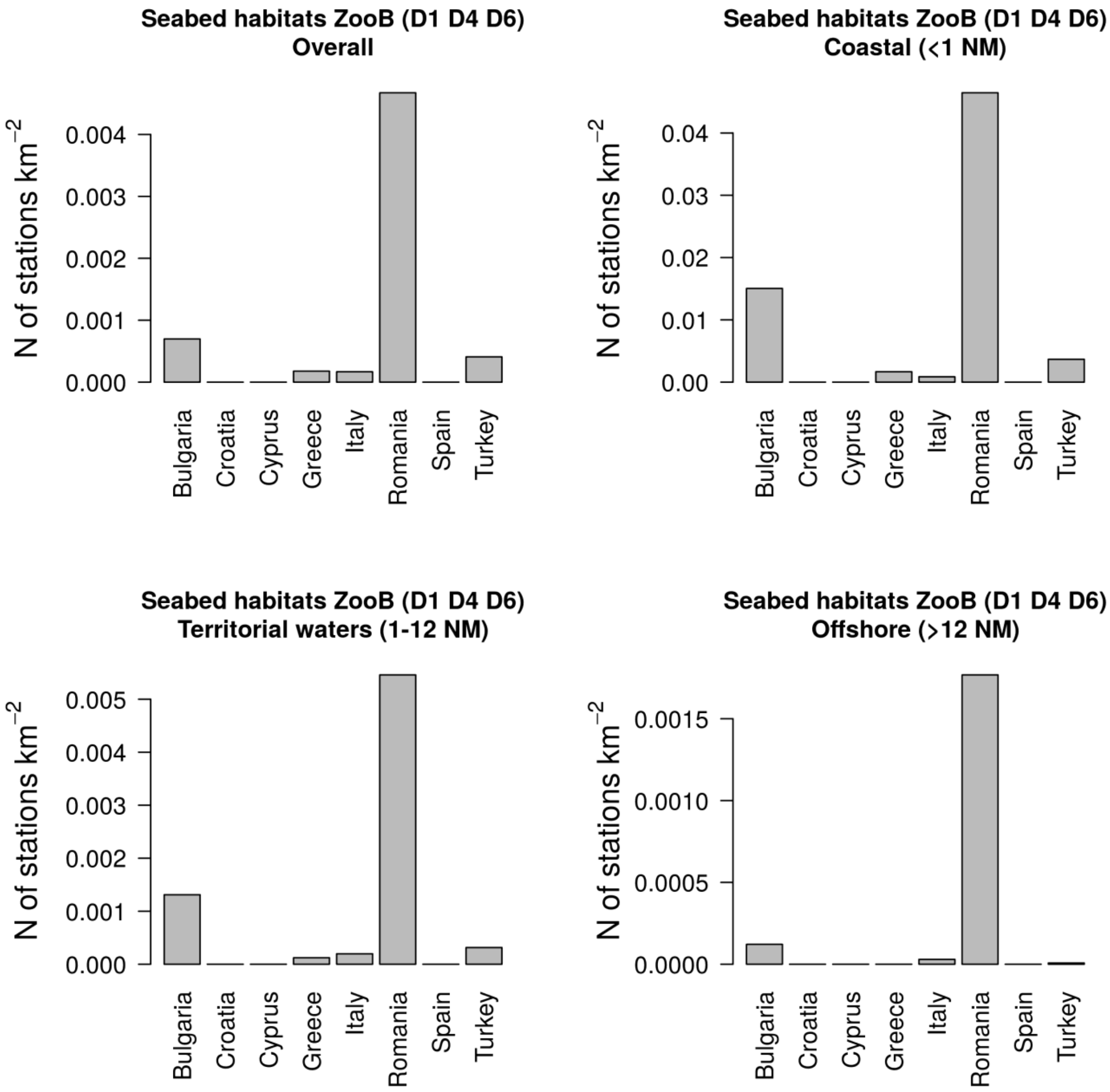


Figure 68. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

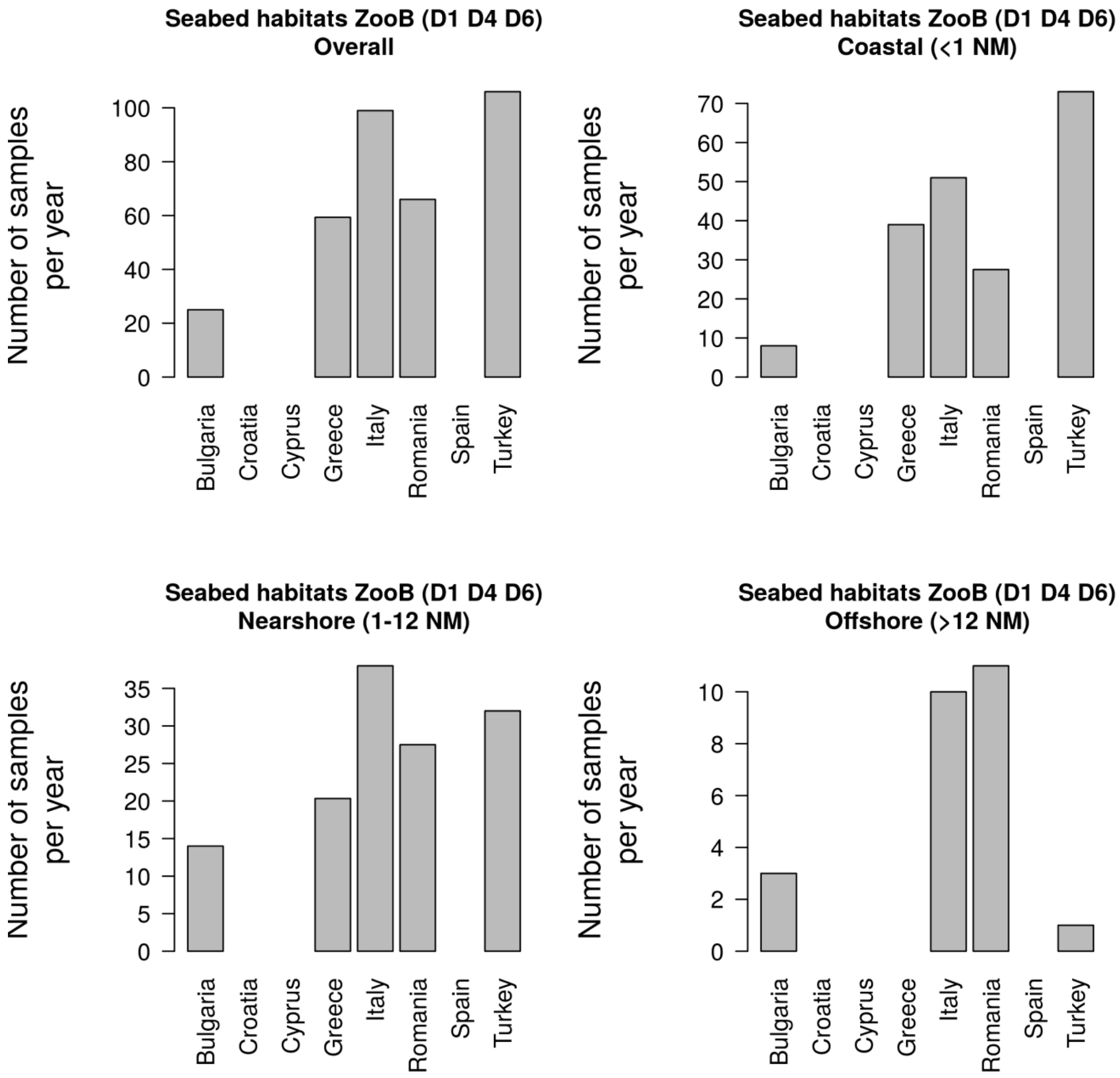


Figure 69. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

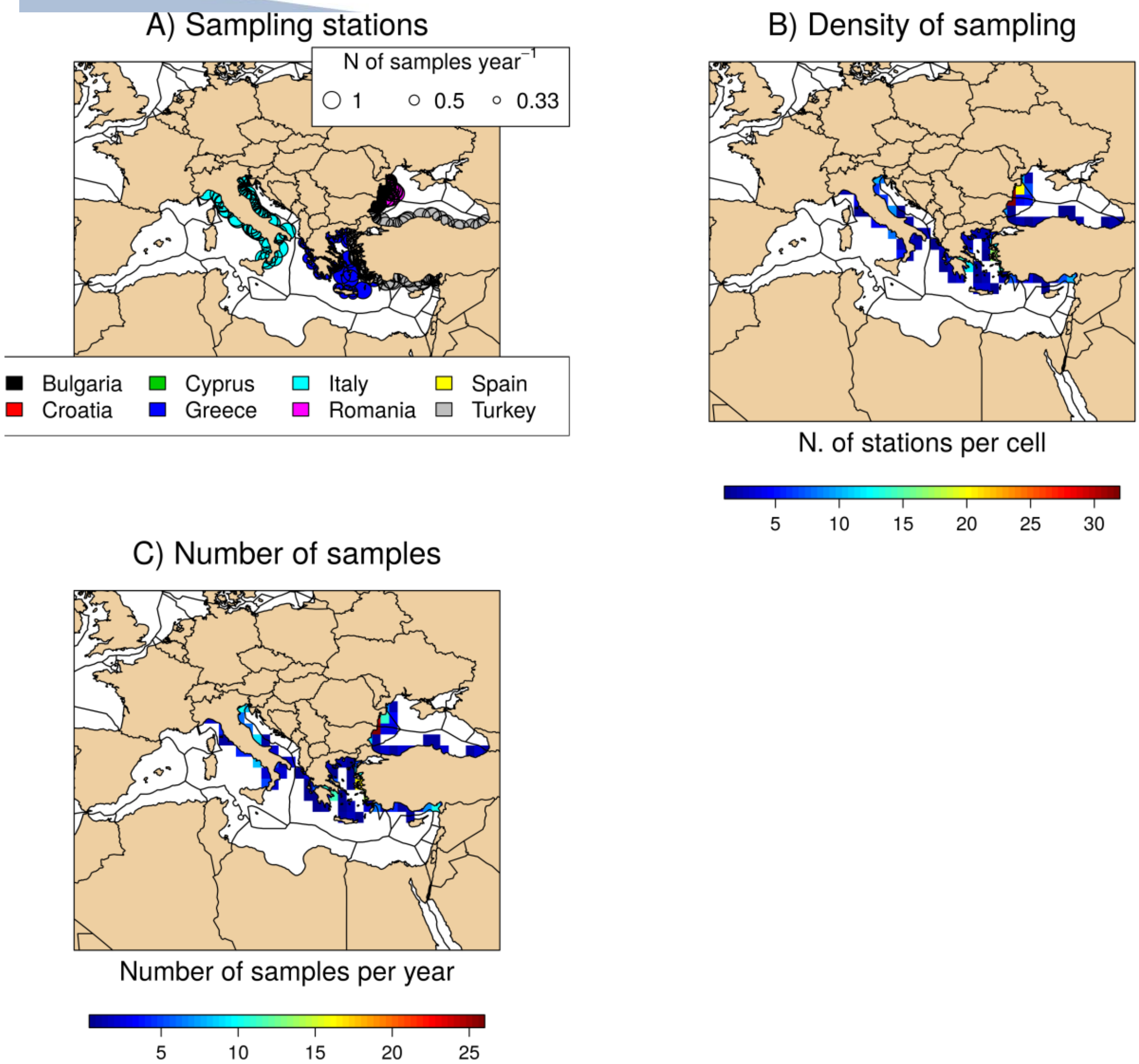


Figure 70. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D1 D4 D6 Water column habitat Phytoplankton

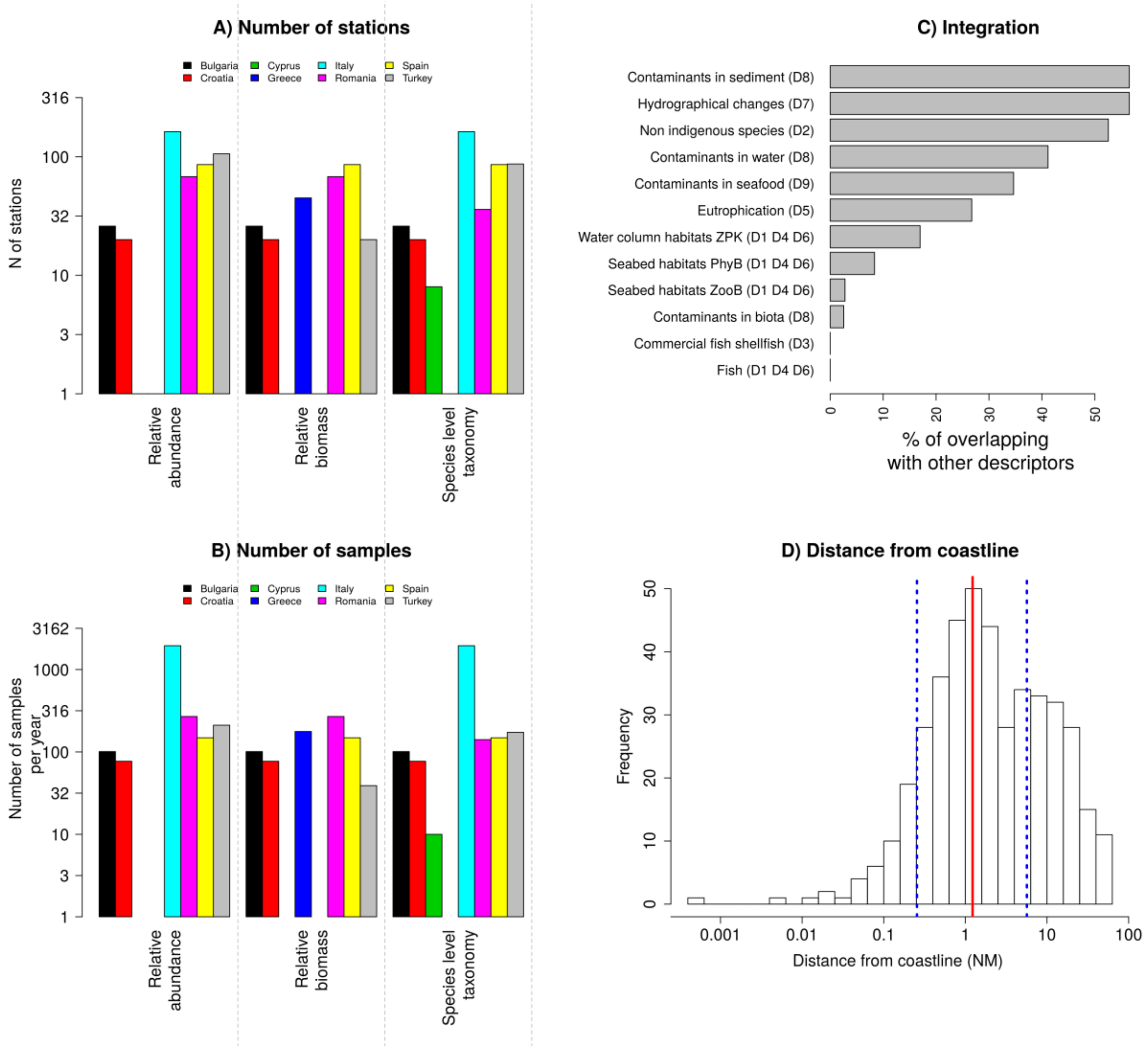


Figure 71. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value; the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

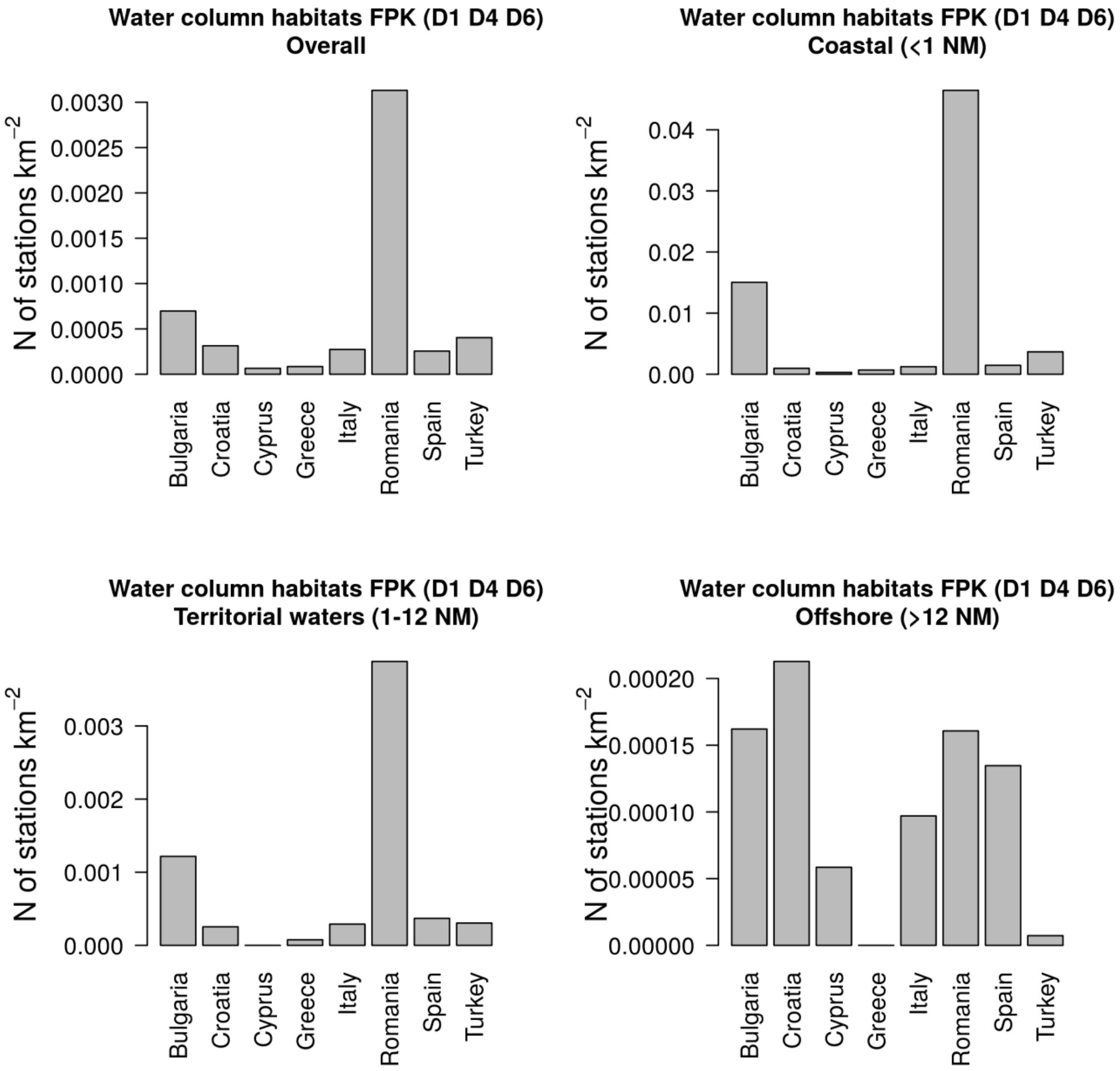


Figure 72. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

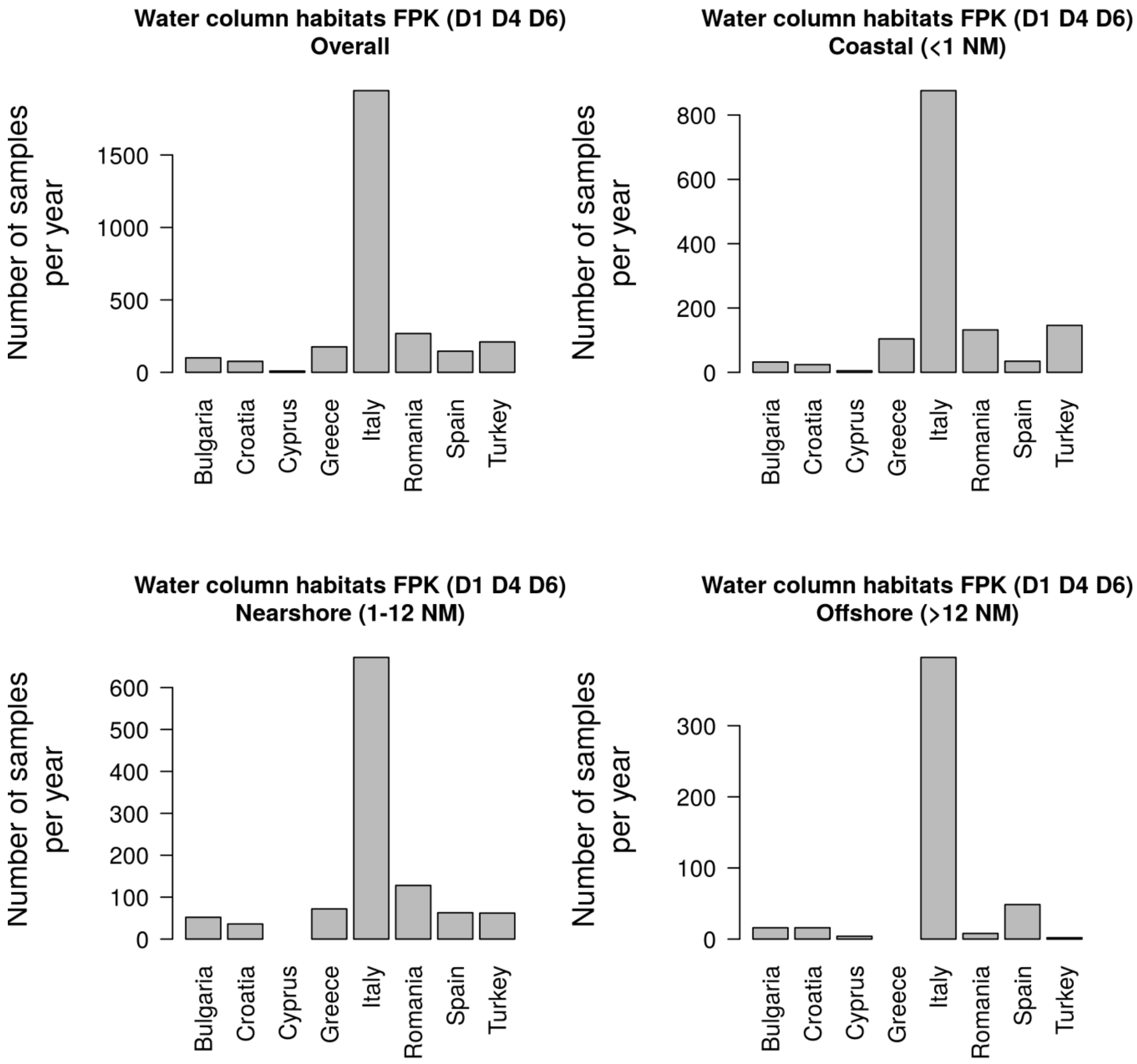


Figure 73. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

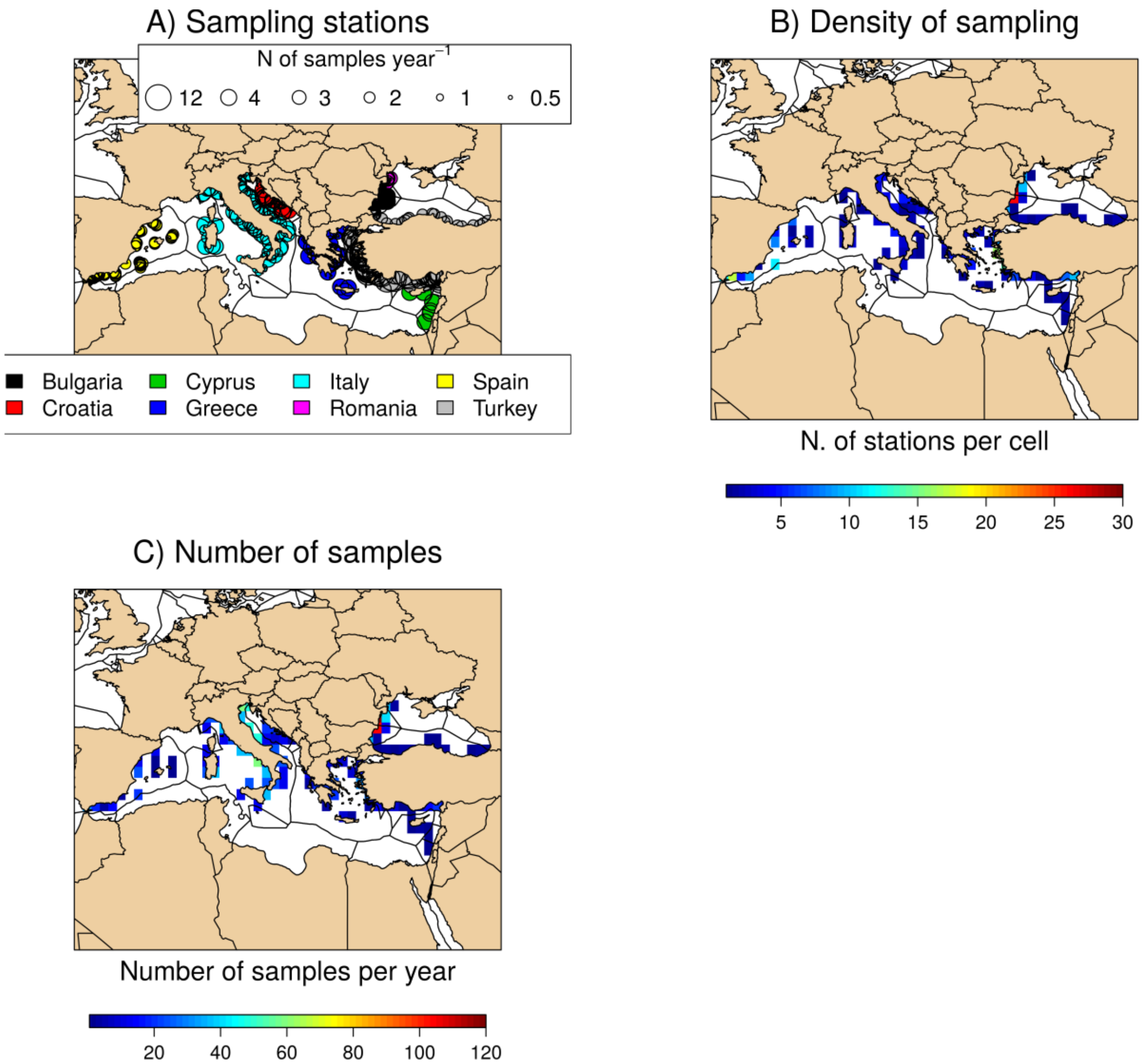


Figure 74. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D1 D4 D6 Water column habitat Zooplankton

