Final Workshop
Instituto Superior Técnico, Lisboa, Portugal
1st June 2023

iFADO
INNOVATION IN THE FRAMEWORK OF THE ATLANTIC DEEP OCEAN

CSIC
Consejo Superior de Investigaciones Científicas

Interreg Atlantic Area
European Regional Development Fund

EUROPEAN UNION

WP4 – Enhanced *in situ* monitoring for MSFD

MSFD assessment: iFADO methodological harmonization for *in situ* monitoring

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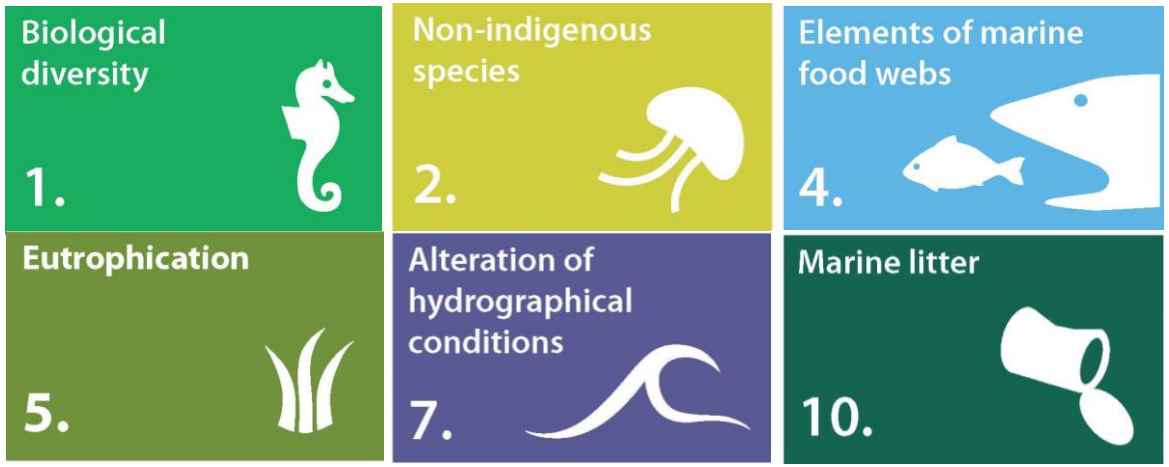
MSFD 2008/56/EC

Marine Strategy Framework Directive (MSFD 2008/56/EC)

- aims to achieve Good Environmental Status (GES) of the EU's marine waters by 2020.
- aims to integrate environmental considerations into relevant policy areas

11 DESCRIPTORS

WP4 IFADO targets =



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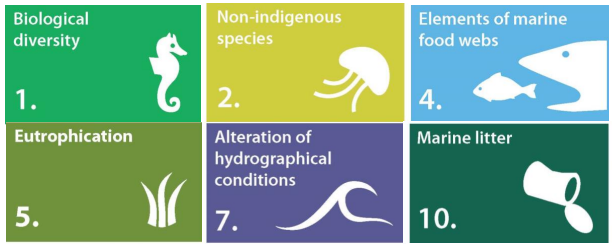
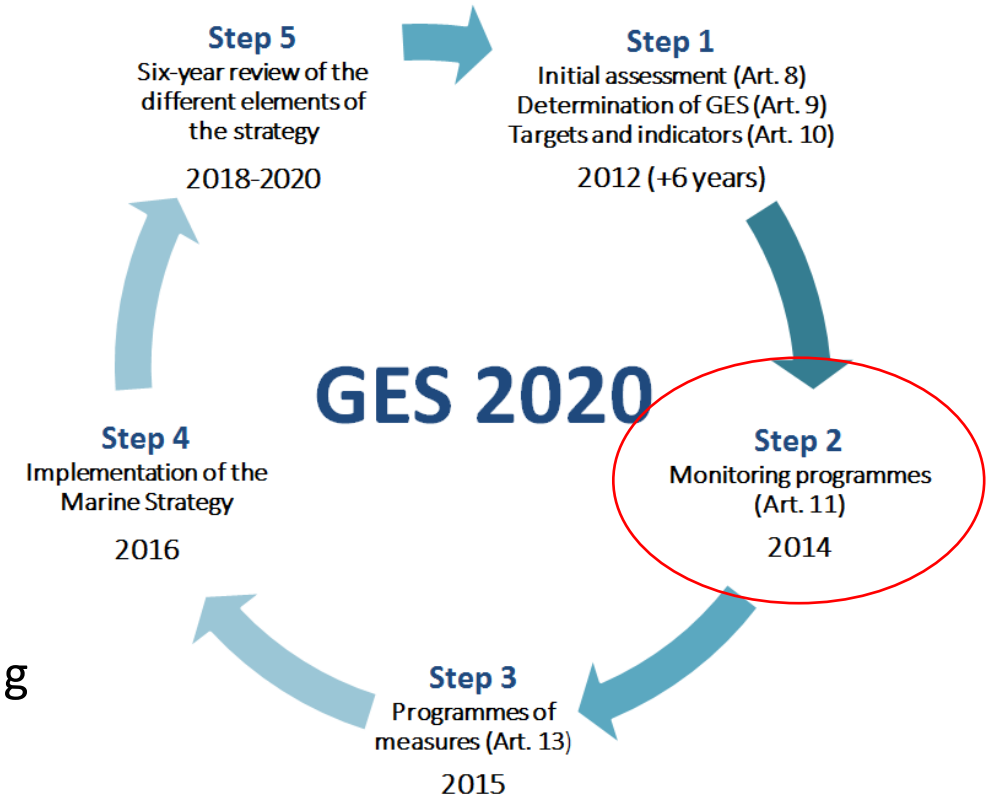