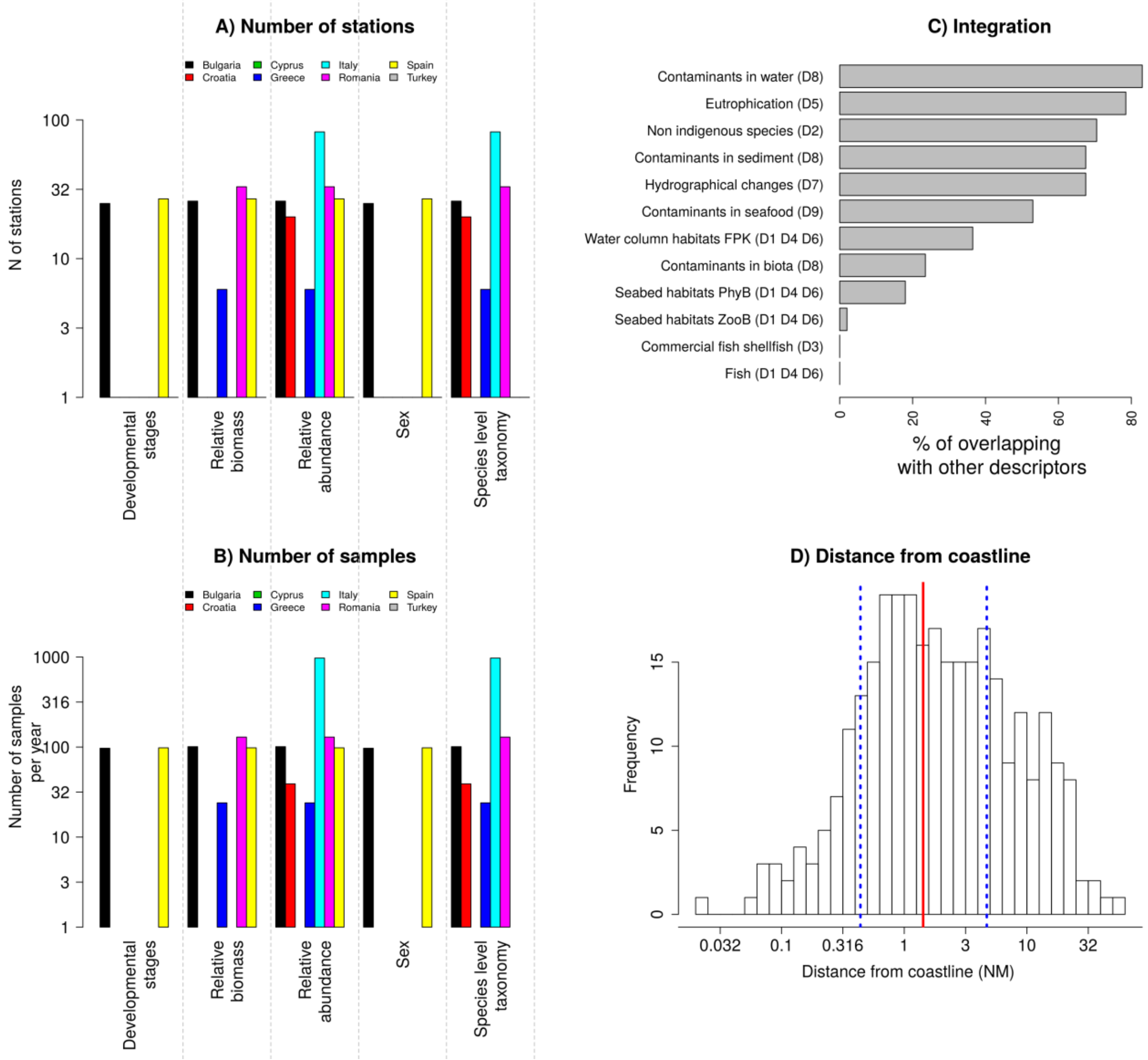


Figure 75. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

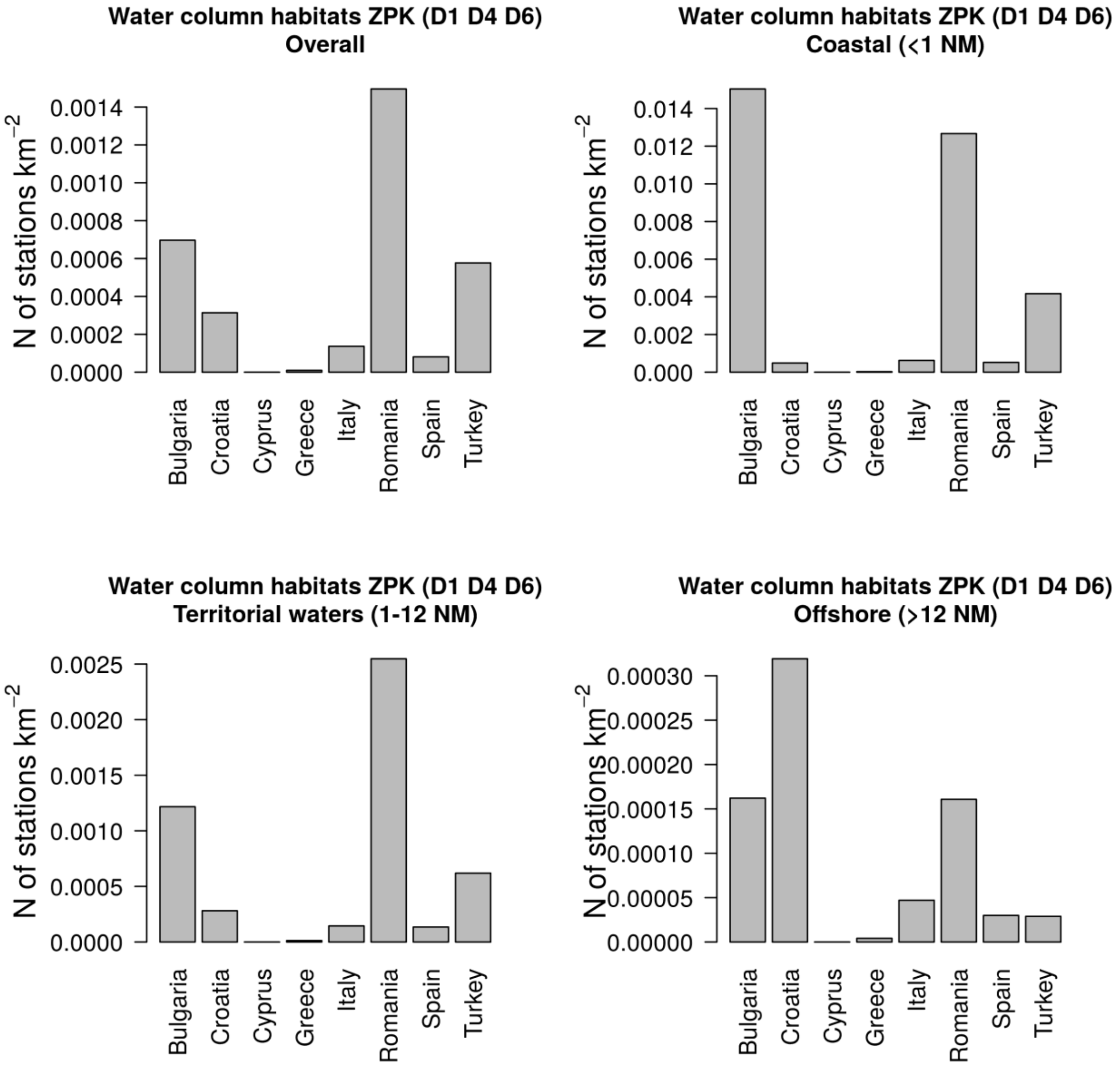


Figure 76. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

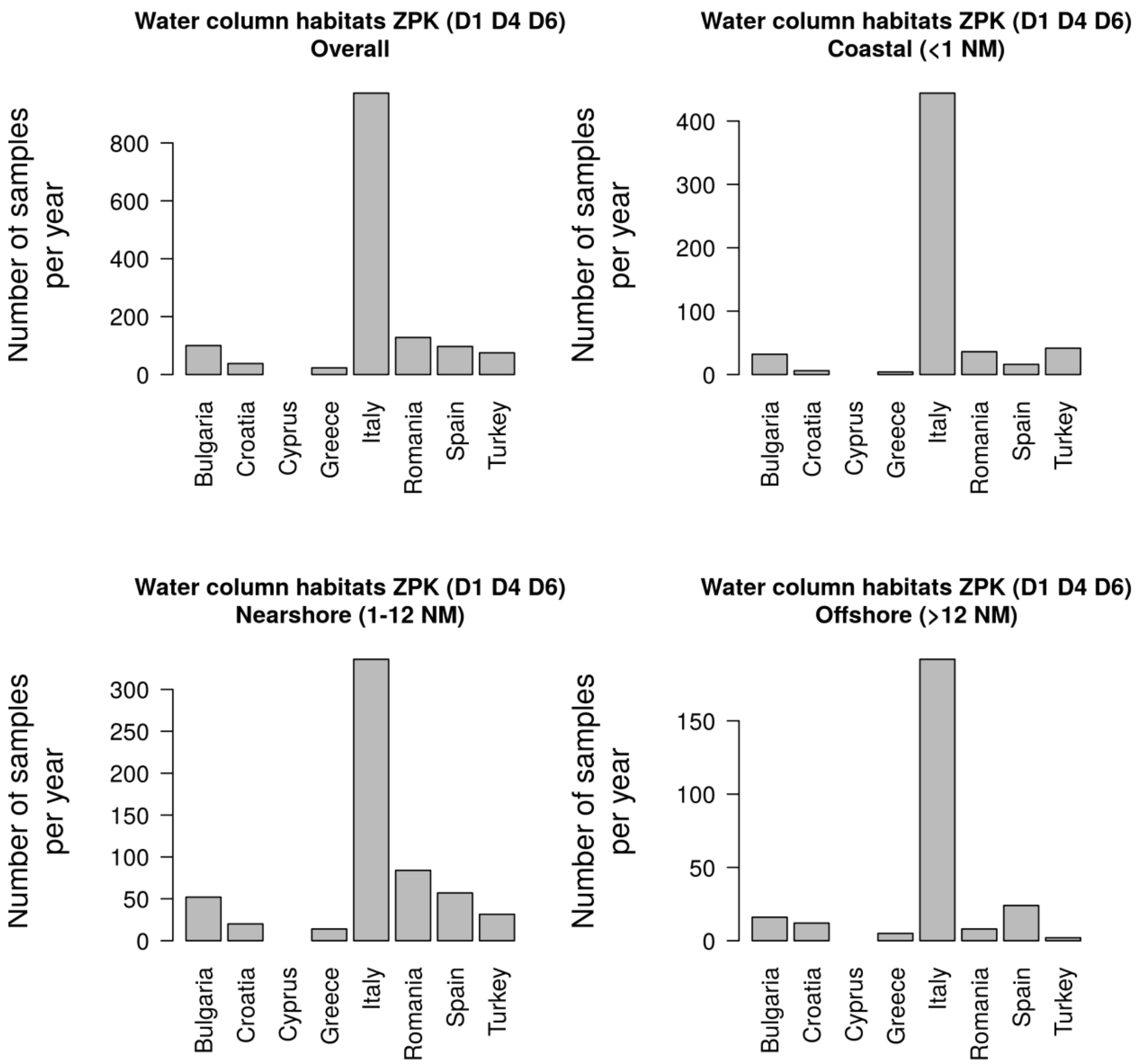


Figure 77. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

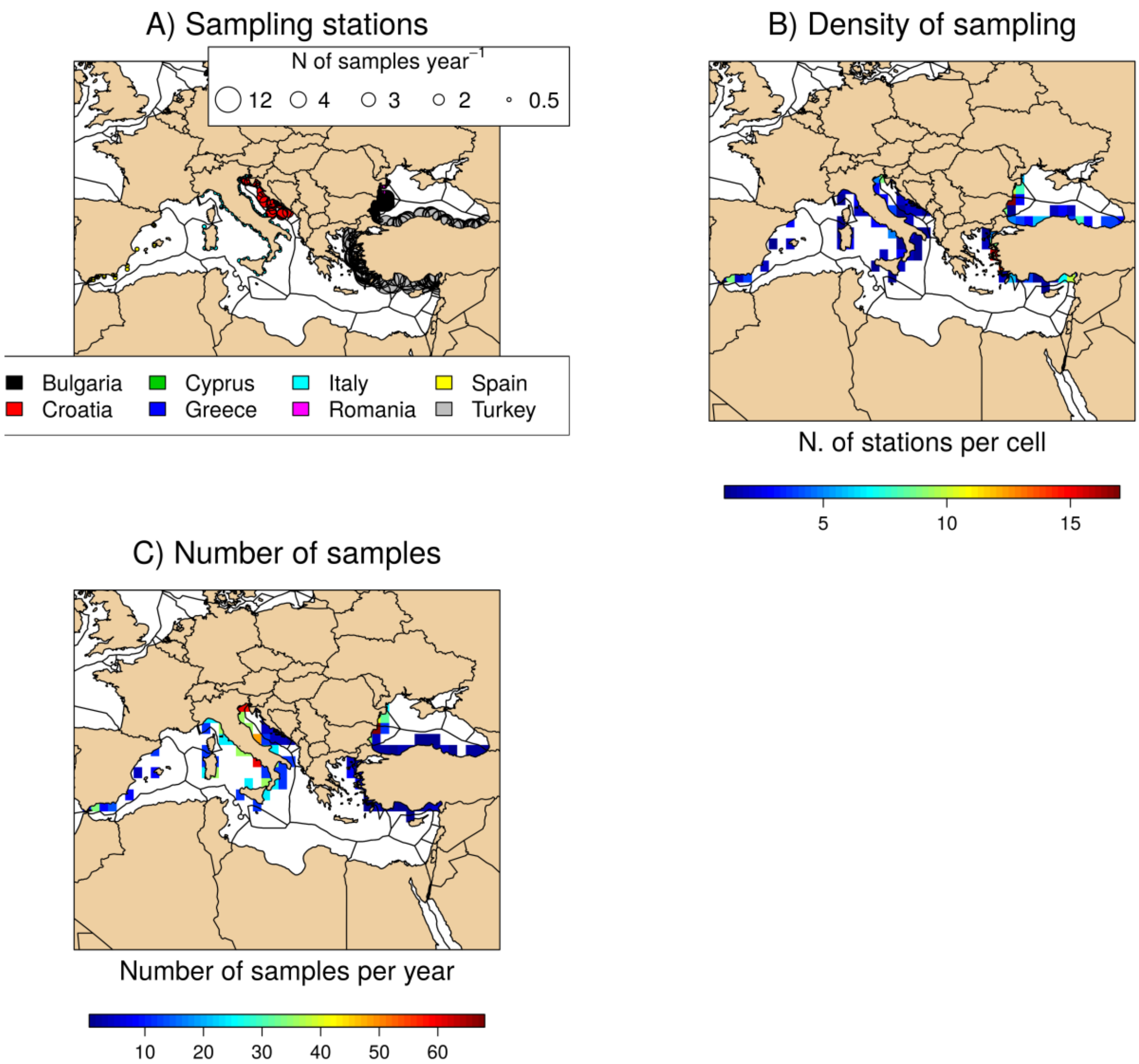


Figure 78. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D2 Non indigenous species

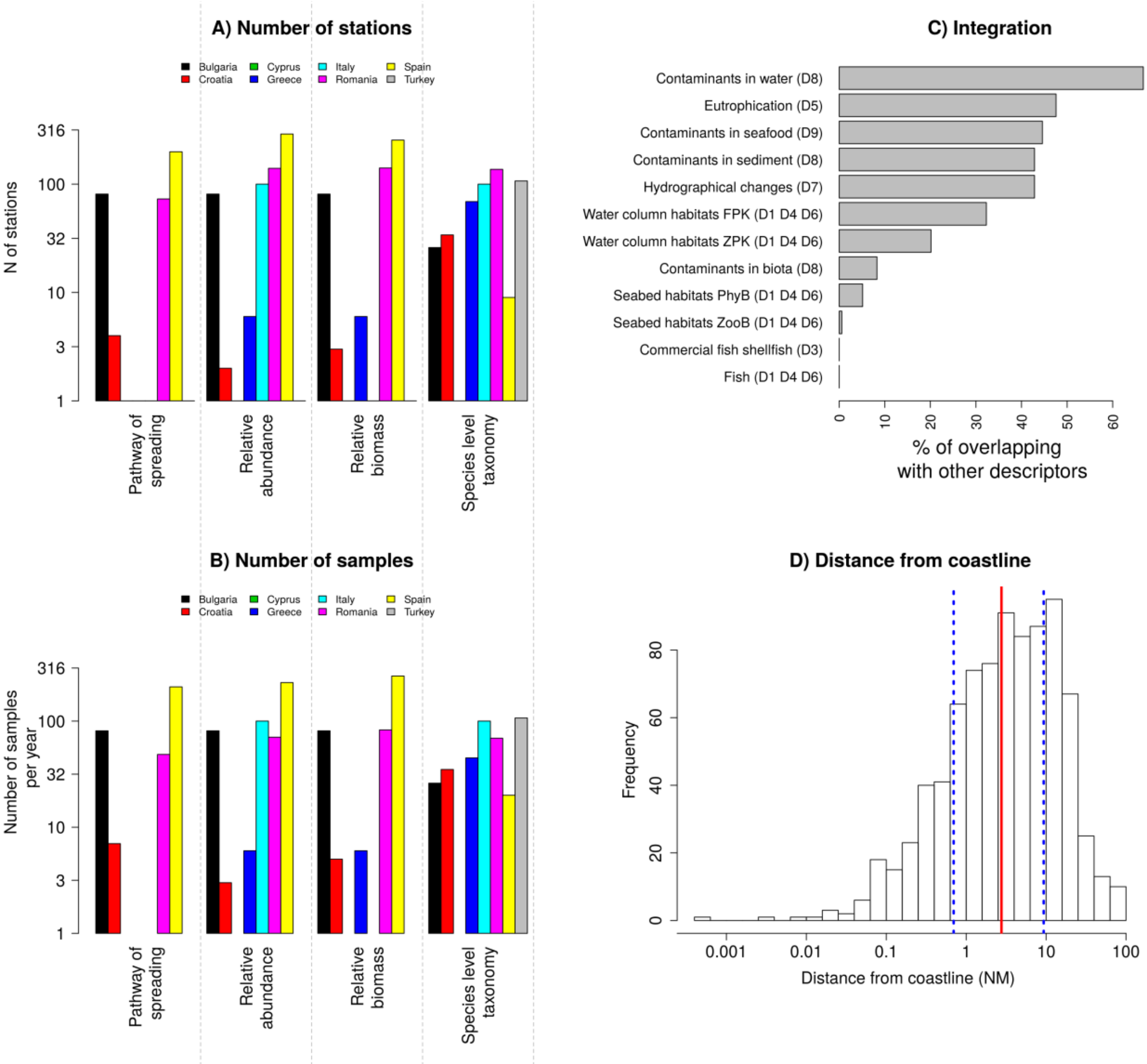


Figure 79. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

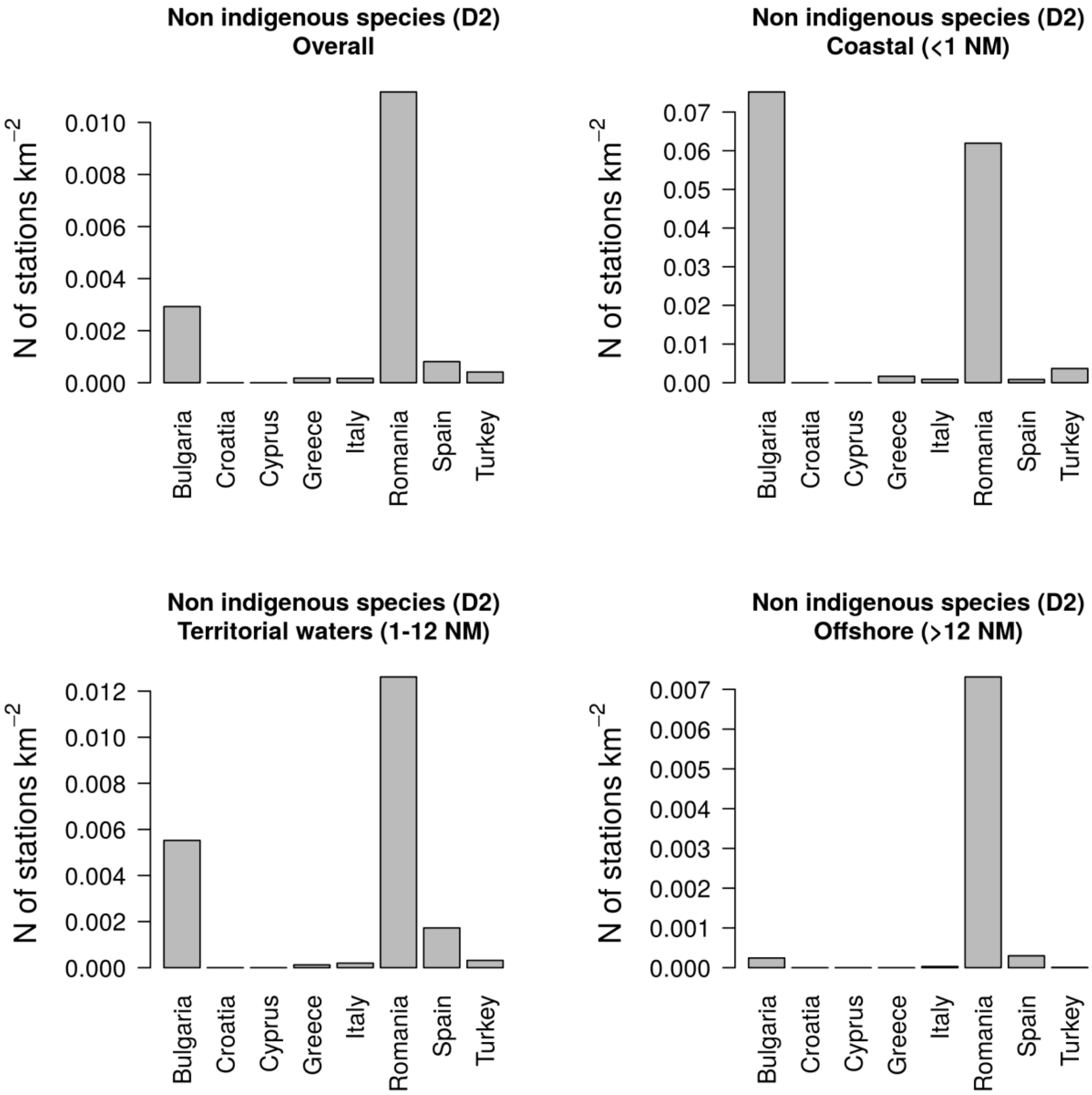


Figure 80. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known

location).