- Reiterative 6-years cycles

- Member States are required by Article 11 of the Marine Strategy Framework Directive (MSFD) to establish and implement **coordinated monitoring programmes**, improving the **comparability of results**



MSFD 2008/56/EC

REVIEW OF THE INITIAL ASSESSMENT REPORTS (2012)

For each descriptor, among Member States:

- Different definitions of GES
- Different indicators
- Different reporting methods
- Different monitoring methods:
    - Different units
    - Different spatio-temporal scales

COMMISSION DECISION (EU) 2017/848 ..

.. laying down **criteria and methodological standards** on GES of marine waters and **specifications and standardised methods for monitoring and assessment**

” … **further development of methodological standards** in close coordination with the establishment of monitoring programmes.”

“ … and Member States collaborate to .. aiming at a clearer, simpler, more concise, more **coherent and comparable set of GES criteria and methodological standards** and … “

“ … emphasised the need for Member States to more systematically build upon standards stemming from Union legislation or, where they do not exist, upon **standards set by Regional Sea Conventions** or other international agreements.”

“… It is necessary to clarify, revise or introduce criteria, **methodological standards, specifications and standardised methods** to be used by Member States, … “

“… Member States should apply the criteria, methodological standards, specifications and **standardised methods for monitoring and assessment** … “

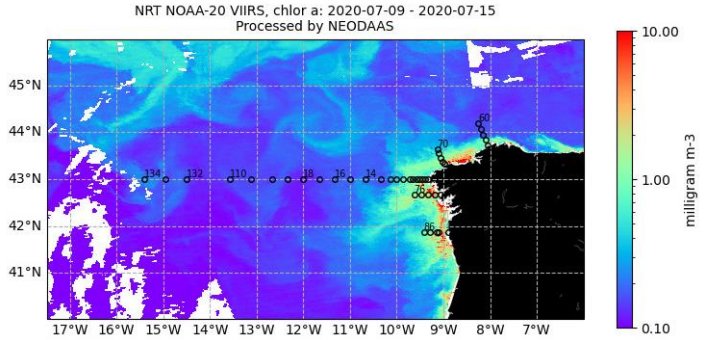
COMMISSION DECISION (EU) 2017/848 ..