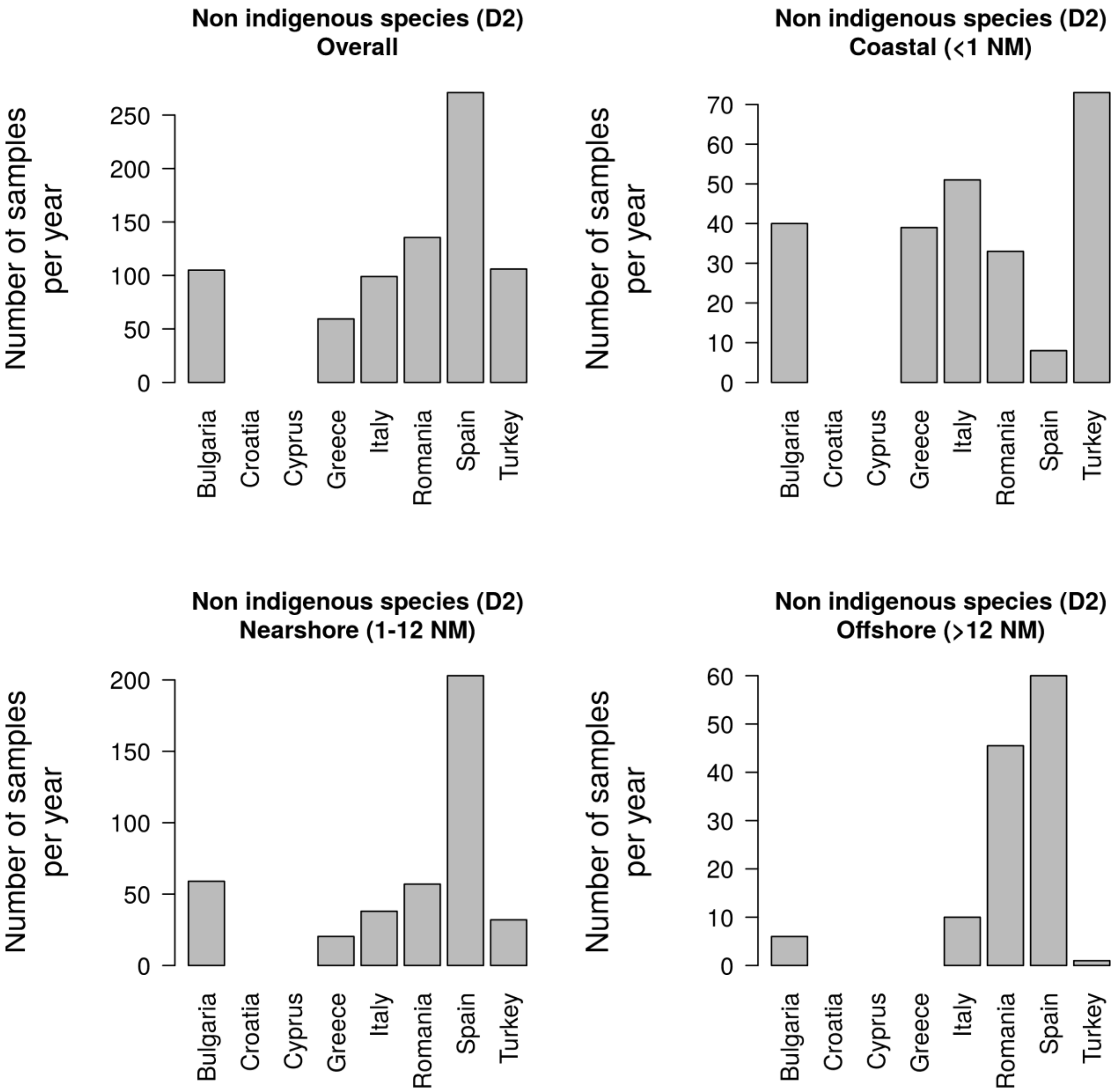


Figure 81. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposedsampling frequency).

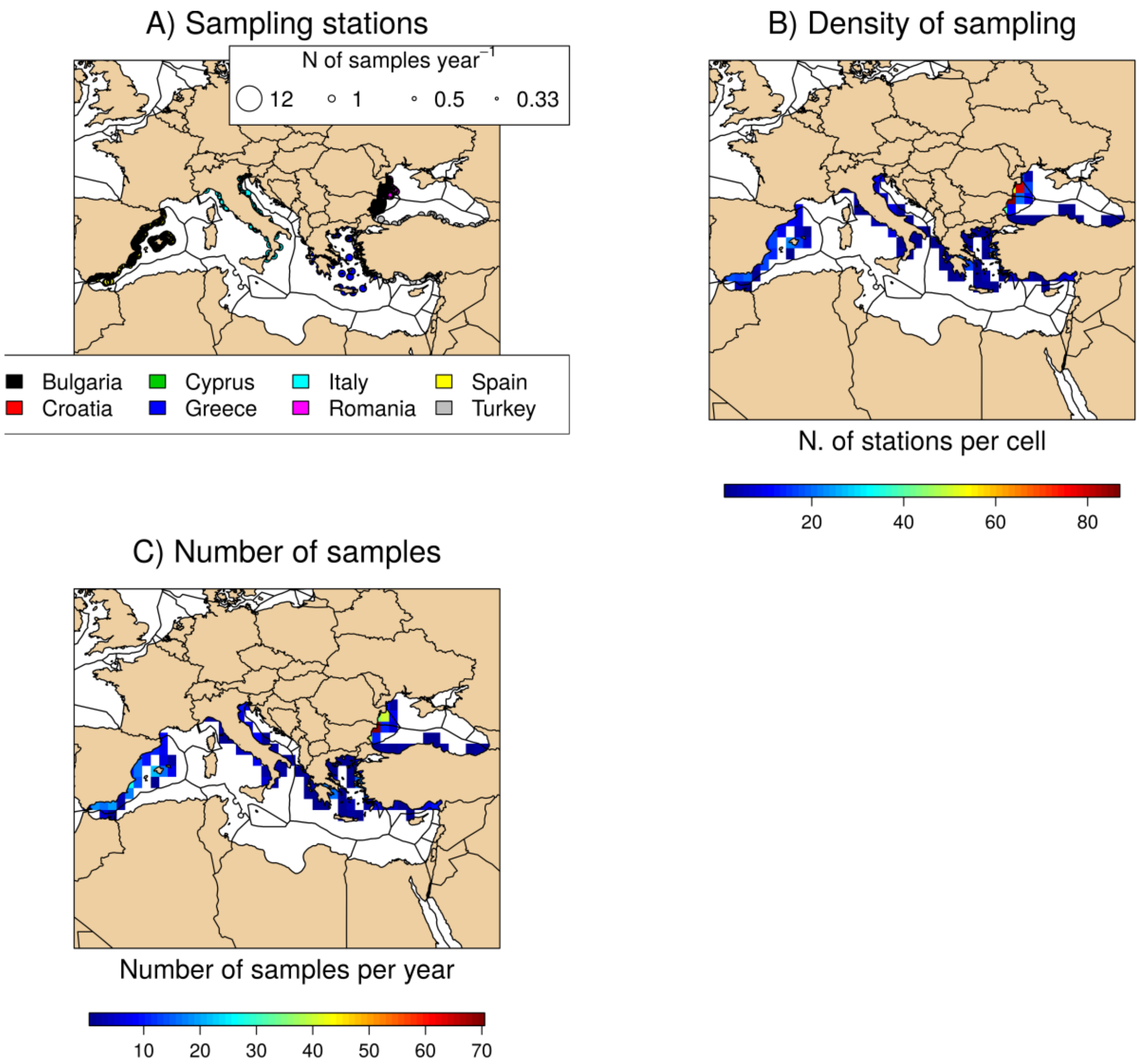


Figure 82. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D3 Commercial fish shellfish

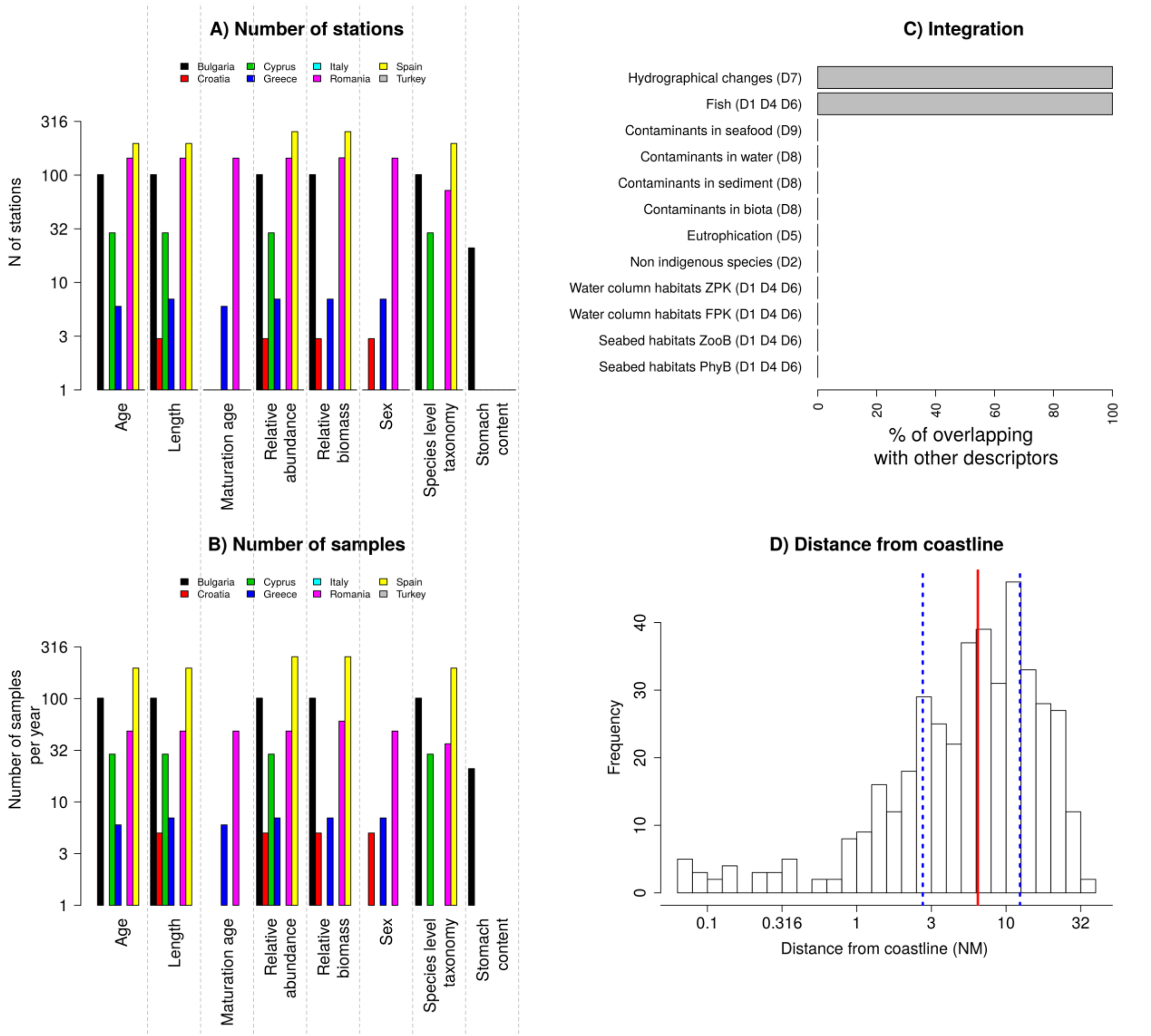


Figure 83. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

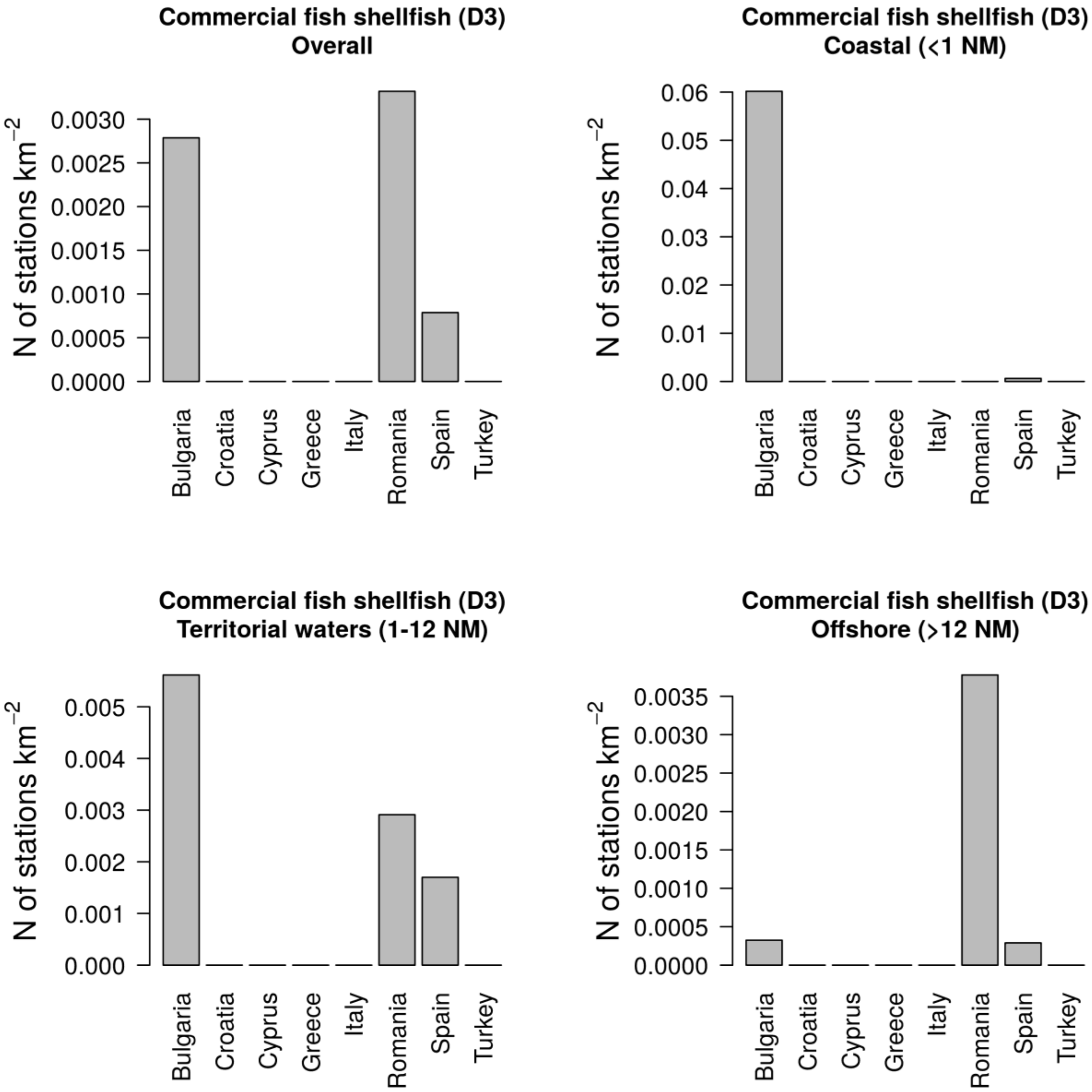


Figure 84. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

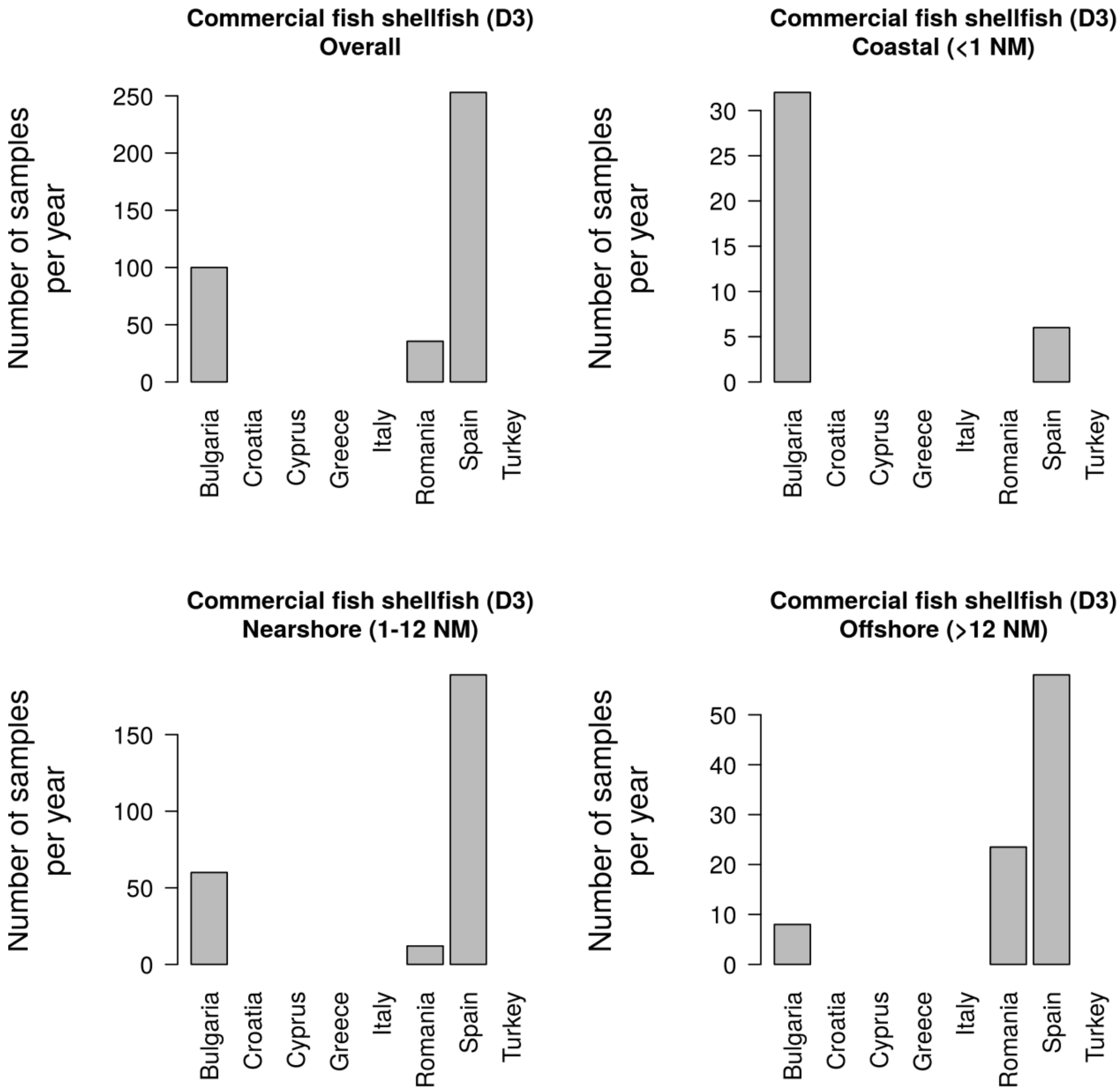


Figure 85. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

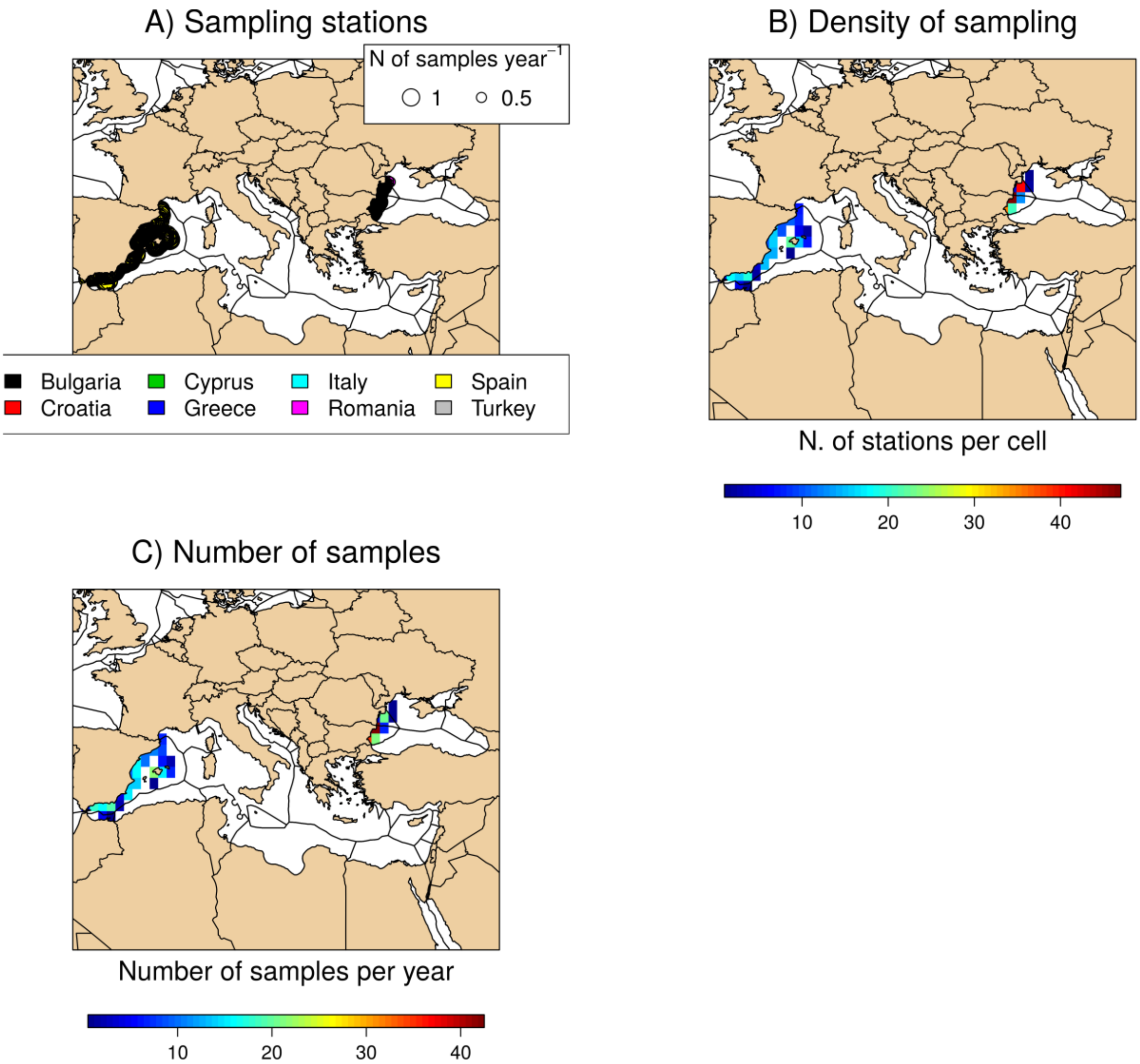


Figure 86. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D5 Eutrophication

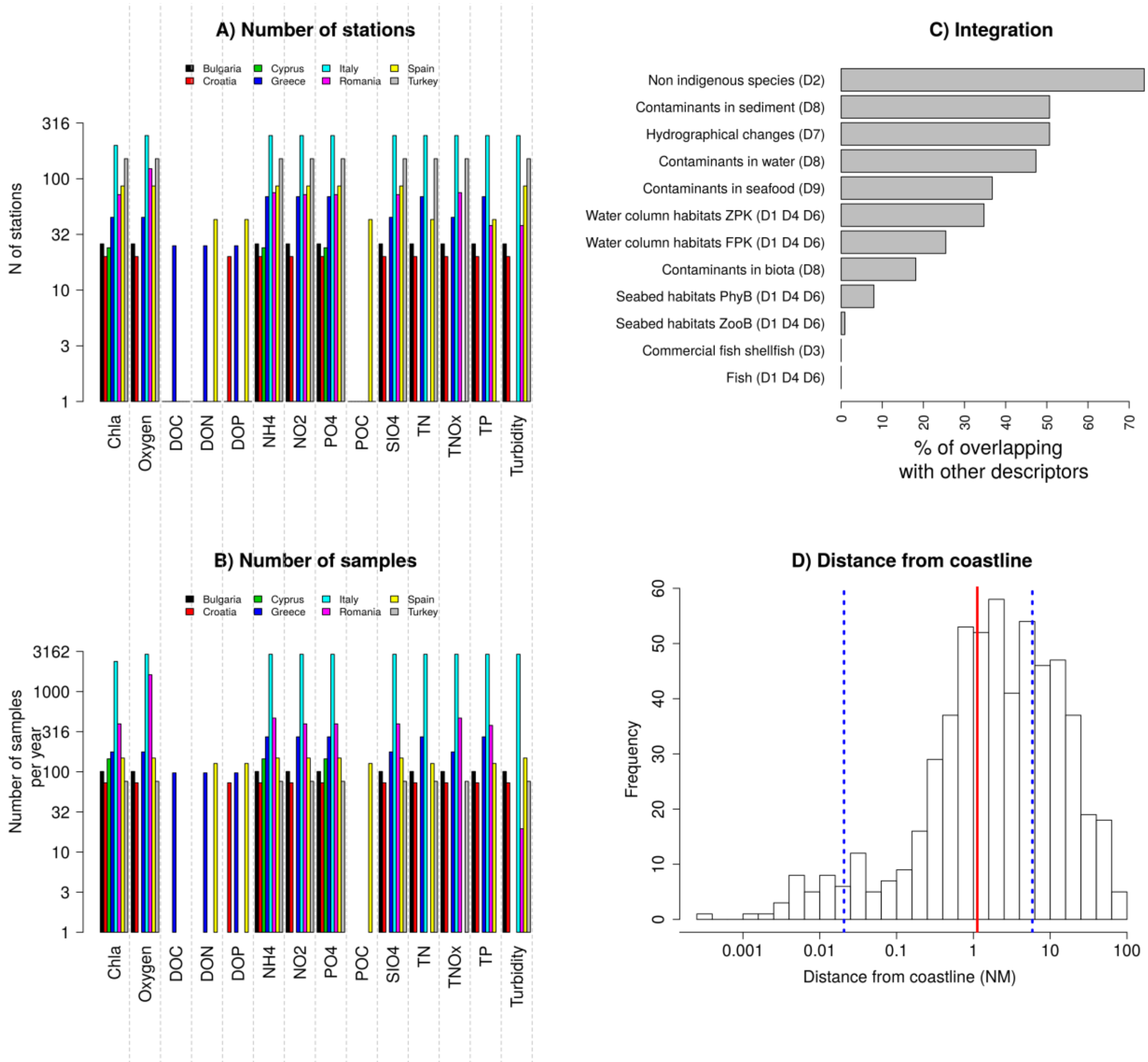


Figure 87. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value; the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