D5. Eutrophication

Chlorophyll a in the water column	D5C2 — Primary: Chlorophyll a concentrations are not at levels that indicate adverse effects of nutrient enrichment. The threshold values are as follows: (a) in coastal waters, the values set in accordance with Directive 2000/60/EC; (b) beyond coastal waters, values consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.	Methodological standards *Scale of assessment:* — within coastal waters, as used under Directive 2000/60/EC, — beyond coastal waters, subdivisions of the region or subregion, divided where needed by national boundaries. *Use of criteria:* The extent to which good environmental status has been achieved shall be expressed for each area assessed as follows: (a) the values achieved for each criterion used, and an estimate of the extent of the assessment area over which the threshold values set have been achieved; (b) in coastal waters, the criteria shall be used in accordance with the requirements of Directive 2000/60/EC to conclude on whether the water body is subject to eutrophication ([1]); (c) beyond coastal waters, an estimate of the extent of the area (as a proportion (percentage)) that is not subject to eutrophication (as indicated by the results of all criteria used, integrated in a manner agreed where possible at Union level, but at least at regional or subregional level). Beyond coastal waters, the use of the secondary criteria shall be agreed at regional or subregional level.

COMMISSION DECISION (EU) 2017/848 ..

D5. Eutrophication



Chlorophyll a in the water column

SATELLITE
REMOTE SENSING

Near-surface concentration of chlorophyll-a (mg m^{-3}), calculated using empirical relationships derived from remote sensing reflectances and **in situ measurements of Chla**:

D5C2 — Primary: Chlorophyll a concentrations are not at levels that indicate adverse effects of nutrient enrichment. The threshold values are as follows: (a) in coastal waters, the values set in accordance with Directive 2000/60/EC; (b) beyond coastal waters, values consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.	Methodological standards *Scale of assessment:* — within coastal waters, as used under Directive 2000/60/EC, — beyond coastal waters, subdivisions of the region or subregion, divided where needed by national boundaries. *Use of criteria:* The extent to which good environmental status has been achieved shall be expressed for each area assessed as follows: (a) the values achieved for each criterion used, and an estimate of the extent of the assessment area over which the threshold values set have been achieved; (b) in coastal waters, the criteria shall be used in accordance with the requirements of Directive 2000/60/EC to conclude on whether the water body is subject to eutrophication ([1]); (c) beyond coastal waters, an estimate of the extent of the area (as a proportion (percentage)) that is not subject to eutrophication (as indicated by the results of all criteria used, integrated in a manner agreed where possible at Union level, but at least at regional or subregional level). Beyond coastal waters, the use of the secondary criteria shall be agreed at regional or subregional level.

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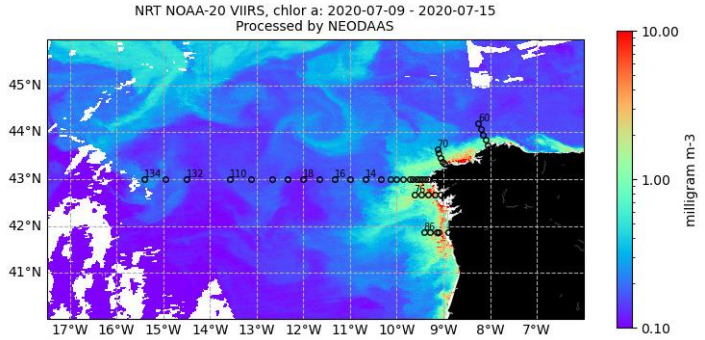

Chlorophyll a in the water column

SATELLITE
REMOTE SENSING

Near-surface concentration of chlorophyll-a (mg m^{-3}), calculated using empirical relationships derived from remote sensing reflectances and **in situ measurements of Chla**:

- Discrete samples:
  - HPLC
  - Long time-series: Spectrofluorometry, Fluorometry

- Continuous measurements:
  - Optical sensors in fixed or mobile platforms (CTD, underway TSG, glider, FerryBox …)

D5C2 — Primary:

Chlorophyll a concentrations are not at levels that indicate adverse effects of nutrient enrichment.

The threshold values are as follows:

(a) in coastal waters, the values set in accordance with Directive 2000/60/EC;

(b) beyond coastal waters, values consistent with those for coastal waters under Directive 2000/60/EC. Member States shall establish those values through regional or subregional cooperation.

Methodological standards

Scale of assessment:

— within coastal waters, as used under Directive 2000/60/EC,

— beyond coastal waters, subdivisions of the region or subregion, divided where needed by national boundaries.