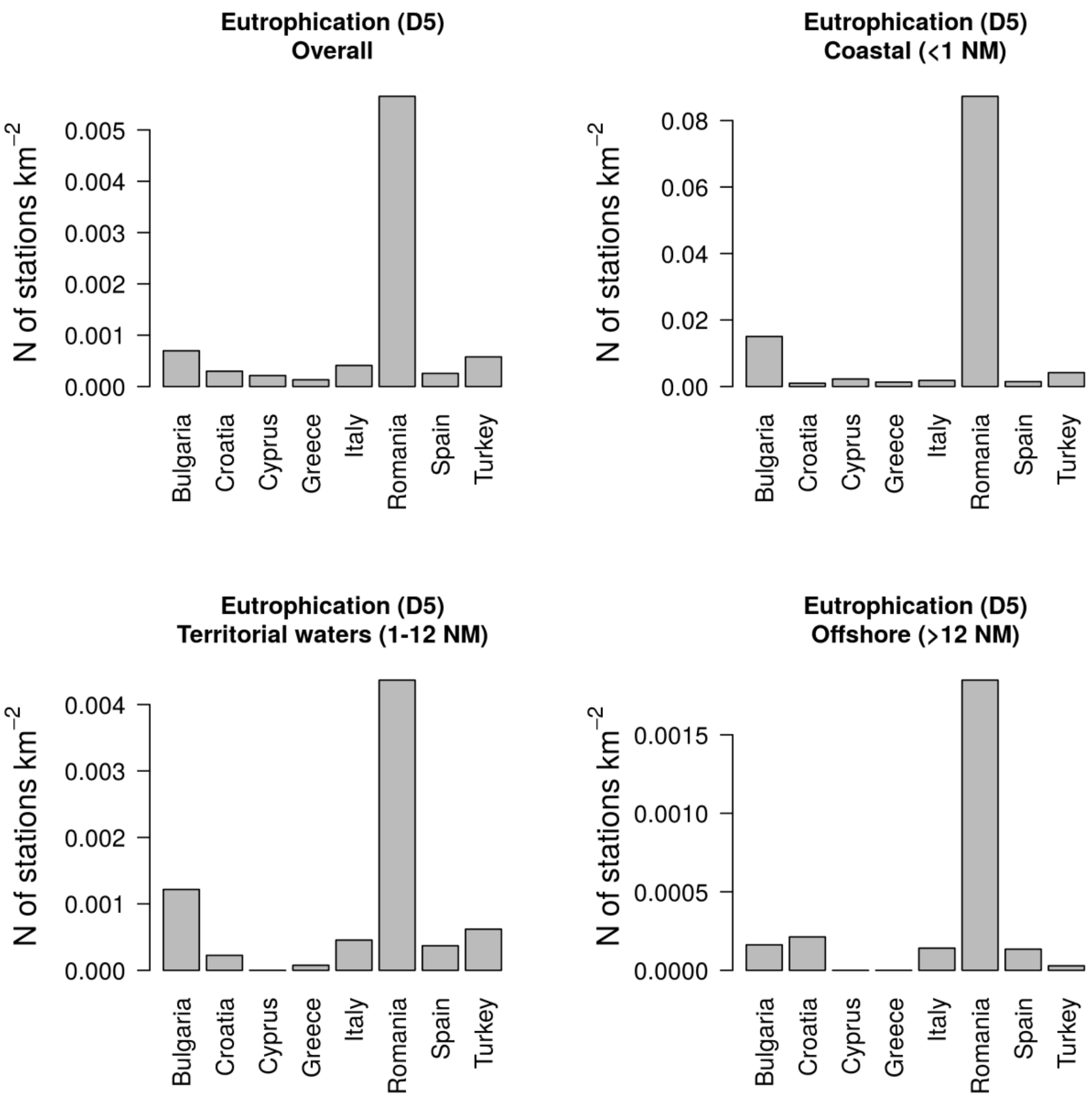


Figure 88. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known

location).

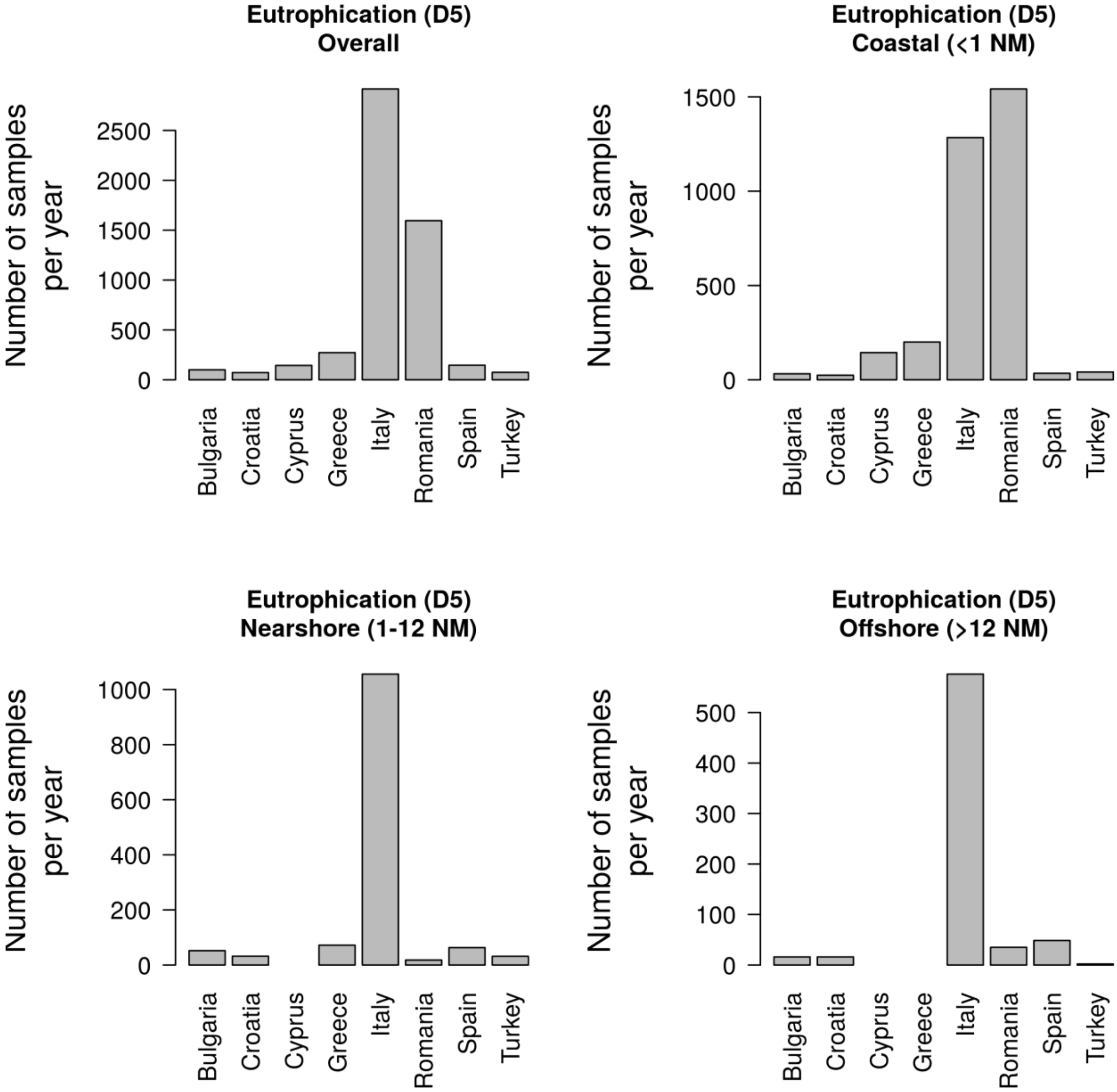


Figure 89. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

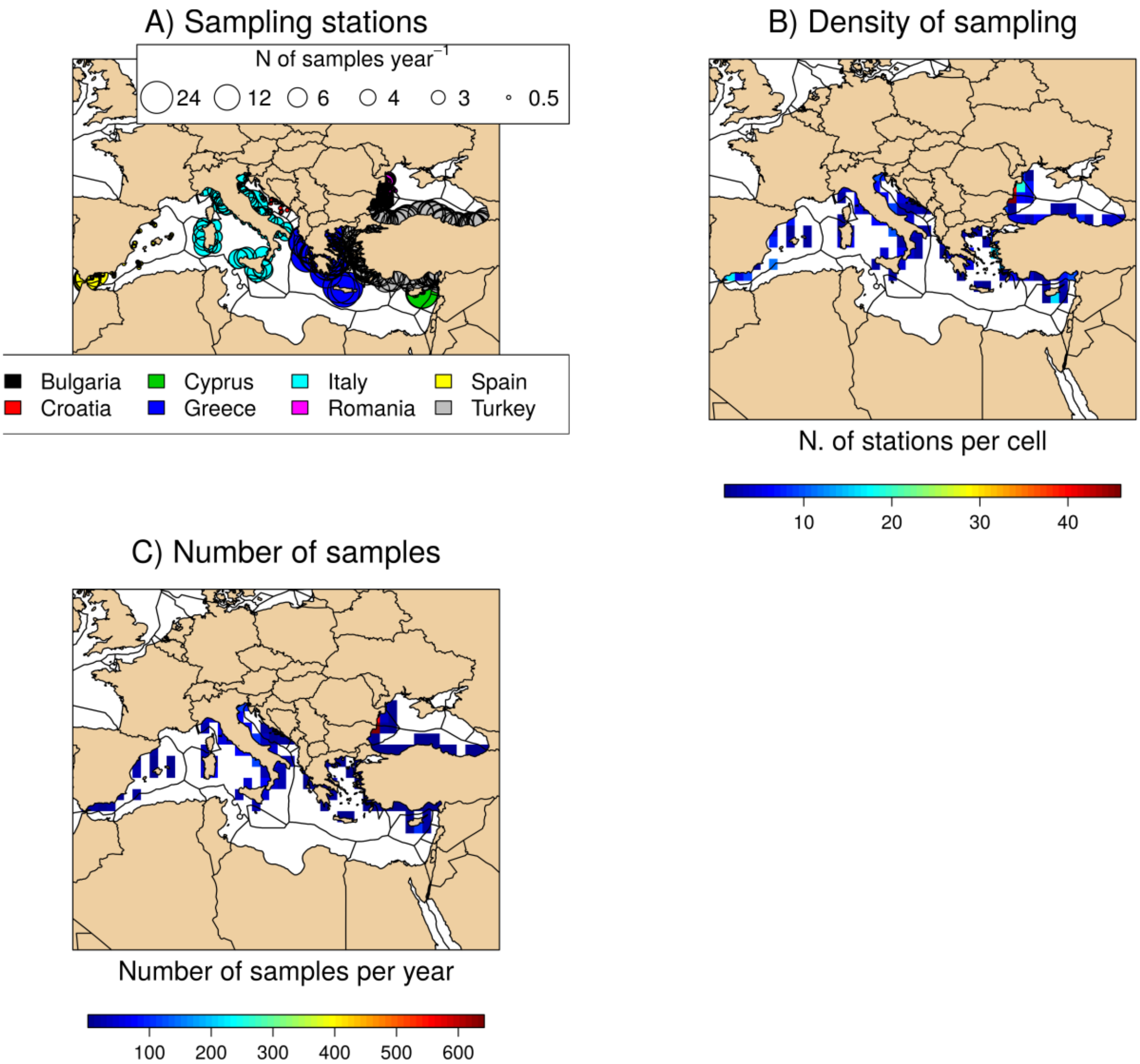


Figure 90. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D7 Hydrographical changes

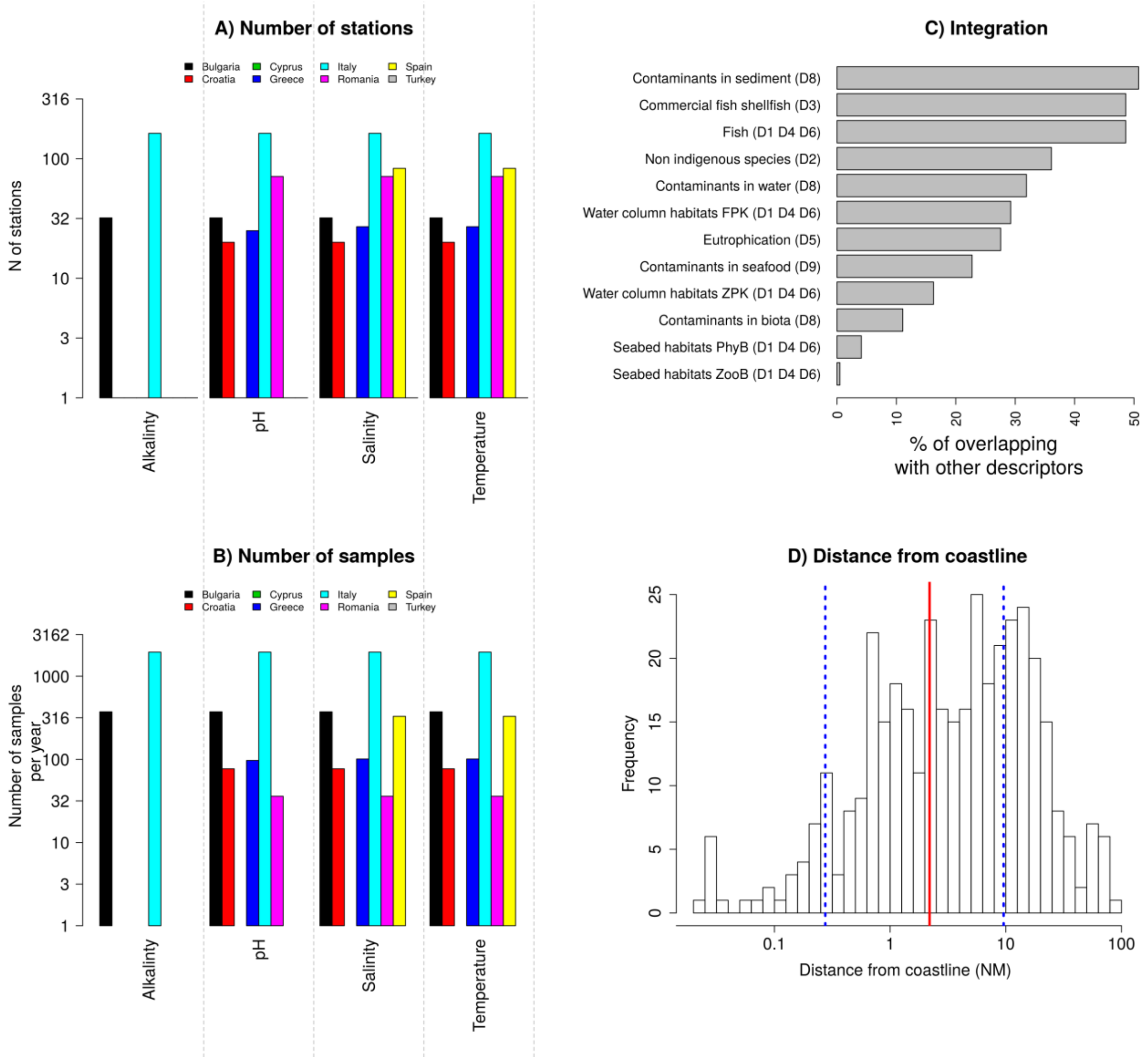


Figure 91. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

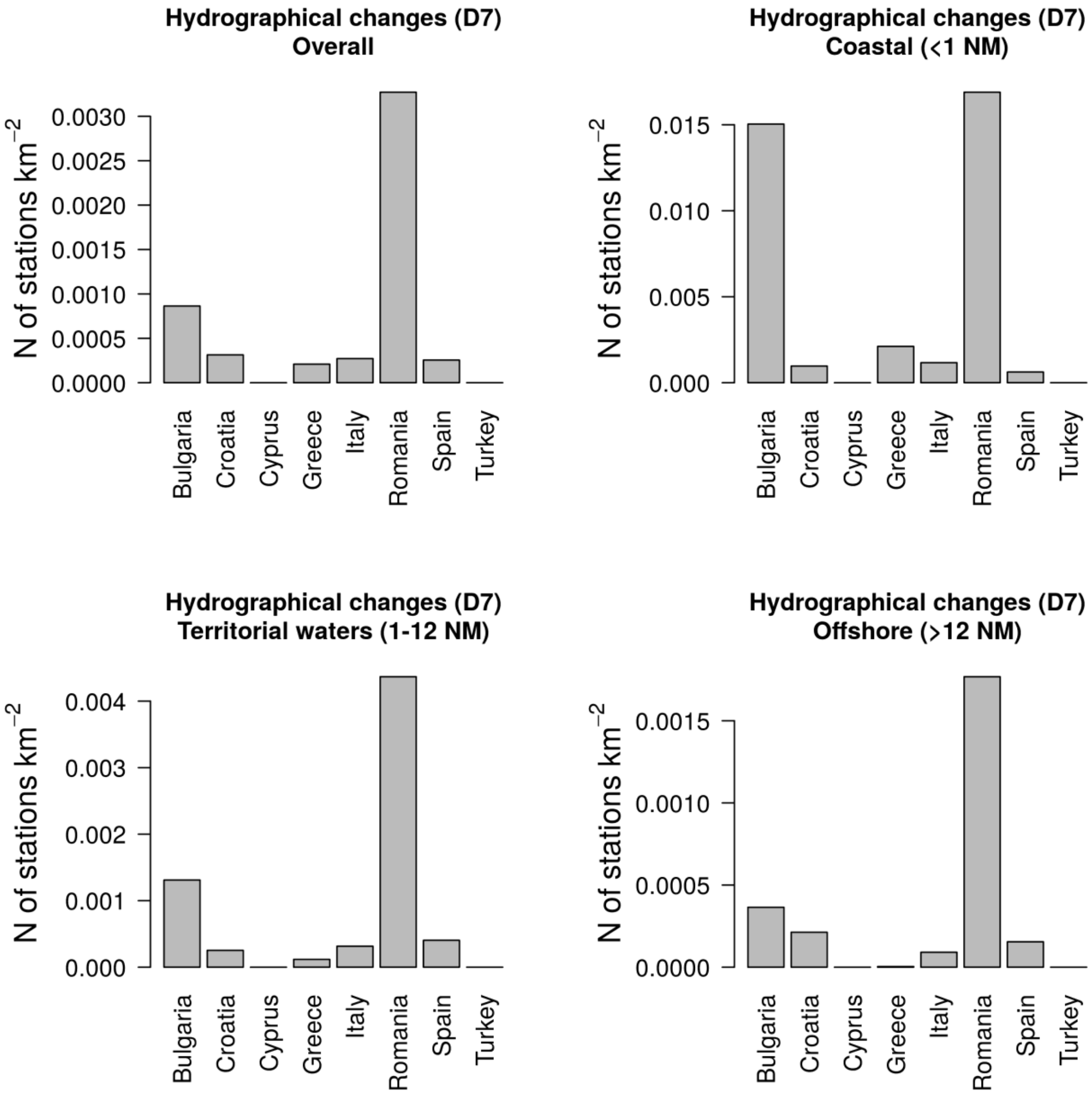


Figure 92. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

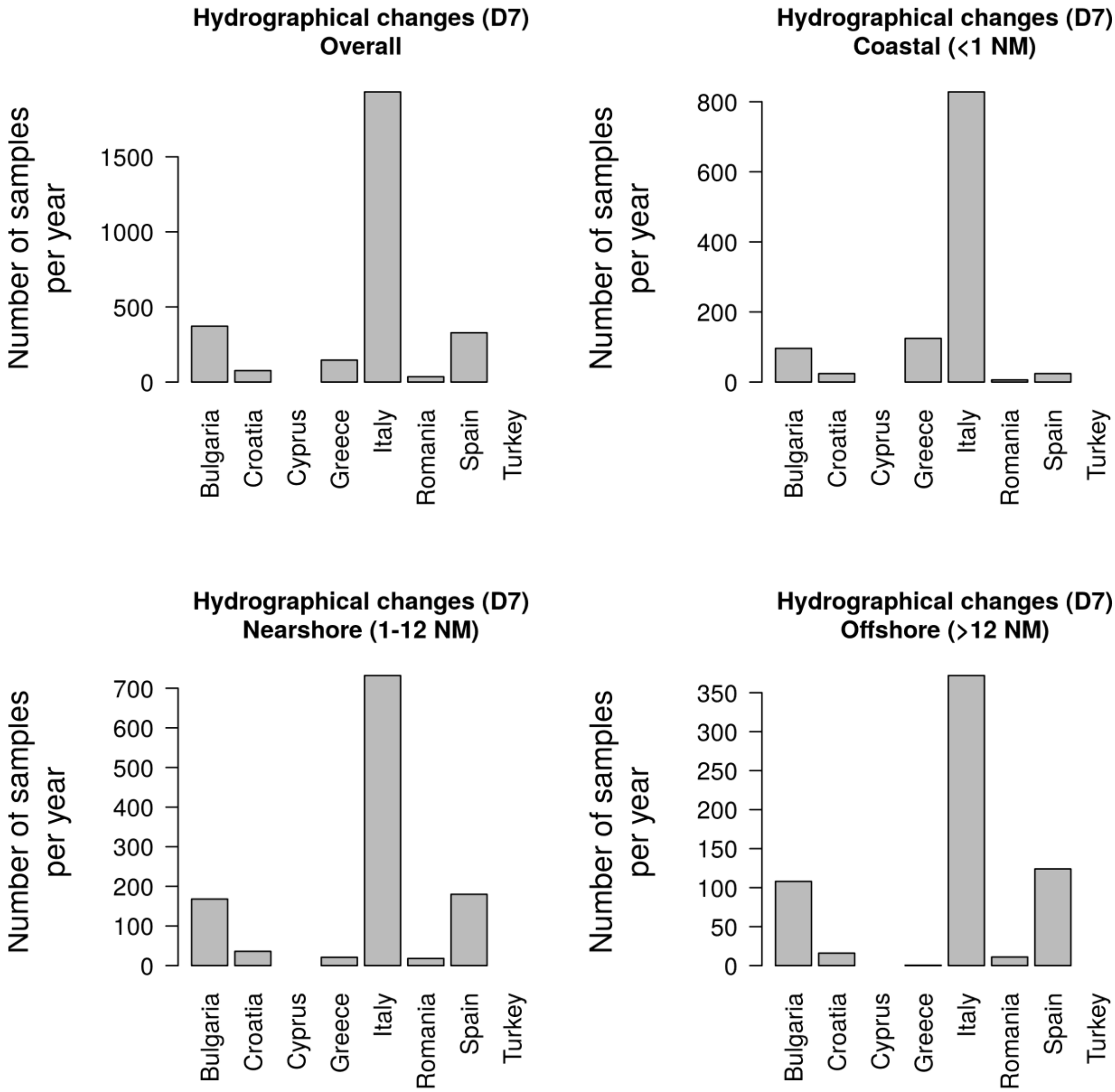


Figure 93. **Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).**

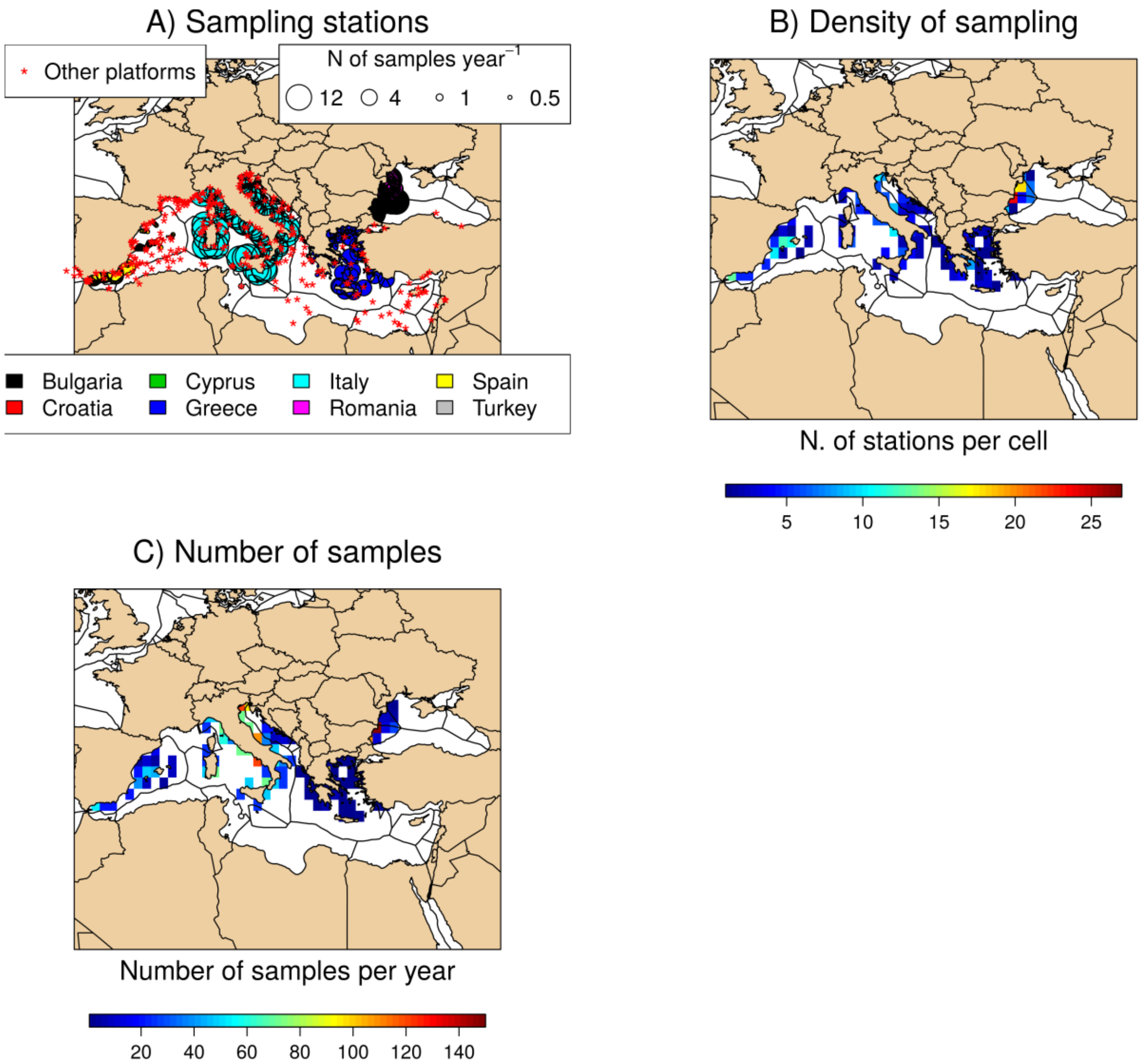


Figure 94. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D8 Contaminants in biota

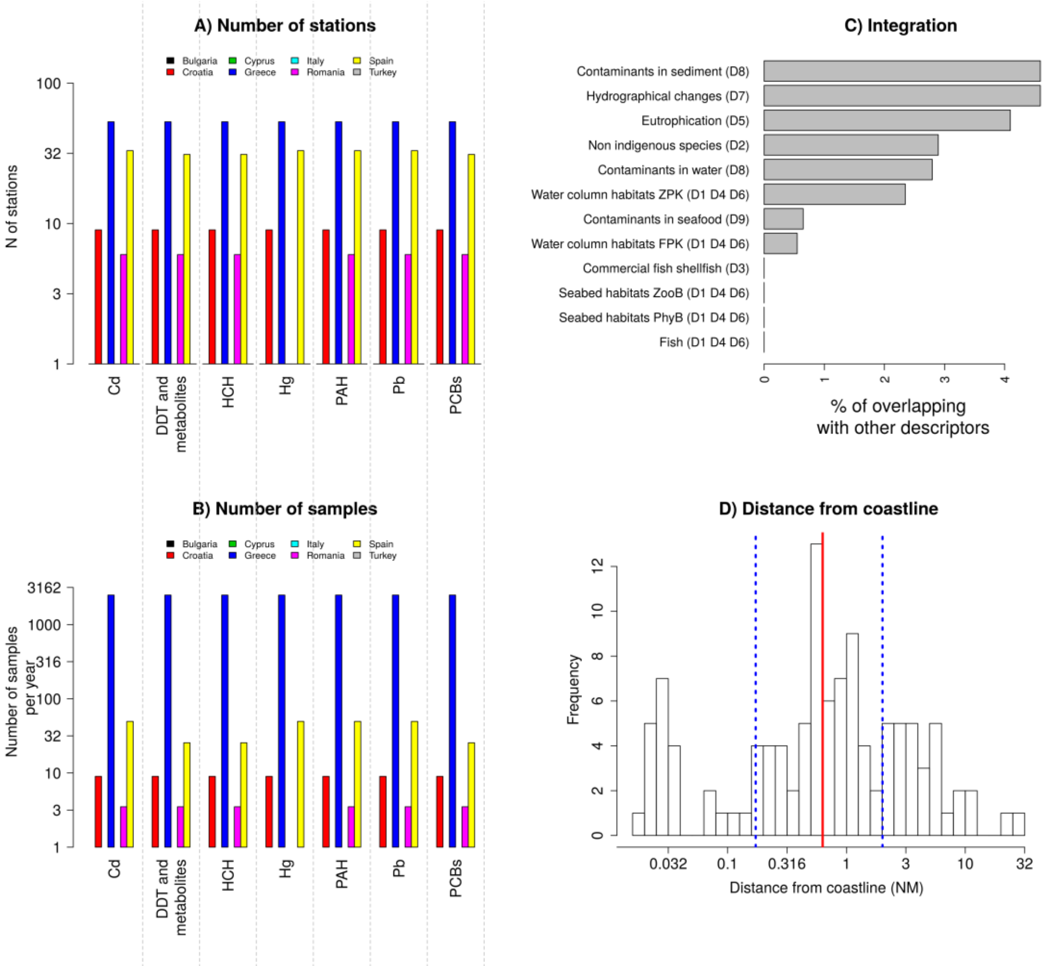


Figure 95. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

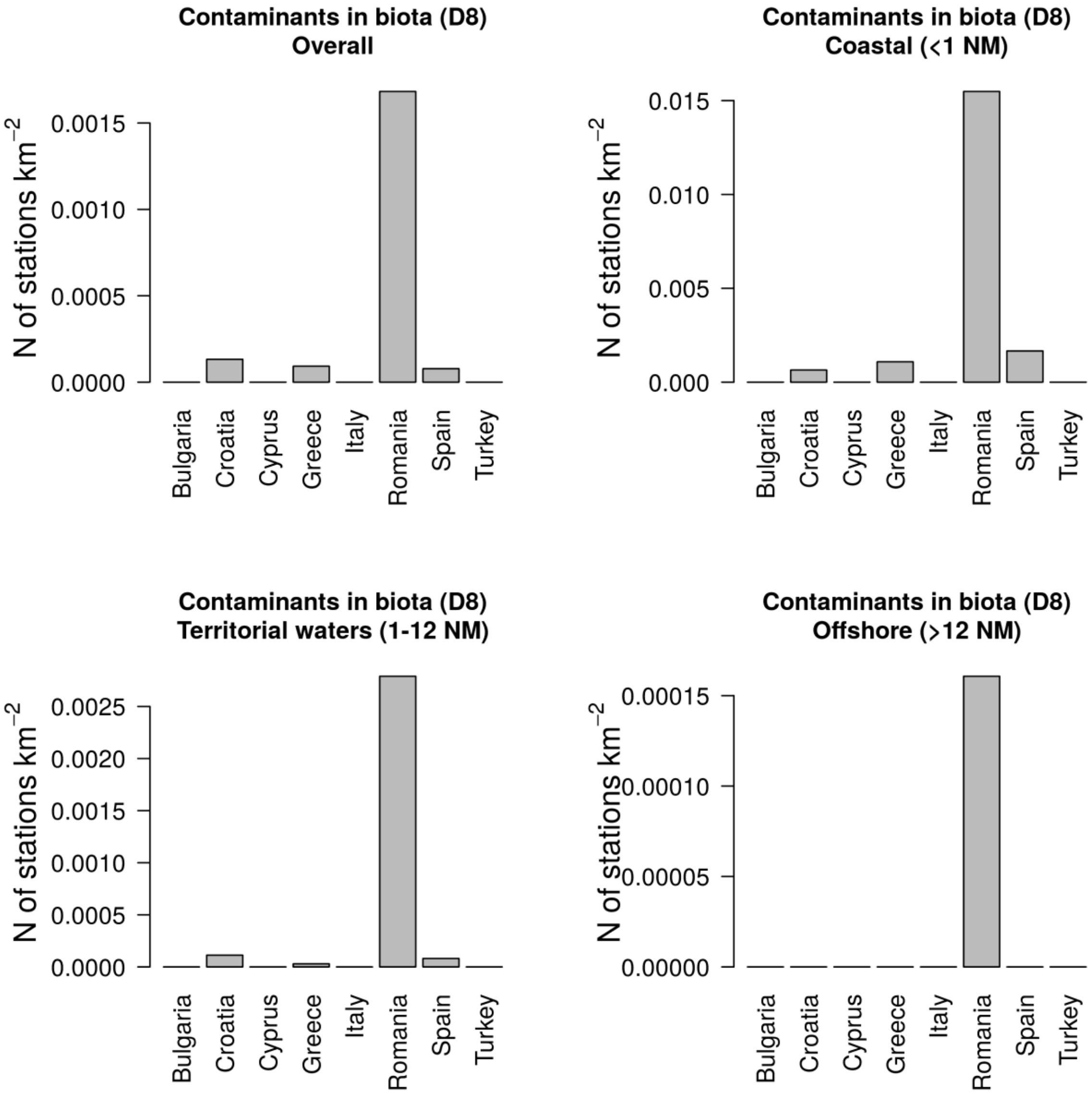


Figure 96. Density of sampling stations (N of station km −2) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

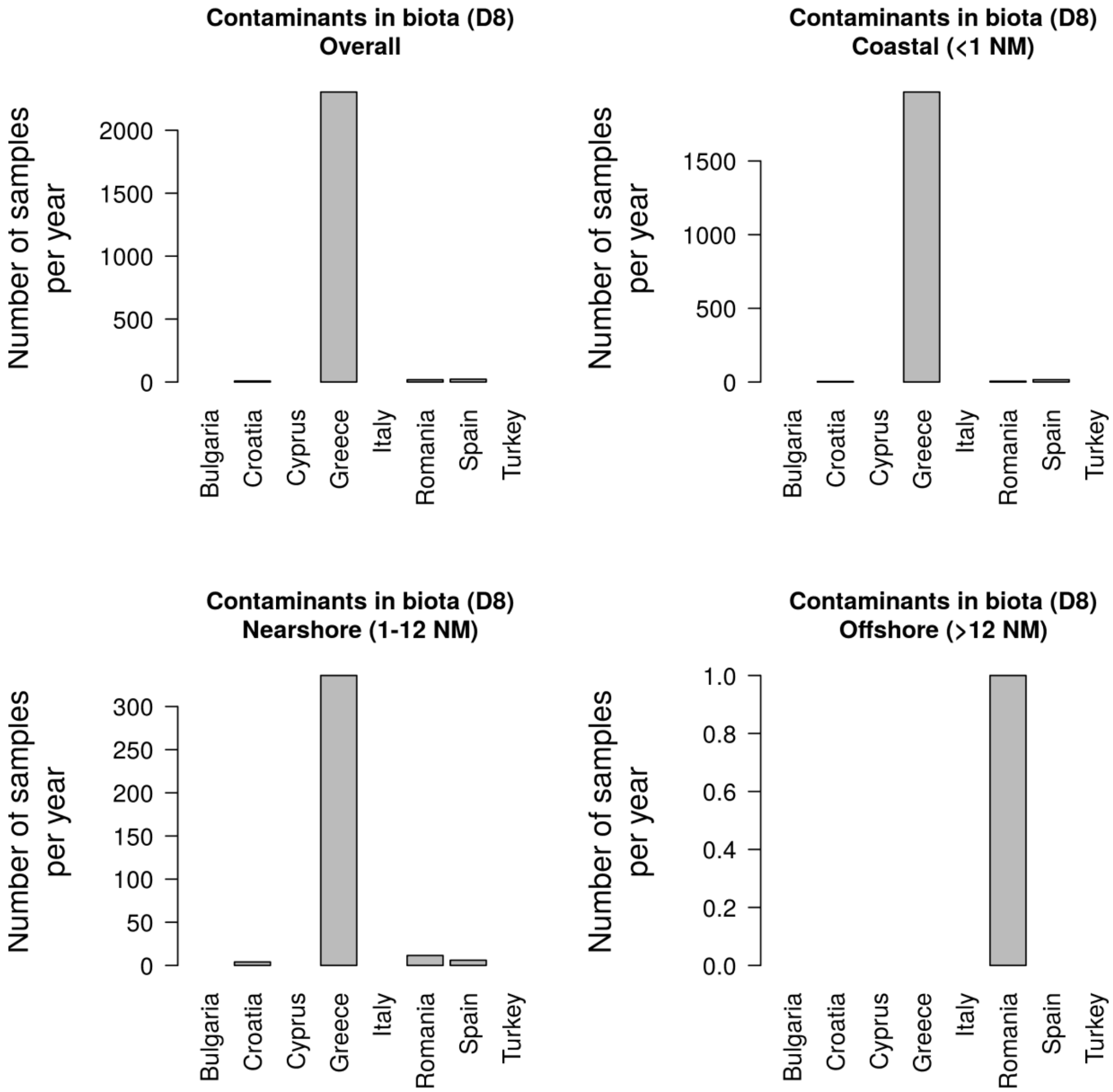


Figure 97. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed sampling frequency).

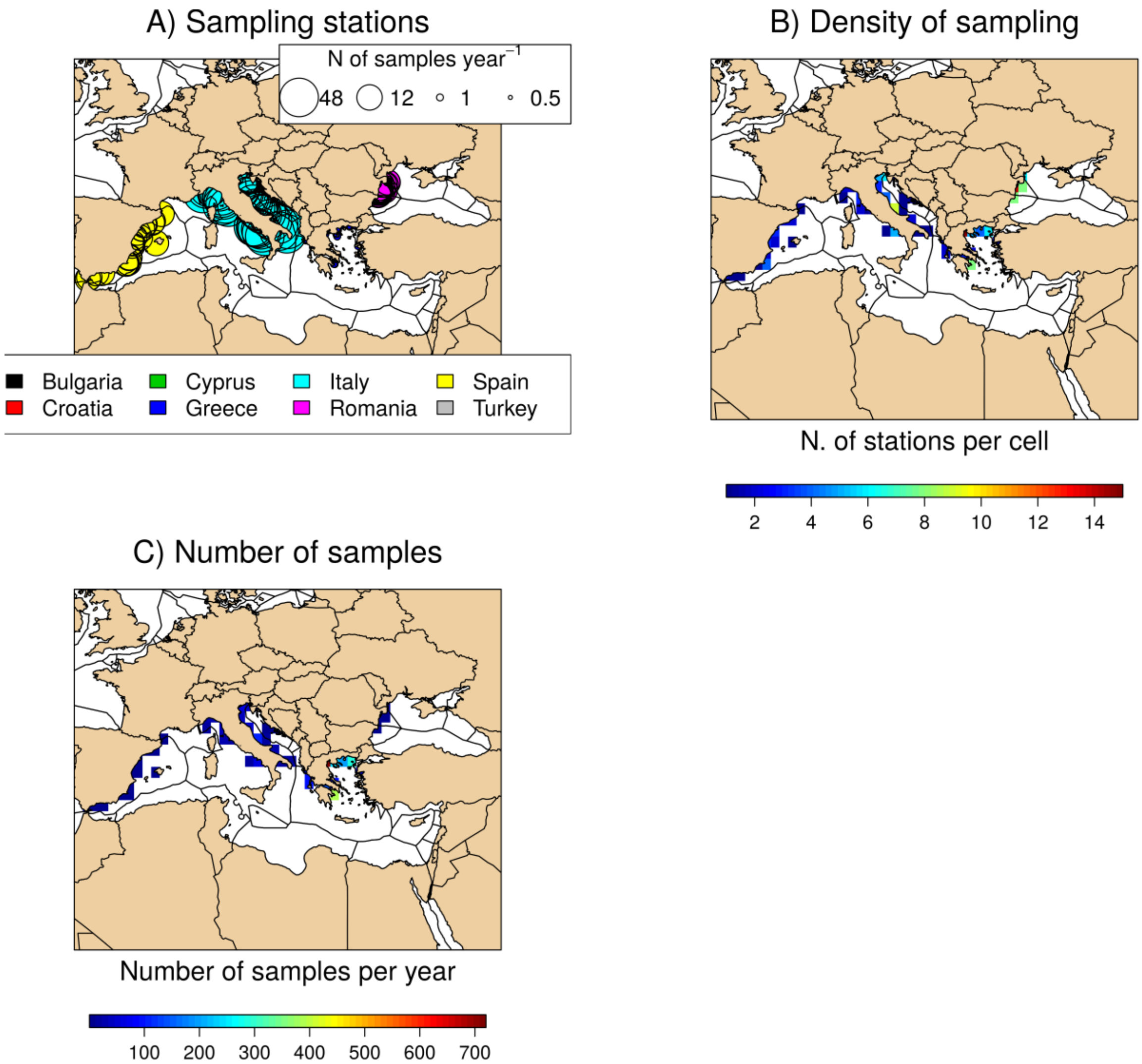


Figure 98. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D8 Contaminants in sediment

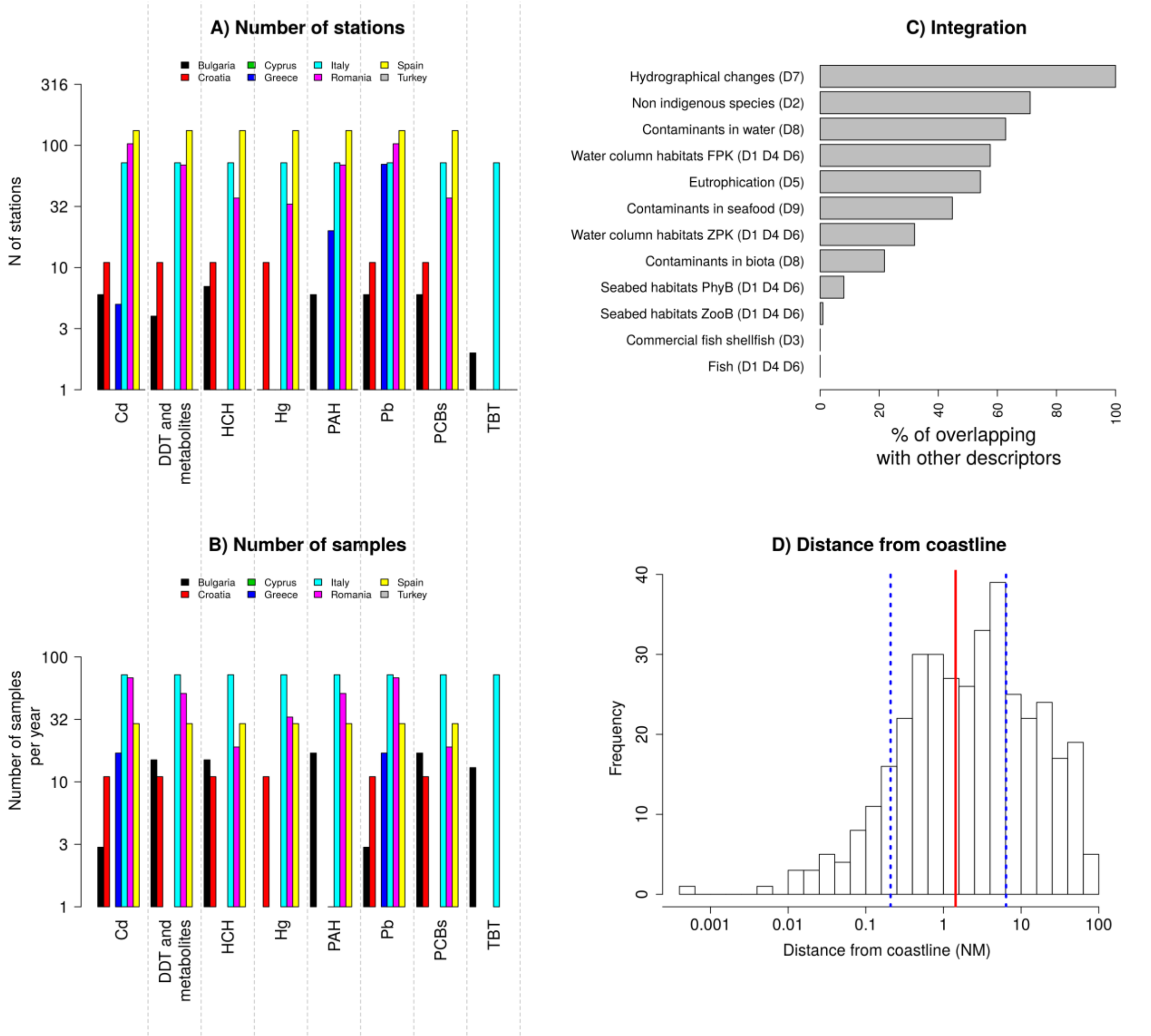


Figure 99. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25th and 75th quantile (stations with known location).