Use of criteria:

The extent to which good environmental status has been achieved shall be expressed for each area assessed as follows:

(a) the values achieved for each criterion used, and an estimate of the extent of the assessment area over which the threshold values set have been achieved;

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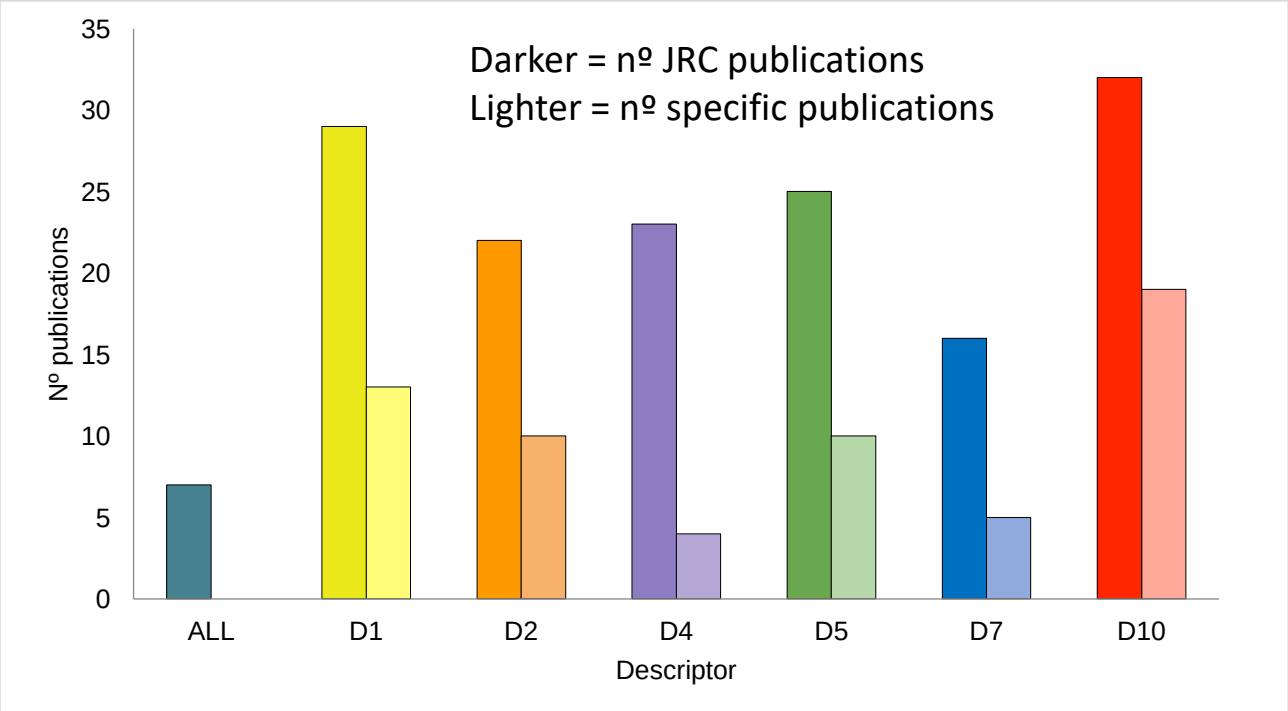
Beyond coastal waters, the use of the secondary criteria shall be agreed at regional or subregional level.

MSFD in offshore and deep waters

EU's JRC repository

- Reports about MSFD Descriptors of interest for WP4
- Available information for deep waters and offshore areas