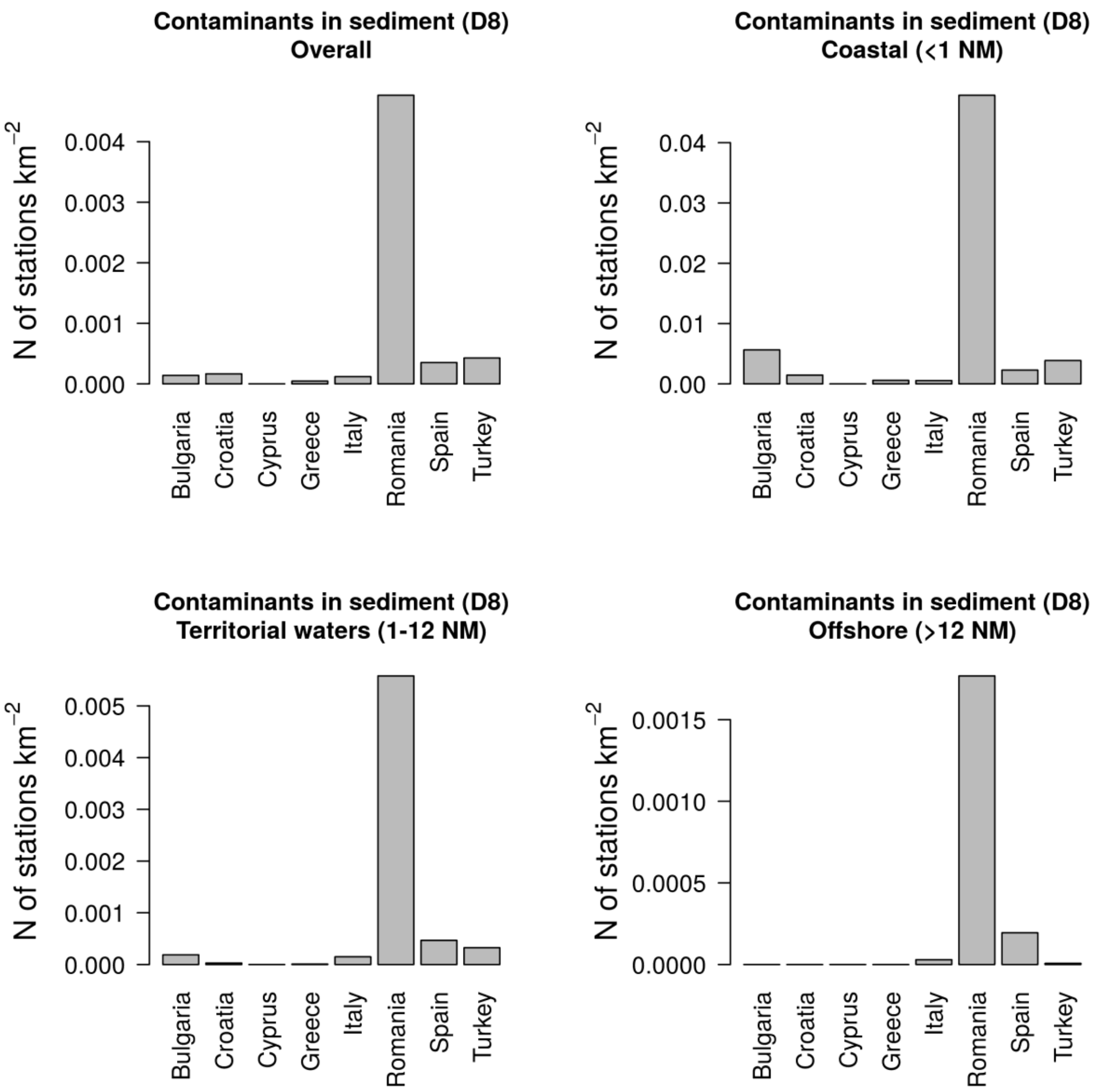


Figure 100. Density of sampling stations (N of station km^{-2}) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

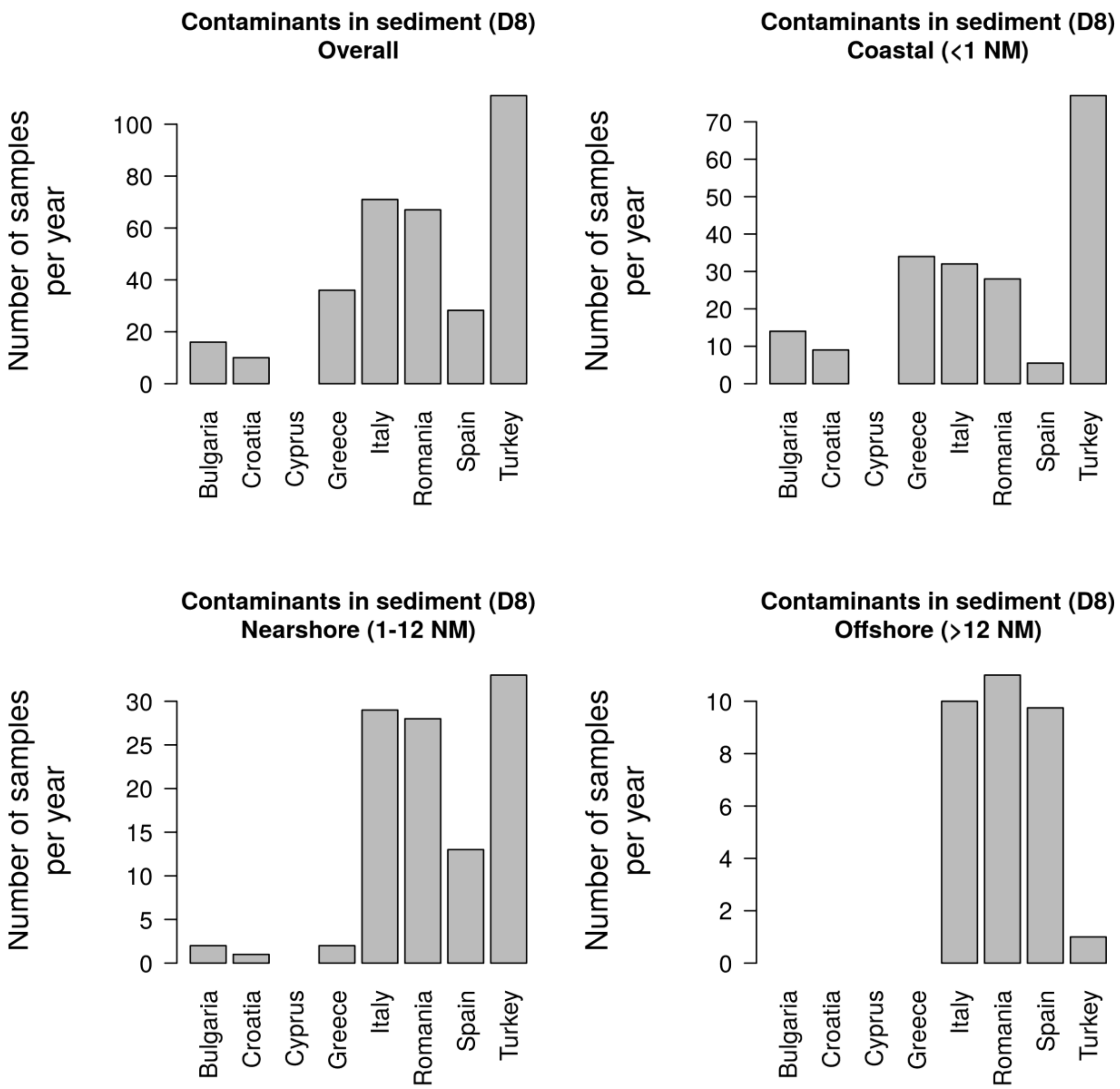


Figure 101. Number of collected samples per year for coastal (<1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed

sampling frequency).

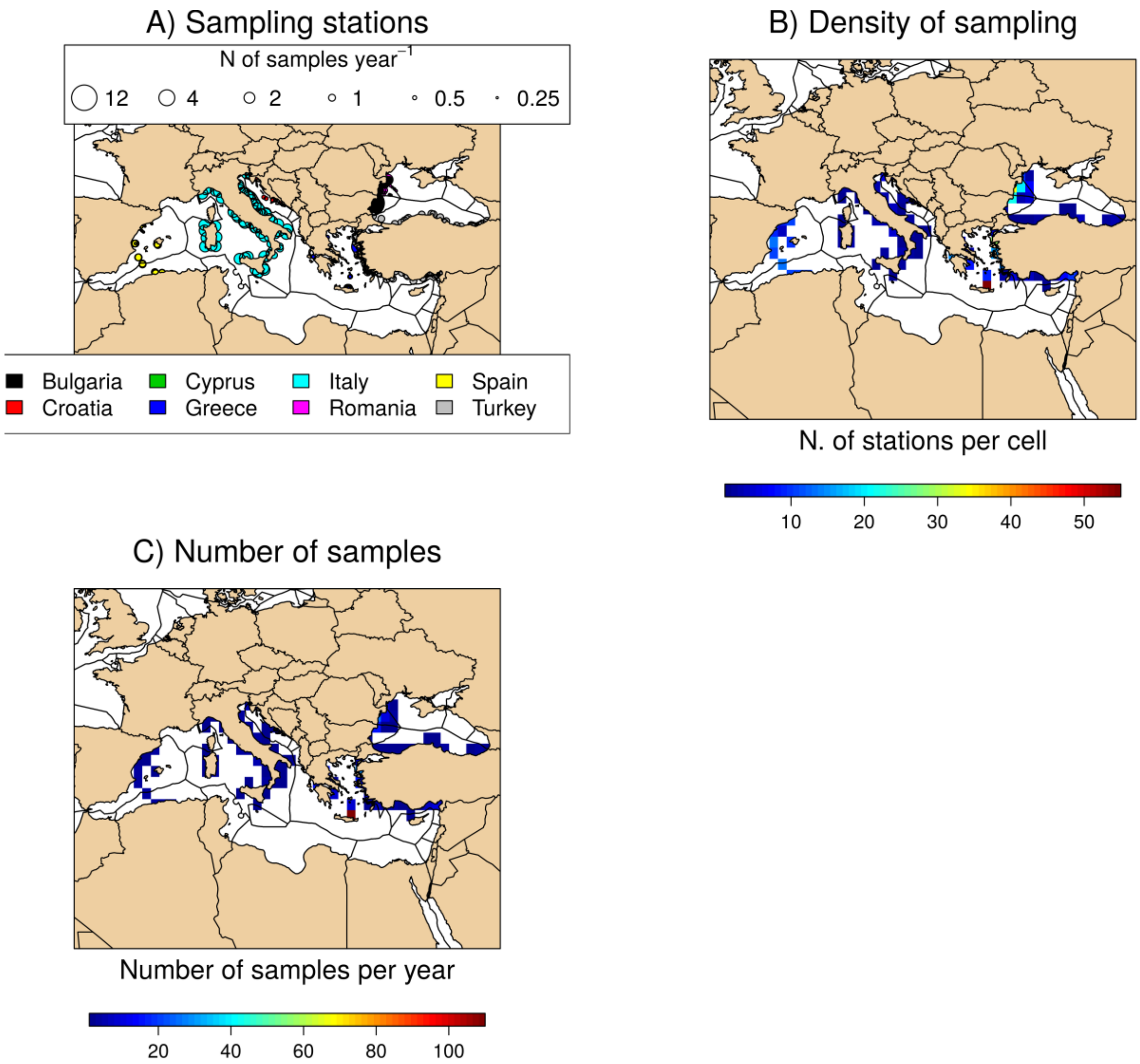


Figure 102. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D8 Contaminants in water

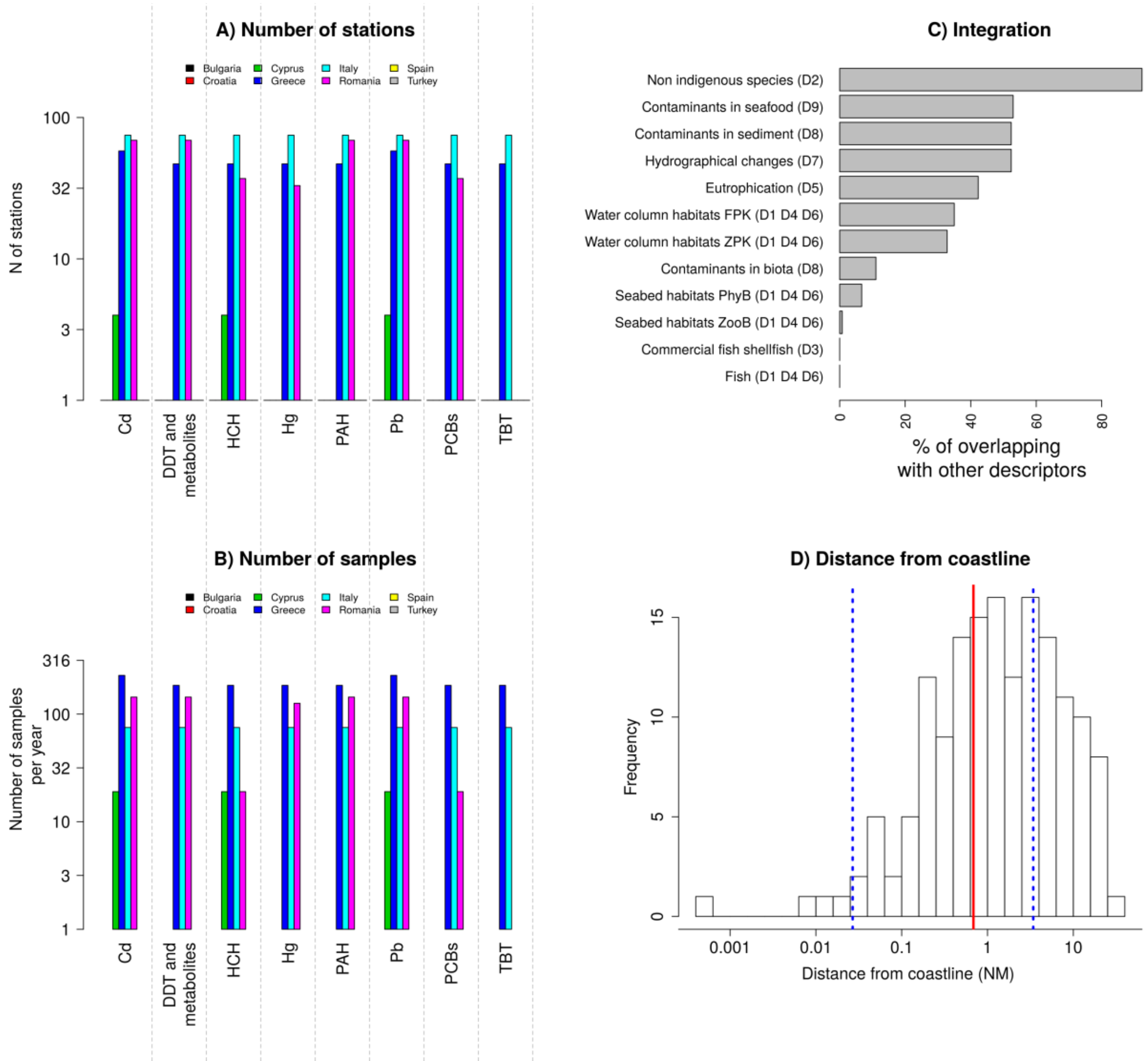


Figure 103. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25th and 75th quantile (stations with known location).

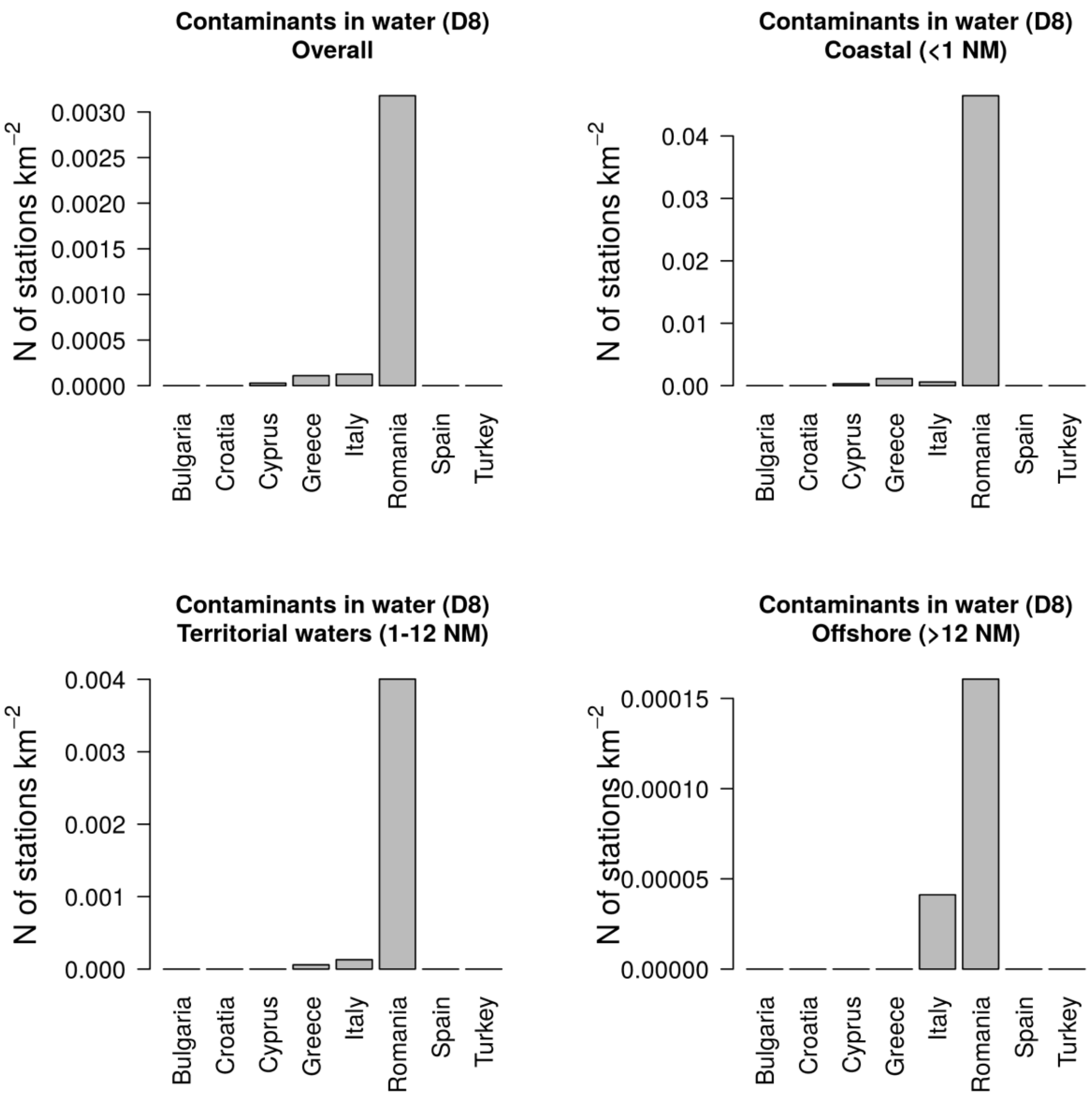


Figure 104. Density of sampling stations (N of station km^{-2}) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

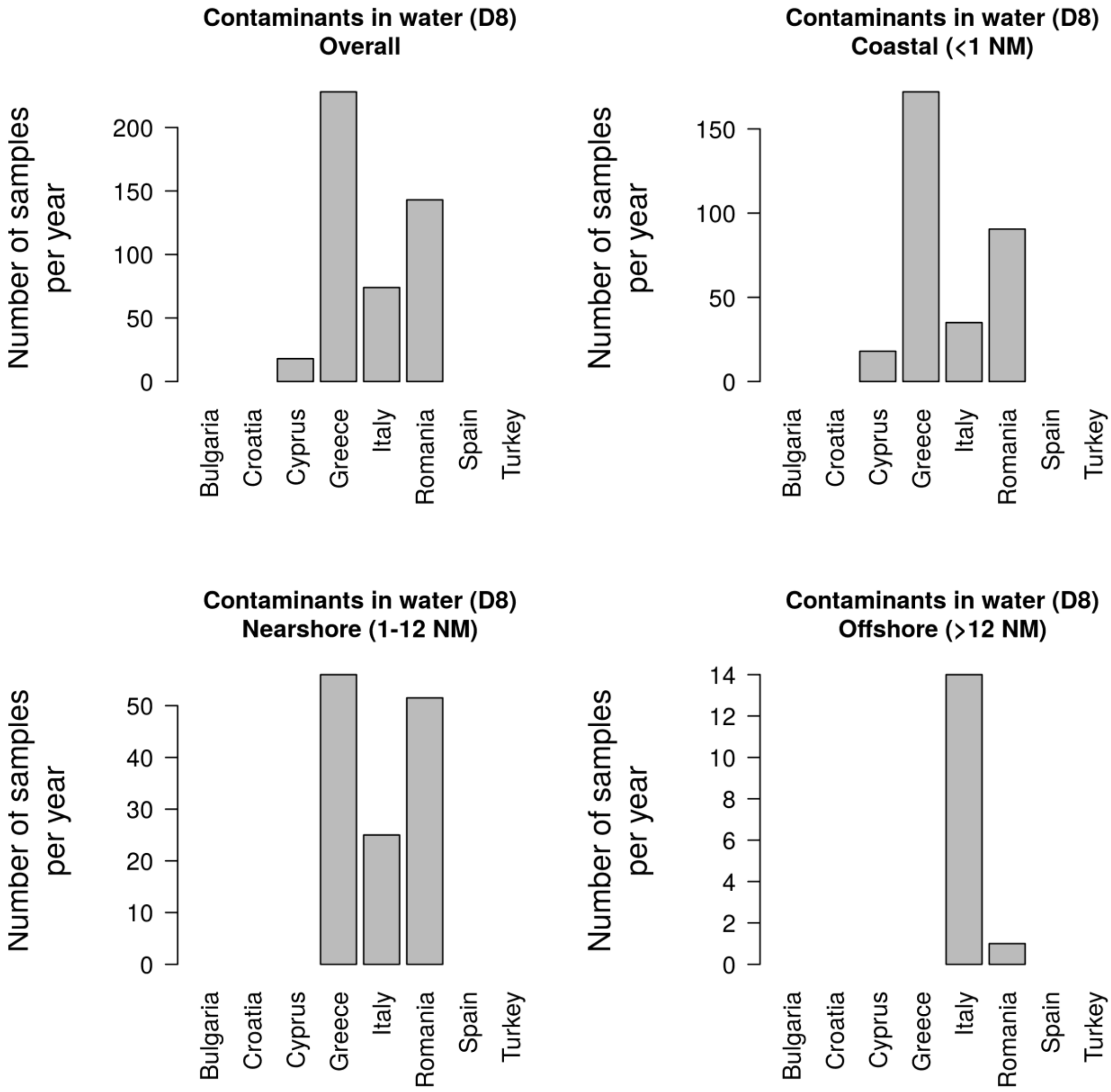


Figure 105. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed

sampling frequency).