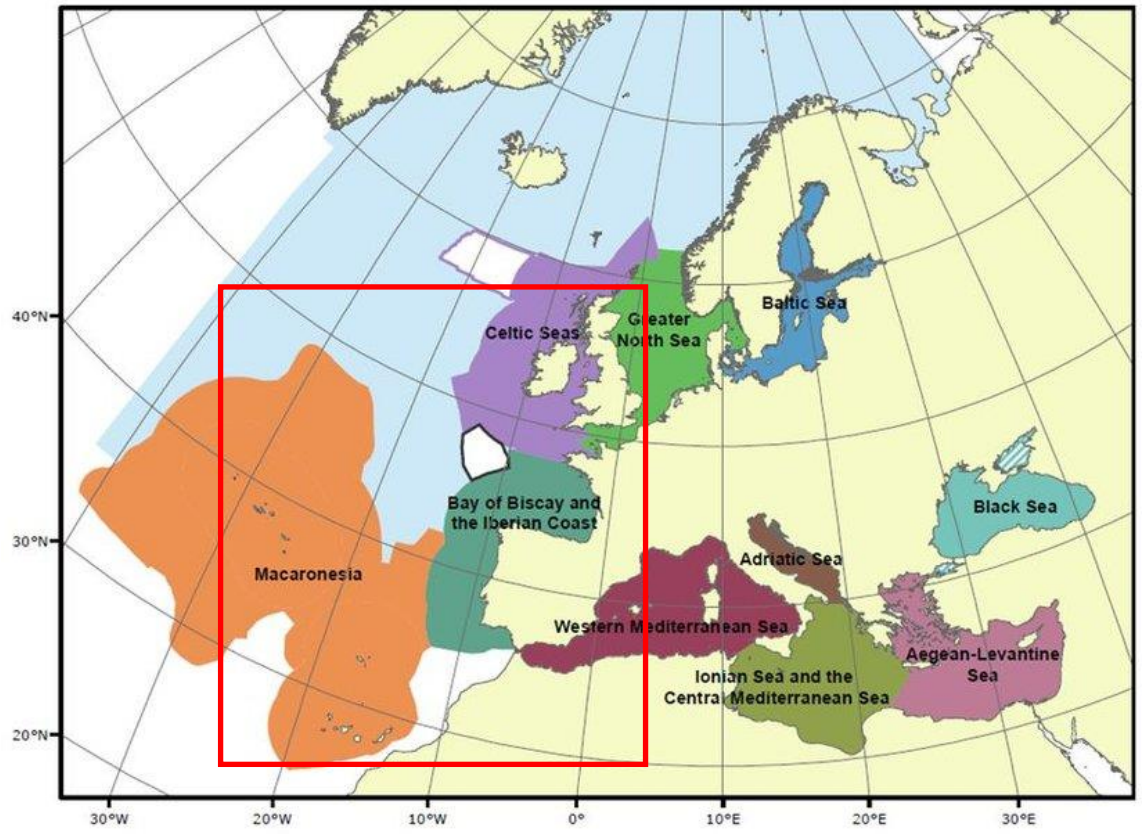


~ 84 reports since 2011
(EU's JRC repository)

- Uneven attention likely due to uneven monitoring capacity for the different descriptors: D1, D2, D4, D5, D7, D10
- Very few available information about deep and offshore environments

D4.2.2 Report on recommendations for the coordination in MSFD monitoring plans in the AA

European Atlantic Area (AA)



- Narrow continental shelf and extensive deep areas
- Great surface area

extremely high costs for detailed monitoring

WORKSHOP Faial (Madeira) 2018

- Coordination of MSFD national monitoring plans in the AA
- Review strategies and gaps in contributions from international projects
- **Improve cost effectiveness through harmonization of methodologies among existing national monitoring programmes.**
- **Providing technical answers for MSFD implementation in AA, especially for the water column in offshore areas.**

D4.2.1 Report on opportunities for harmonised national and international monitoring

Methods harmonization for *in situ* monitoring

Annex 1 = Compilation of **11 SOPs (Standard Operating Procedures)** for **harmonised transnational practices in the Atlantic Area**

Marine Institute
Foras na Mara

CSIC

UNIVERSIDADE da MADEIRA

U LISBOA | UNIVERSIDADE DE LISBOA

National Oceanography Centre

SOP1. **CTD-rosette** sampler system for seawater sampling at discrete deths

SOP2. Quantification and identification of prokaryotic and small eukaryotic populations by **flow-cytometry**

SOP3. **Phytoplankton pigments** composition and concentration

SOP4. Trios RAMSES **Hyperspectral radiometer**

SOP5. **Phytoplankton sampling**, inverse filtration, and samples preservation

SOP6. **FlowCAM®** imaging analysis for phytoplankton biomass and diversity determination

SOP7. **ECOTAXA**: an application web for the semi-automatic counting and identification of plankton samples

SOP8. **Plankton sampling**: nets, mesh-filtration, and sample preservation

SOP9. **ZooScan** imaging analisis for mesozooplankton biomass and diversity determination

+

SOP10. **Microplastics** sampling from sediments collected with Megacores
SOP11. **FlowCAM® Macro** analysis of **faecal pellets** in samples collected with sediment traps

https://www.oceanbestpractices.org **IOC-UNESCO Ocean Best Practices System**

TARGET	SIZE CLASS	SAMPLING		ANALYSIS	MEASURED VARIABLES	DERIVED VARIABLE	MSFD Criteria	SOP / REFERENCE
HYDROGRAPHY					Conductivity + Temperature	Salinity	D7C1	SOP1
					Pressure	Depth		
MICROORGANISMS (flagellates, bacterias)		CTD-rosette + preservation		Flow-Cytometry	Abundance + Biovolume	Biomass	D5C2* D4C2*	SOP1 + SOP2
					Functional groups	Functional diversity	D1C6* D2C1,C2,C3* D4C1* D5C2*	
PHYTOPLANKTON	Total / Size-fractionated	CTD-rosette + filtration		Spectrofluorometry / Fluorometry	[Chl a]	Biomass	D5C2	SOP1 + SOP3
				HPLC		Productivity	D4C4	
				HPLC	[Accessory pigments] + [Carotenoids]	Functional diversity	D1C6 D4C1	
		Hyperspectral radiometer			Radiance + Irradiance	[Chl a]	D4C4 D5C2, C3	SOP4
		CTD-rosette		^{14}C uptake rate	Primary production	Productivity	D4C4	Marañón et al. (2004)
	Pico- (< 2 µm) Nano (2-20 µm)	CTD-rosette + preservation		Flow-Cytometry	Abundance + Biovolume	Biomass	D1C6 D4C2,C3 D5C3	SOP1 + SOP2
					Functional groups	Functional diversity	D1C6* D4C1* D5C2*	
	Nano- (2-20 µm) Micro (> 20 µm)	CTD-rosette + inverse filtration + preservation		FlowCam	Abundance + Biovolume	Biomass	D4C2,C3 D5C3	SOP1 + SOP5 + SOP6
				FlowCam + ECOTAXA	Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1 D5C3	SOP1 + SOP5 + SOP6 + SOP7
	Micro- (40-200 µm)	Bongo net + filtration + preservation		Inverted microscope	Abundance	Biomass	D4C2,C3	SOP 8 + Karlson et al. (2010)
					Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1	
ZOOPLANKTON	Micro- (40-200 µm)	Bongo-type net + mesh filtration		Inverted microscope	Abundance	Biomass	D4C2	SOP8 + Karlson et al. (2010)
					Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1	
	Meso- (200-2000 µm)	WP2 net + mesh filtration	MOCNESS (multi-depth sampling)	ZooScan	Abundance + Area	Biomass	D4C2	SOP7 + SOP8 + SOP9
	Macro- (>2000 µm)			ZooScan + ECOTAXA	Abundance + Community composit.	Diversity	D1C6 D2C1 D4C1	

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MSFD 2008/56/EC

D1 BIODIVERSITY (PELAGIC HABITATS)

D1C6: The **condition of the habitat type**, including its biotic and abiotic structure and its functions (e.g. its typical **species composition** and their **relative abundance**, absence of particularly sensitive or fragile species or species providing a key function, **size structure of species**), is not adversely affected due to anthropogenic pressures.

D2 NON-INDIGENOUS SPECIES

D2C1: The **number of non-indigenous species** which are **newly introduced** via human activity into the wild, per assessment period (6 years), measured from the reference year as reported for the initial assessment under Article 8(1) of Directive 2008/56/EC, is minimised and where possible reduced to zero.

D2C2: **Abundance and spatial distribution of established non-indigenous species**, particularly of invasive species, contributing significantly to **adverse effects on particular species groups or broad habitat types**.

D2C3: **Proportion of the species group or spatial extent of the broad habitat** type which **is adversely altered due to non-indigenous species**, particularly invasive non-indigenous species.

D4 FOOD WEBS

D4C1: The **diversity** (**species composition** and their **relative abundance**) of the trophic guild is not adversely affected due to anthropogenic pressures.

D4C2: The **balance of total abundance (biomass)** between the trophic guilds is not adversely affected due to anthropogenic pressures.