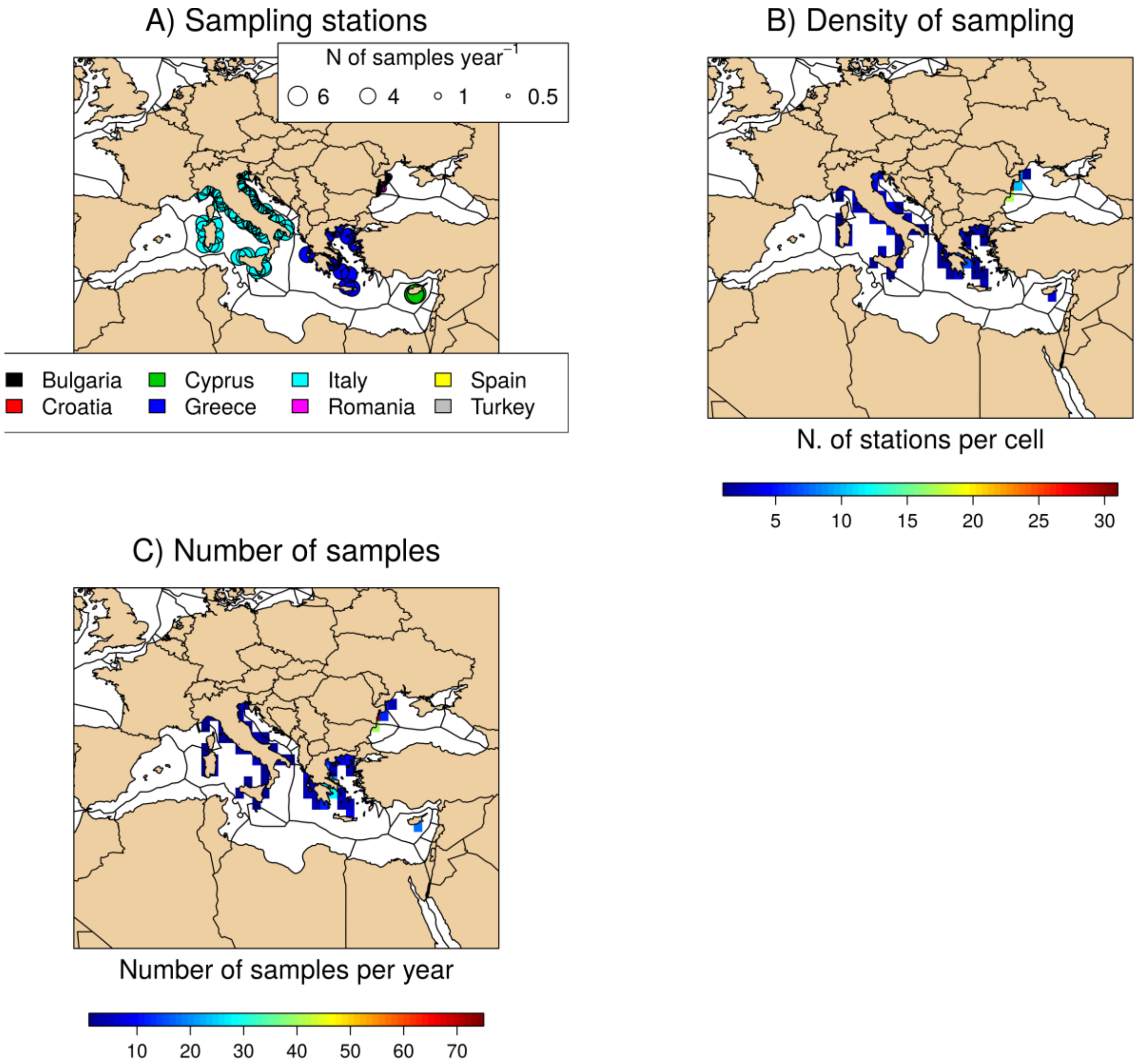


Figure 106. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

D9 Contaminants in seafood

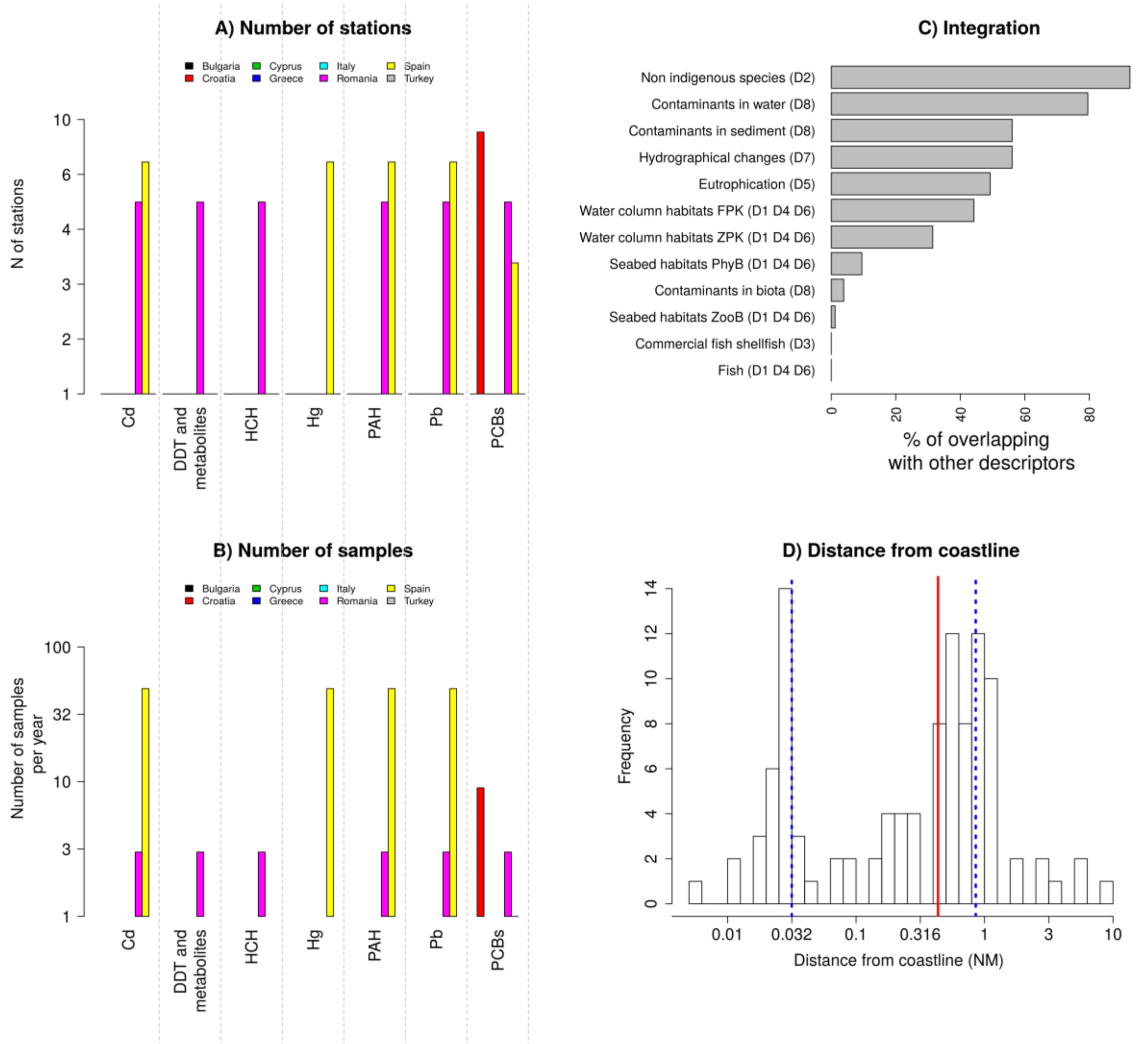


Figure 107. A) Measured parameters (N of stations), divided by MS (full dataset); B) number of collected samples per year (stations with known proposed sampling frequency); C) Overlap with other descriptors (% of stations for the target descriptor in which others descriptors are measured, stations with known location); D) Distribution of sampling stations distances from the coastline (NM). The red vertical line indicates the median value, the left and right blue broken vertical lines indicate respectively the 25 th and 75 th quantile (stations with known location).

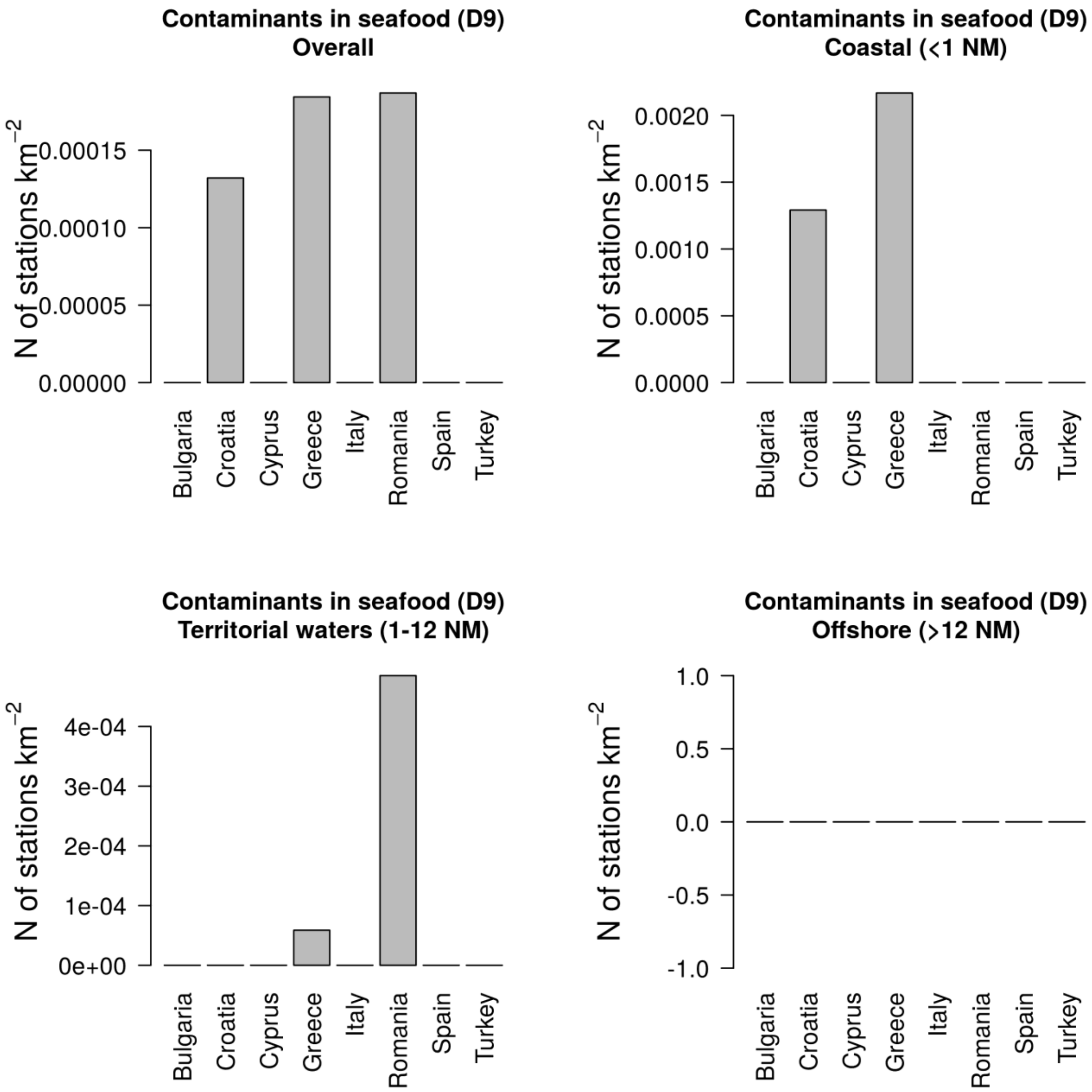


Figure 108. Density of sampling stations (N of station km^{-2}) for the overall dataset, coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location).

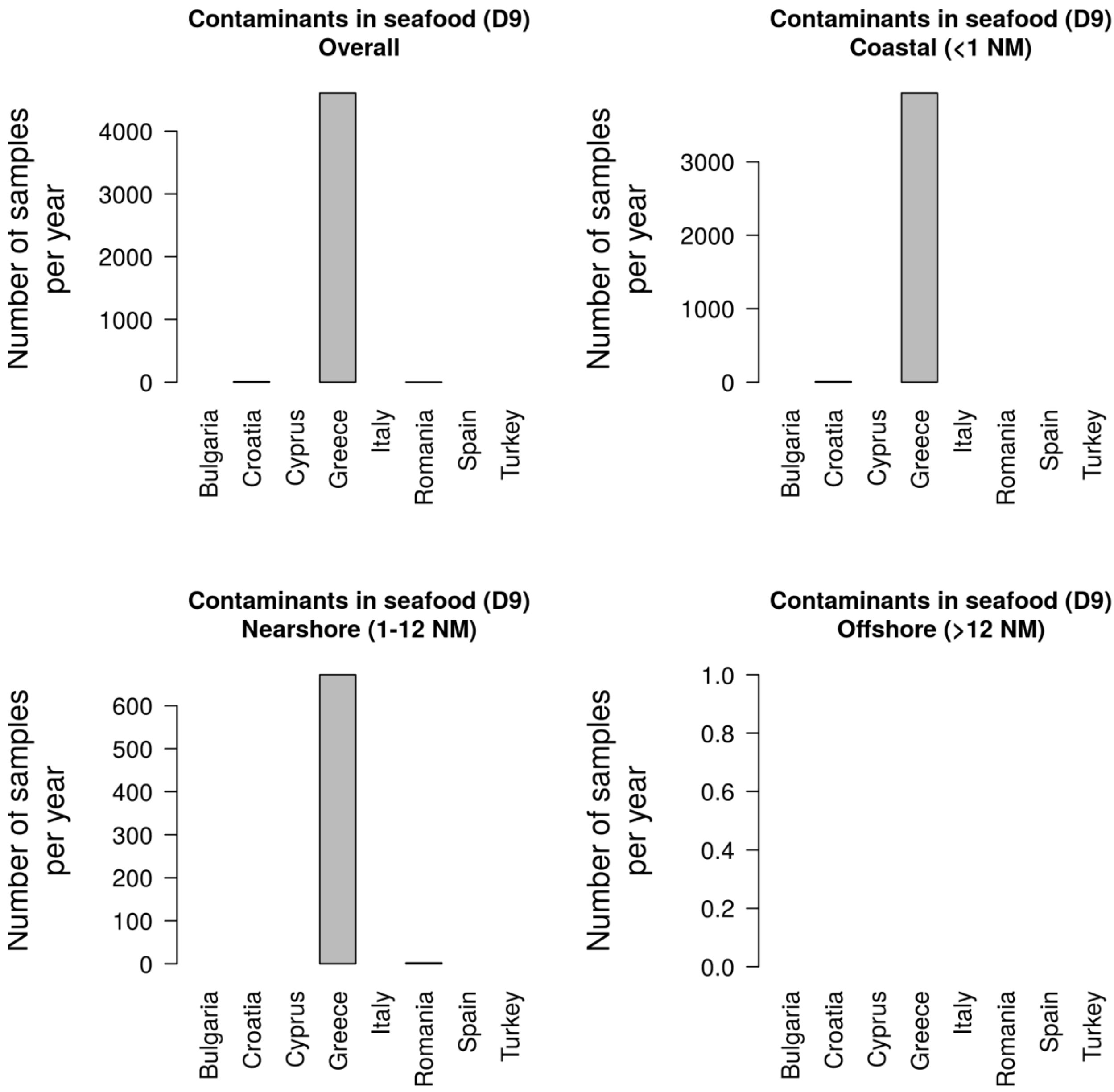


Figure 109. Number of collected samples per year for coastal (< 1 NM of distance from coast), nearshore (between 1 and 12 NM of distance from coast) and offshore (> 12 NM of distance from coast) areas, divided by MS (stations with known location and proposed

sampling frequency).

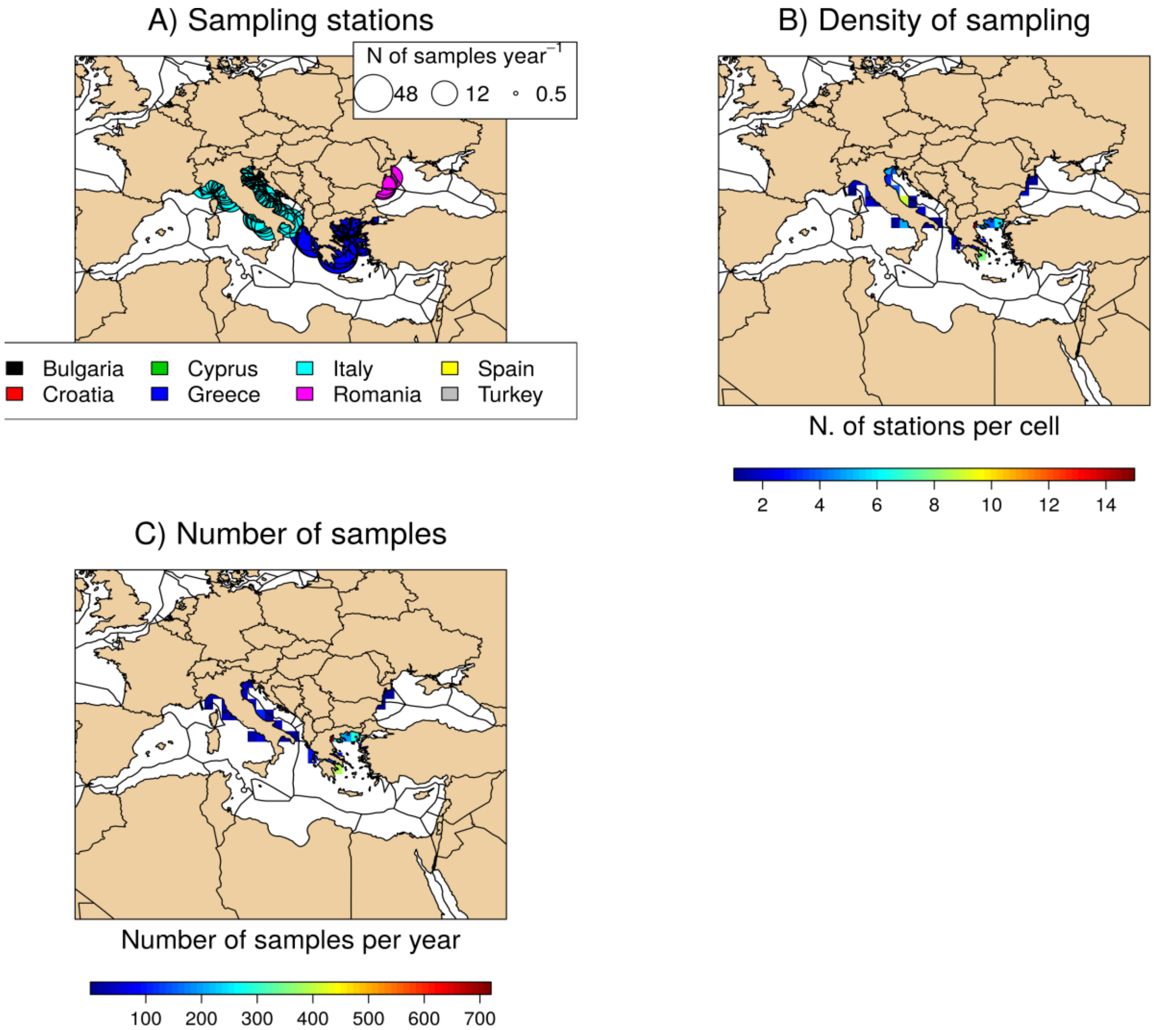


Figure 110. The existent monitoring stations and frequencies distributions have been plotted on a raster map. The scales considered in this preliminary phase are: density of sampling stations, number of yearly collected samples. An arbitrary grid is used with a uniform mesh size of 1 decimal degree (stations with known location and proposed sampling frequency).

5. OCEAN OBSERVING SYSTEMS

HCMR contributors: Reizopoulou Sofia, Kyriakidou Chara, Chalkiopoulos Antonis, Perivoliotis Leonidas, Korres Gerasimos, Nikos Streftaris, Kalliopi Pagou

1. INTRODUCTION

The aim of this section is to assess the opportunities to use and develop the infrastructure including platforms, buoys, remote sensing tools etc., and assess the MSFD elements-indicators covered into multidisciplinary programmes (e.g. remote sensing of chlorophyll-a can contribute to attributes for Descriptor 5).

The available data on ocean observing platforms in the Mediterranean were collected from MyOcean Mediterranean In-situ Thematic Assembly Center (TAC, Figure 109). TAC collects data from the Operational Oceanography data providers along the Mediterranean Sea. The ocean observing systems for the Black Sea were collected from MyOcean (http://www.myocean.eu), whereas additional information has been collected from the IRIS-SES consortium partners. The detailed information and gap analysis on observing systems from the PERSEUS project's Deliverable Nr. 3.1 "Review of ocean observing systems in the SES and recommendations on upgrades to serve PERSEUS needs", Poulain et al., 2013, has also been considered. The catalogues on the existing observing capacities for the Mediterranean and Black Seas in June 2014 are shown in Annex I.

2. Ocean observing systems in the Mediterranean and Black Sea

Moored and free floating buoys can measure a large variety of physical, chemical and biological variables such as salinity, temperature, turbidity, dissolved oxygen, trace metals, pCO2 and others, depending on the number of instruments they can handle, and the data are transmitted in real time to land based observatories (Zampoukas et al., 2012). Table 26 shows the platform types in the Mediterranean and Black Sea.

Table 26. Platform types in the Mediterranean and Black Sea

Platform types
MO = Mooring or Fixed buoys
PF = Argo floats
GL = Gliders
DB = Drifting buoys

The ARGOS buoy network provides data from buoys which are periodically sinking to depth and transmit the data when surfacing. More specifically the Argo floats are mobile autonomous platforms moving freely, horizontally with the currents and vertically in the water column. Sub-surface currents can be estimated from their sub-surface displacements while drifting at the parking depth during the cycle period (1 to 10 days). Water properties such as pressure, temperature, salinity, oxygen, chlorophyll and nitrate concentrations, and optical properties are measured when the floats are profiling up to the surface.

Surface drifters usually measure the surface currents, and they move freely with the currents, but due to wind and currents they can slip above the sea surface. Most drifters transmit data on sea surface temperature, voltage, drogue presence indicator, etc.). Autonomous gliders have recently become an operational technology for Oceanography and they can help to fill the gaps between shipboard sampling and satellite imagery. Gliders follow an up-and-down profile through the water providing data on temporal and spatial scales such as temperature, oxygen, conductivity, however they also measure variables such as nutrients, contaminants, and also phytoplankton biomass.. They provide profiles, such as ARGO floats do, however the time and location of the glider observations is remotely controllable. They can provide valuable information for ocean climate and can also support video cameras to record pelagic organisms or support detectors of acoustic signals. The European Gliding Observatories (EGO; http://www.ego- network.org/) promotes the use of gliders in the marine environment.

The number of ocean observing systems and their locations in the Mediterranean and Black Sea are shown in Figure 111 and Figure 112.

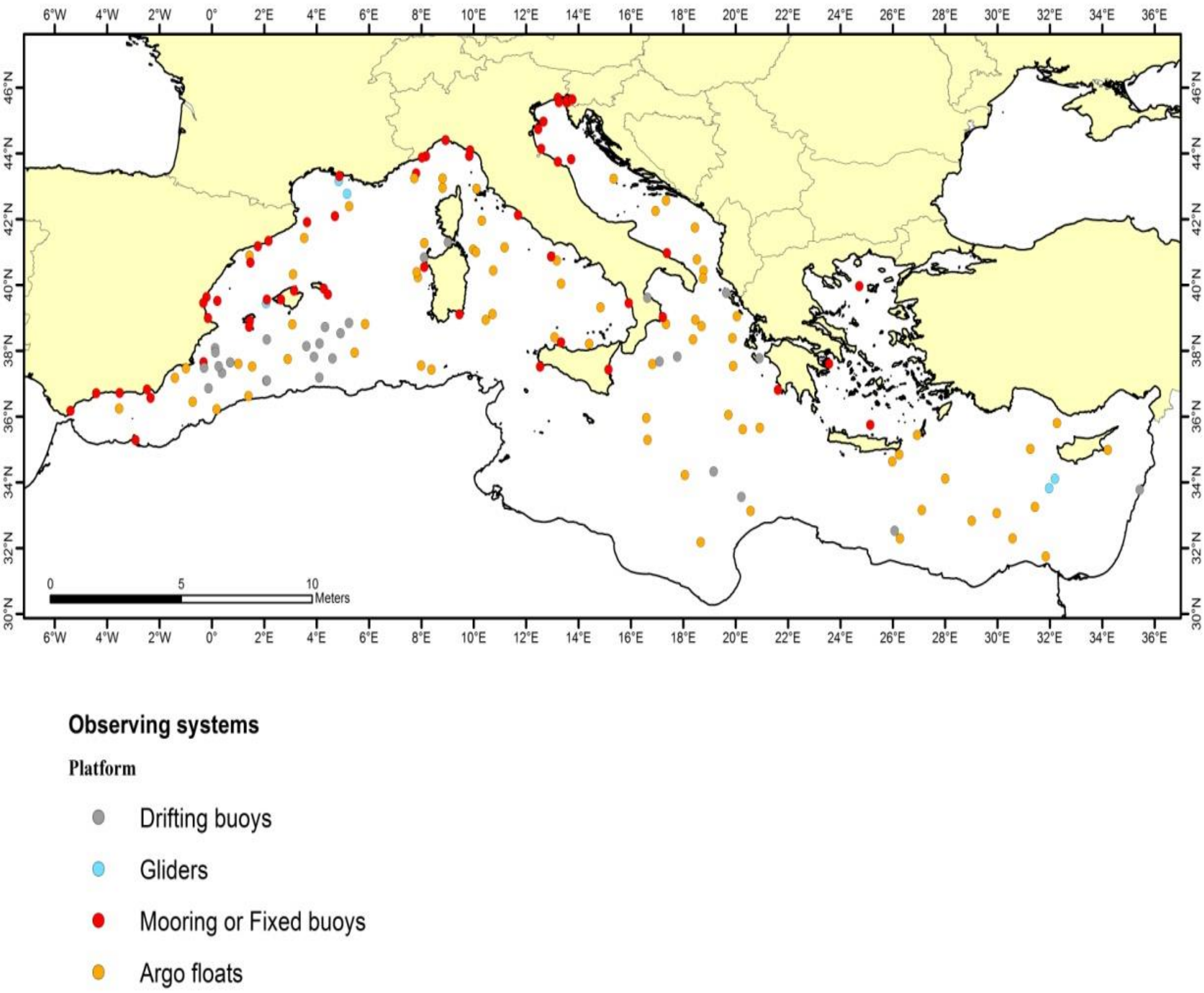


Figure 111. Ocean observing systems availability and their locations in the Mediterranean

Sea - July 2014.

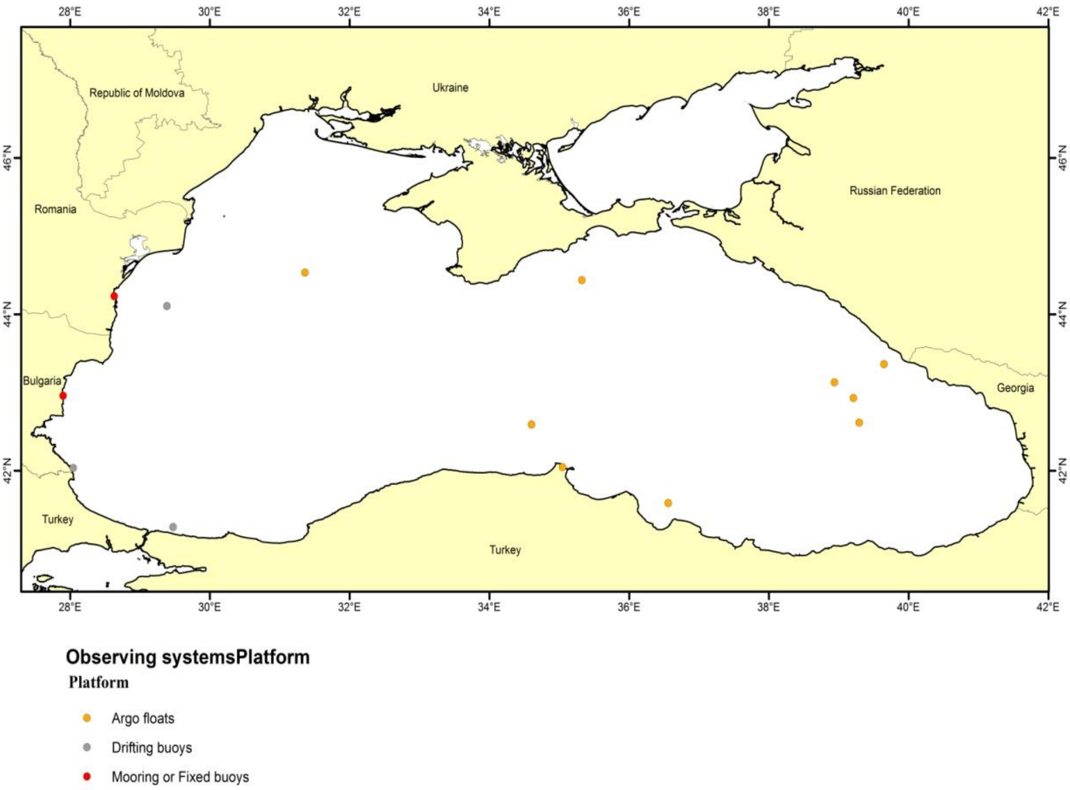


Figure 112. Ocean observing systems availability and their locations in Black Sea – July 2014

3. Ship of Opportunity / Ferry Box

Ships of opportunity can be fitted with various instrumentations to collect data related to physical, chemical and biological oceanography. As an alternative to the often expensive and time consuming research vessels, merchant fleet and specifically ferries offer a regular line sampling frequency across a wide range of water types.