D4C3: The **size distribution of individuals** across the trophic guild is not adversely affected due to anthropogenic pressures

D5 EUTROPHICATION

D5C1: **Nutrient concentrations** are not at levels that indicate adverse eutrophication effects.

D5C2: **Chlorophyll a concentration** are not at levels that indicate adverse effects of nutrient enrichment.

D5C3: The number, spatial extent and duration of **harmful algal bloom events** are not at levels that indicate adverse effects of nutrient enrichment.

D5C4: The **photic limit** (transparency) of the water column is not reduced, due to increases in suspended algae, to a level that indicates adverse effects of nutrient enrichment.

D5C5: The concentration of **dissolved oxygen** is not reduced, due to nutrient enrichment, to levels that indicate adverse effects on benthic habitats or other eutrophication effects.

D7 HYDROGRAPHICAL CONDITIONS

D7C1: Spatial extent and distribution of permanent alteration of **hydrographical conditions** (e.g. changes in wave action, currents, salinity, temperature) to the seabed and **water column**, associated in particular with physical loss of the natural seabed.

TARGET	SIZE CLASS	SAMPLING	ANALYSIS	MEASURED VARIABLES	DERIVED VARIABLE	MSFD Criteria	SOP / REFERENCE
HYDROGRAPHY				Conductivity + Temperature	Salinity	D7C1	SOP1
				Pressure	Depth		
MICROORGANISMS (flagellates, bacterias)		CTD-rosette + preservation	Flow-Cytometry	Abundance + Biovolume	Biomass	D5C2* D4C2*	SOP1 + SOP2
				Functional groups	Functional diversity	D1C6* D2C1,C2,C3* D4C1* D5C2*	
PHYTOPLANKTON	Total / Size-fractionated	CTD-rosette + filtration	Spectrofluorometry / Fluorometry	[Chl a]	Biomass	D5C2	SOP1 + SOP3
			HPLC		Productivity	D4C4	
			HPLC	[Accessory pigments] + [Carotenoids]	Functional diversity	D1C6 D4C1	
	Nano- (2-20 µm) Micro (> 20 µm)	CTD-rosette + inverse filtration + preservation	FlowCam	Abundance + Biovolume	Biomass	D5C2* D4C2,C3 D5C3	SOP1 + SOP5 + SOP6
			FlowCam + ECOTAXA	Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1 D5C3	SOP1 + SOP5 + SOP6 + SOP7
	Micro- (40-200 µm)	Bongo net + filtration + preservation	Inverted microscope	Abundance	Biomass	D4C2,C3	SOP 8 + Karlson et al. (2010)
				Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1	
ZOOPLANKTON	Micro- (40-200 µm)	Bongo-type net + mesh filtration	Inverted microscope	Abundance	Biomass	D4C2	SOP8 + Karlson et al. (2010)
				Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1	
	Meso- (200-2000 µm) Macro- (>2000 µm)	WP2 net + mesh filtration	MOCNESS (multi-depth sampling) ZooScan	Abundance + Area	Biomass	D4C2	SOP7 + SOP8 + SOP9
			ZooScan + ECOTAXA	Abundance + Community composit.	Diversity	D1C6 D2C1 D4C1	

TARGET	SIZE CLASS	SAMPLING	ANALYSIS	MEASURED VARIABLES	DERIVED VARIABLE	MSFD Criteria	SOP / REFERENCE
PHYTOPLANKTON	Total / Size-fractionated	CTD-rosette + filtration	Spectrofluorometry / Fluorometry	[Chl a]	Biomass	D5C2	**SOP1 + SOP3**
			HPLC		Productivity	D4C4	