In the Ferrybox program (http://www.ferrybox.org) automated instrument packages are operated on ships of opportunities. These instruments range from the simple "Continuous Plankton Recorder (CPR)" with its single purpose of collecting plankton samples during regular ship cruises (included in PERSEUS, WP3 activities planned) up to the most recent sophisticated "FerryBoxes" with an ensemble of different sensors and biogeochemical analysers (Poulain et al., 2013). There is only one Ferrybox program in the Mediterranean recently activated in Greece in the route from Piraeus to Heraclion in Crete (Figure 111).

Figure 113. Active Ferrybox line in the Mediterranean Sea: From Perseus Deliverable Nr. 3.1 "Review of ocean observing systems in the SES and recommendations on upgrades to serve PERSEUS needs" (Poulain et al., 2013)

In addition the CIESM PartnerSHIPS programme aims is to develop a network of ships of opportunity (Figure 114 or Figure 112) for automated monitoring of the surface waters of the Mediterranean, using a complex of different physical, chemical sensors to measure the physical and biogeochemical parameters of the Mediterranean (temperature, salinity, oxygen and pCO2, chlorophyll, etc.).

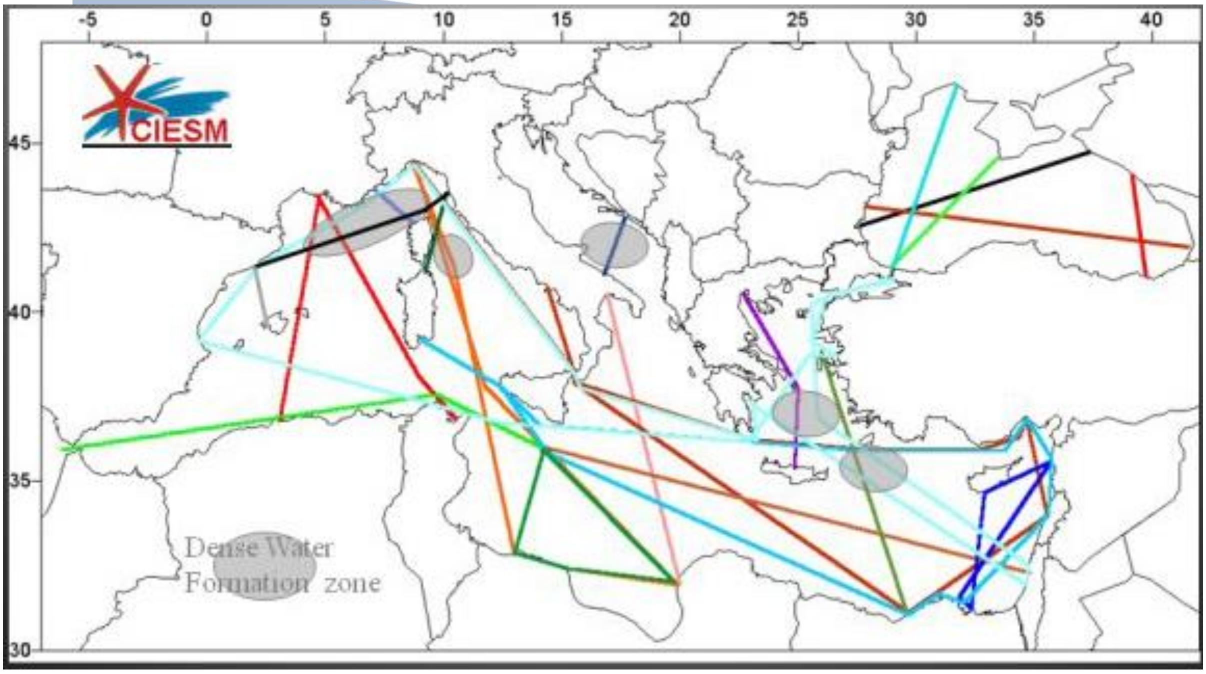


Figure 114. CIESM PartnerSHIPS potential network.

Since May 2010, the CIESM PartnerSHIPS project operates between Genoa (Italy), Malta and Libyan harbours (Figure 113).

Figure 115. CIESM PartnerSHIPS route operated in mid-December 2010.

4. Satellite Remote Sensing

Earth observing satellites can monitor the physical properties of the ocean, such as surface temperature, wave height, surface winds, as well as the ocean colour measurements of phytoplankton pigment concentrations.

The Mediterranean satellite observing is part of the MOON Scientific Strategic Plan with the following aiming to provide Near Real Time (NRT) regional satellite data products to be assimilated in the MOON modelling forecasting systems, and to improve the quality of the oceanic observations in the Mediterranean and Black Sea. This observing system delivers a large variety of satellite observations and it is now one of the components of the European GMES Marine Core service developed by MyOcean (Poulain et al., 2013, Figure 114 & Figure 115).

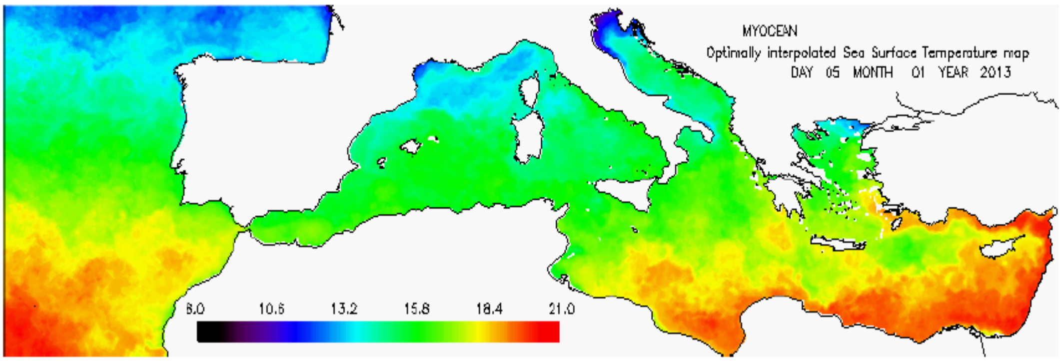


Figure 116. Example of sea surface temperature (SST) data: From Perseus Deliverable Nr. 3.1 "Review of ocean observing systems in the SES and recommendations on upgrades to serve PERSEUS needs" (Poulain et al., 2013).

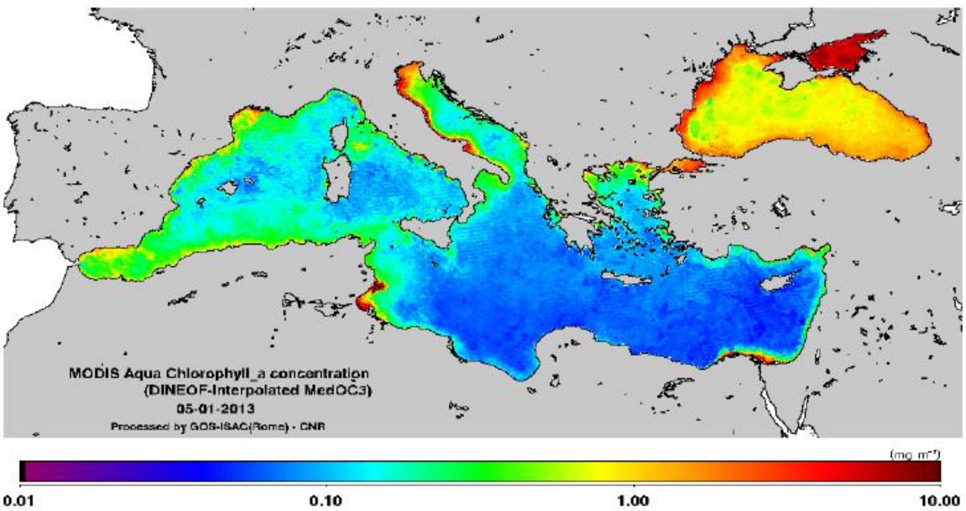


Figure 117. Example of chlorophyll concentration L4 covering the Mediterranean and Black Sea: from Perseus Deliverable Nr. 3.1 "Review of ocean observing systems in the SES and recommendations on upgrades to serve PERSEUS needs" (Poulain et al., 2013).

Other products of interest include total suspended matter, pigmented fraction of dissolved organic matter. The data can be accessed freely through space agencies or via specific web sites such as the Environmental Marine Information System from the Joint Research Centre (http://emis.jrc.ec.europa.eu) (Zampoukas et al., 2012).

5. Other approaches to be considered in MSFD monitoring

Underwater video & Imagery

Video cameras can be used on ferries, ships of opportunity etc. to provide images of the water column and seabed, collecting information on the seafloor and water column macro-organisms, litter or monitor other types of impacts.

Underwater acoustics

Hydroacoustics (echo sounding or sonar), is commonly used for detection, assessment, and monitoring of underwater physical and biological characteristics. Sonars can be used for the detection of animal and plant populations and provide some information on their abundance, size, behavior and distribution. Advances in acoustic technology, and especially data analysis software, have made this survey method even more powerful, through the use of high-resolution sonar imaging, used for habitat mapping, while the combination of different hydroacoustic methods (e.g. multi beam sonar and side scan sonar) enables the spatial classification of the seafloor and its vegetation.

Recording of sounds produced by marine mammals can also provide information on their population abundance, their movements and location of their habitats (Zampoukas et al., 2013).

Continuous Plankton Recorder (CPR)

CPR (Figure 116) is a plankton-sampling instrument towed from ships at a depth of approximately 10 metres. The plankton is filtered and CPR samples are analyzed in the laboratory in two ways. A semiquantitative estimation of phytoplankton biomass, the Phytoplankton Colour Index (PCI), can be determined and also microscopic analysis for species identification and abundance of phytoplankton and zooplankton taxa can be performed (Warner & Hays, 1994). The instrument can sample larger areas and provide biomass data and taxonomic information necessary for many indicators. CPR can also been used to monitor microlitter in the water column (Thompson et al., 2004).

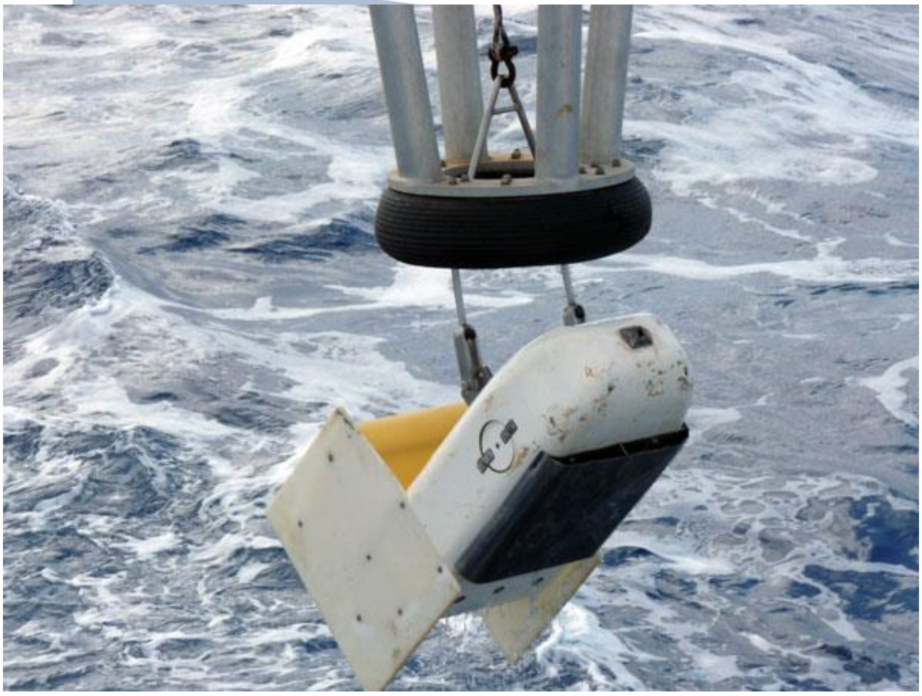


Figure 118. CPR of the Okeanos Explorer (Image of NOAA Okeanos Explorer Program and NMFS, http://oceanexplorer.noaa.gov/okeanos/explorations/.

6. Gaps

The analysis on the spatial coverage of operation of autonomous mobile platforms (drifters, floats and gliders) showed that the southern areas of the Mediterranean Sea and the entire Black Sea are under-sampled and denser observations are required. This gap can be partially filled by involving scientists from North African countries in new observational programs. For the Black Sea more integrated programs among the Black Sea countries are needed (Poulain et al., 2013).

Currently the present remote sensing products in the Mediterranean and Black Sea are limited to core variables (e.g. SST, SSH, CHL, etc). The development of remote sensing datasets more suitable to evaluate the ecosystem attributes relevant for the MSFD Descriptors (e.g. productivity, biological diversity, turbidity, etc) is required, and is part of the activity planned in PERSEUS WP4.

7. References

- Poulain et al., 2013. Review of ocean observing systems in the SES and recommendations on upgrades to serve PERSEUS needs. PERSEUS Deliverable Nr. 3.1
- Thompson R.C., Olsen Y., Mitchell R.P., Davis A., Rowland S.J., John A.W.G., McGonigle, D. & Russell, A.E. 2004.Lost at sea: where is all the plastic? Science 304: 838. (doi:10.1126/science.1094559
- Warner A.J. & Hays G.C. 1994. Sampling by the continuous plankton recorder survey. Progress InOceanography 34: 237- 256
- Zampoukas, N., H. Piha, E. Bigagli, N. Hoepffner, G. Hanke, A.C. Cardoso, 2012. *Monitoring for the Marine Strategy Framework Directive: Requirements and Options*. JRC Scientific and Technical Report. EUR 25187 EN–2012. http://publications.jrc.ec.europa.eu/ repository/handle/111111111/23169

Appendix II
(See excel files in the incorporated to the document memory device)

IRIS-SES

Integrated regional monitoring implementation strategy in the South European Seas

www.iris-ses.eu

PART 3

E-LEARNING

Project coordinator: Dr. Kalliopi Pagou
Grant Agreement:
07.0335/ 659540 / SUB / C

1. E-Learning strategy of IRIS-SES

Premise

MSFD innovative and holistic approach extends previous schemes of monitoring coastal and aquatic ecosystems in the light to assessing their Environmental Status and the departure from Good Environmental Status to a wide range of both descriptors and indicators, which had not been addressed before or, if addressed, not in the light of GES. It is requiring: a. new or updated monitoring strategies, methodologies and techniques; b. accounting for new or additional components of natural variability of selected descriptors and indicators, of their synchrony, covariance or connectivity; c. considering new or additional external drivers of both natural variability and human induced perturbation, as well as an increased importance of multiple stressors; and, d. deeper consideration and understanding of the relevance of hierarchical, spatial and temporal scales and cross-scale dynamics. Moreover, MSFD and the related monitoring actions have relevance for a number of different stakeholders, from scientists, to technical staff of environmental protection agencies, to natural resource managers and decision makers up to general public and young generations, as costumers of good and services of tomorrow ecosystems.

Training on all new issues posed by the innovative approach of MSFD and involving the methodological and practical aspects listed above is essential for the scientific stakeholder community as well as for the whole community of technicians and researchers actively involved in the MSFD monitoring programs at the different national or regional levels. However, training and communication tools are also relevant for the other categories of MSFD stakeholders in order to strengthen their awareness on the overall importance of implementing MSFD programs and reinforce their willingness to pay for marine ecosystems matching the GES requirement.

IRIS-SES Strategy

The Rationale for developing in IRIS-SES a specific e-Learning activity, based on the premises listed in the previous section, is to offer to a whole stakeholder community of the MSFD a platform, which is going to remain active also after the completion of the project, where different training tools are made available to target groups, with the aim of covering in the long run key descriptors and indicators of the MSFD monitoring programme, the different methodological and technical aspects involved with monitoring. Training tools should be adapted to the different target groups having also a role in disseminating to the general public the importance of monitoring the ecological/environmental status of our seas as well as the

relevance and characteristics of MSFD descriptors.

According to this rationale, the IRIS-SES strategy towards an effective, long lasting, continuously updated e-Learning system has the following key components:

Develop an online e-Learning platform with short interviews and power-point 'lessons' on the IRIS-SES website open-access and open-resource to any interested stakeholder http://iris-ses.eu/outreach/multimedia/e-learning/;

Link the online e-Learning Platform to the LifeWatch e-Learning resources and facilities with the agreement of LifeWatch hosting the IRIS-SES e-Learning resources on the respective LifeWatch working area after IRIS-SES completion, supporting further updates from the IRIS-SES community and other projects IRIS-SES has linked to in a network of collaboration;

Mobilise scientists of all partners involved as well as colleagues from other projects in the IRIS-SES project developed network into e-training actions;

Define a range of e-training tools targeting different stakeholder groups, including short TED-like and long lecture-like presentations

A general description of strategy and organisation of the IRIS-SES e-Learning action is reported in the following Figure 119.

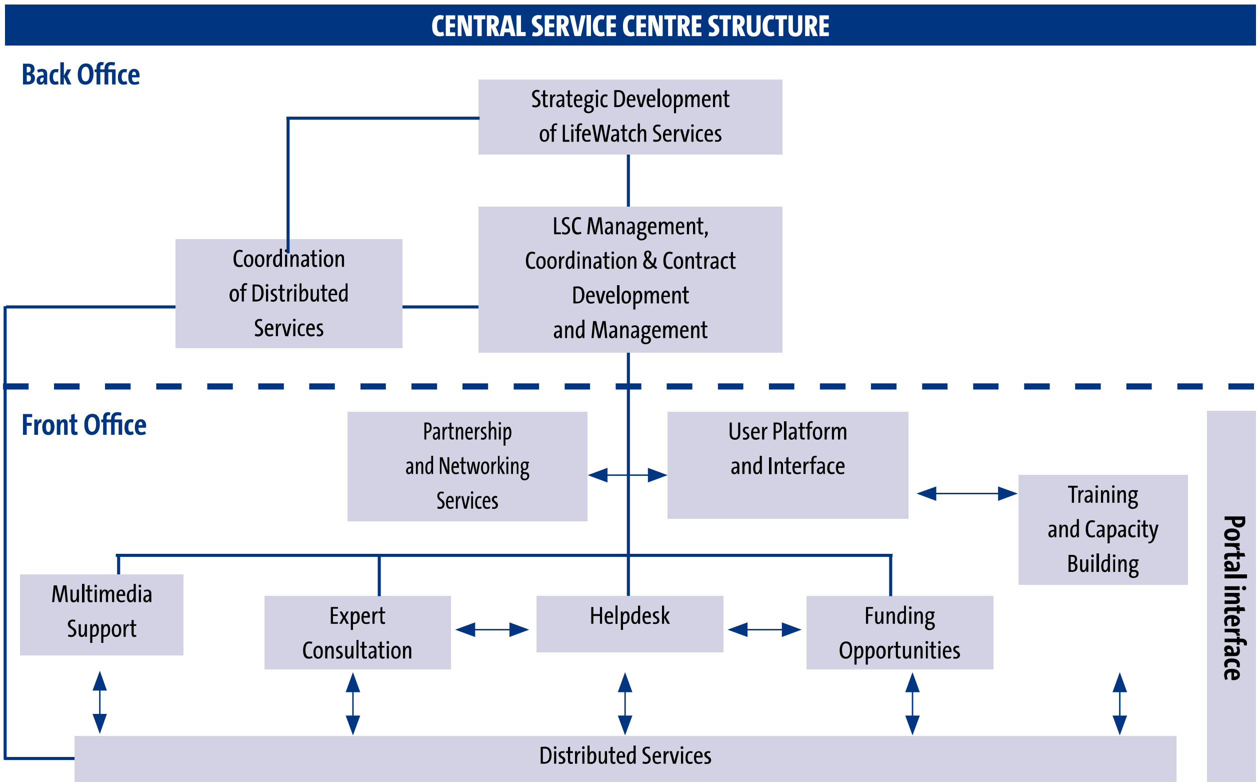


Figure 119. Lifewatch platform.

The organisation plan was structured into steps. During a first phase (step 1) IRIS-SES partners have been asked for their specific contribution. Potential topics (i.e. main themes & titles), targets (i.e. students, academics, stakeholders) and modalities (short talks & long lessons) had been defined and remain as slots of e-Learning resources which will be updated also after IRIS-SES completion from other initiatives, projects, infrastructure, JPI contributions. Etc through the Life Watch.

After discusion with the scientists involved on the best potential outcome and taking also into account technical limitations it had been decided that Contributions will materialised into two modes, as follows:

Short talks (max 5 minutes TED like interviews on the theme and title indicated by the author)

Long lessons (max 45 minutes of power-point structured lesson with an audio record)

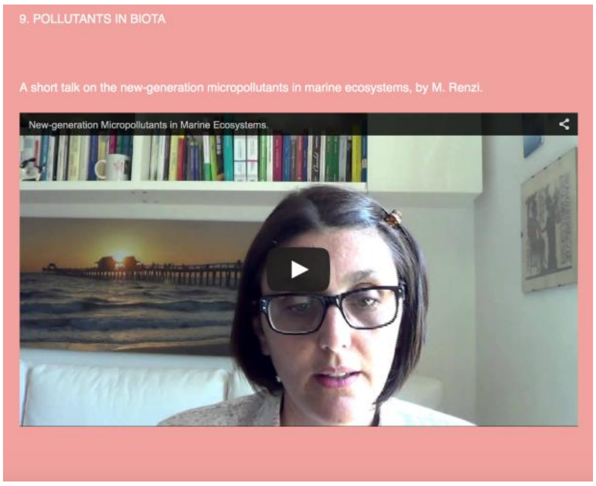
In a second phase (Step 2) contributions were collected, edited and eventually standardised and made available on the IRIS-SES e-Learning section of the website (http://iris-ses.eu/outreach/multimedia/e-learning/) and will be made available on the LifeWatch e-Learning section after the IRIS-SES completion.

2. IRIS-SES E-learning material

http://iris-ses.eu/outreach/multimedia/e-learning/

The e-learning material published on the IRIS-SES website, is constituted by thirteen didactic interventions, nine as short talks and four as long lesson covering topics under Descriptors: D1 Biodiversity, D2 Alien Species, D4 Food webs, D5 Eutrophication, D6 Sea bed integrity and D8 Contaminants, and D10 Marine Litter. Most of them are already available on the projects website while the rest are at the final editing stage and will become available soon.

The nine TED-like short talks are dealing respectively on morpho-functional diversity, as body size diversity, in marine ecosystems (D1), zooplankton biodiversity and pelagic food web dynamics in Adriatic (D1, D4), alien species treats to marine Mediterranean ecosystems (D2), nutrient dynamics, phytoplankton dynamics and harmful algal blooms in the Black Sea (D5), smart monitoring for phytoplankton blooms – remote sensing (D1, D5, bioturbation and sea bed integrity (D6), interactions of biotic and abiotic components of the sea bed ecosystem (D6), problems raising from new nano-particle contaminants, critical pressures from POPs concentration in marine ecosystems and organism responses and contaminant bio-magnification (D8), and the ecological impact of marine litter (D10). They all are presenting the different cases and topics with scientific rigour while using images, examples and languages accessible to a wide range of stakeholders, including young generation and students of intermediate and high schools.



The four long lessons are dealing with the issues of eutrophication (Nutrients in the NW Black Sea (D5)) and the increasing contamination of marine ecosystems (POPs in the NW Black Sea (D8), Chemical pollution and trophic webs (D5, D8), and importance of assessing contaminant-related biomarker responses in marine organisms (D8).

All material will be kept available and updated even after the IRIS-SES completion on the LifeWatch Service Centre e-Learning area, where a section dealing with concept, methodologies, services and tools to support MSFD will be implemented with the contribution of the USalento IRIS-SES partner, in charge of the Activity 2 coordination in the project.