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MSFD 2008/56/EC

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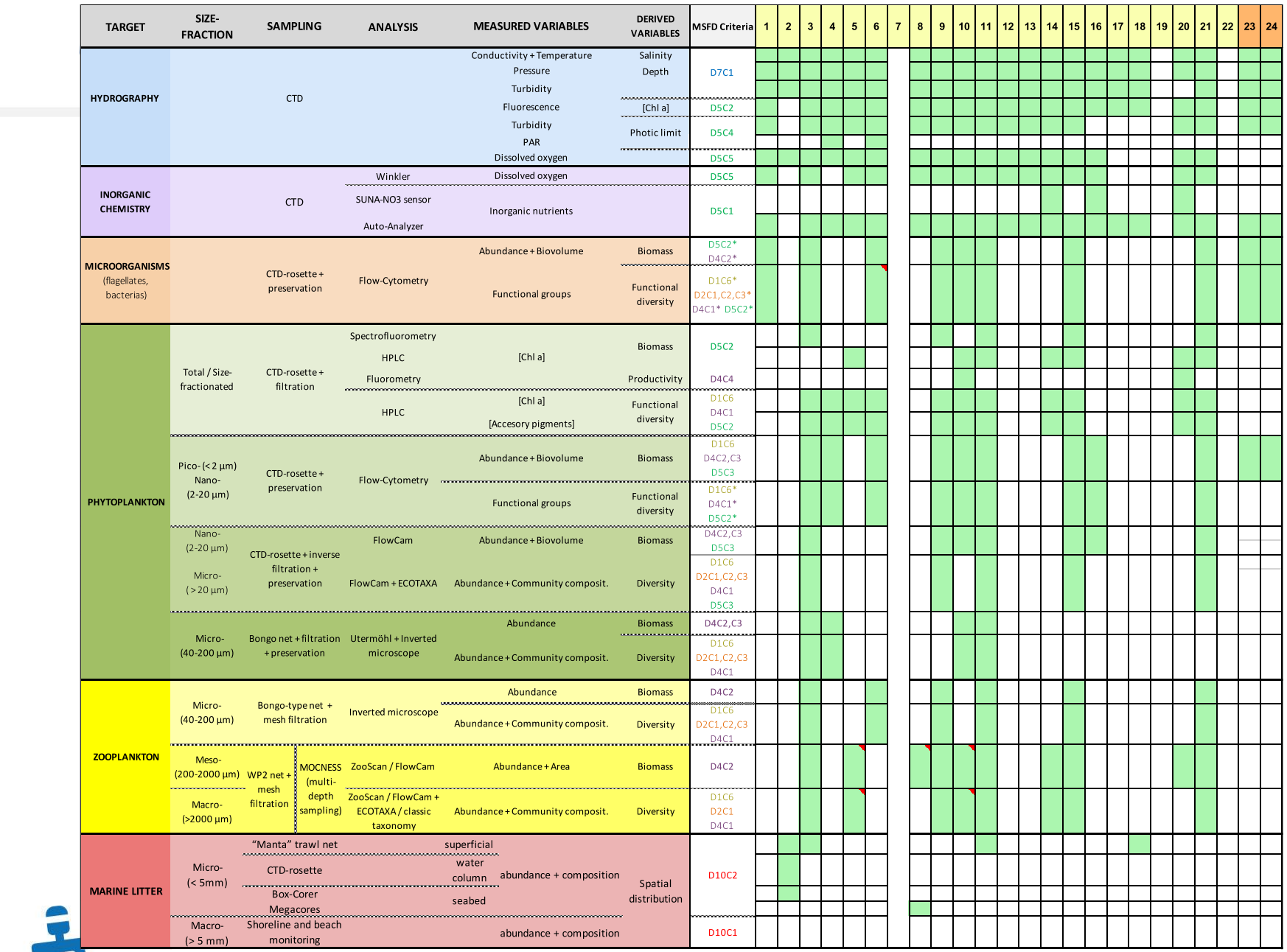
D7C1: Spatial extent and distribution of permanent alteration of **hydrographical conditions** (e.g. changes in wave action, currents, salinity, temperature) to the seabed and **water column**, associated in particular with physical loss of the natural seabed.

TARGET	SIZE-FRACTION	SAMPLING	ANALYSIS	MEASURED VARIABLES	DERIVED VARIABLES	MSFD Criteria
HYDROGRAPHY		CTD		Conductivity + Temperature	Salinity	
				Pressure	Depth	D7C1
				Turbidity		
				Fluorescence	[Chl a]	D5C2
				Turbidity	Photic limit	D5C4
				PAR		
				Dissolved oxygen		D5C5
INORGANIC CHEMISTRY		CTD	Winkler	Dissolved oxygen		D5C5
			SUNA-NO3 sensor	Inorganic nutrients		D5C1
			Auto-Analyzer			
MICROORGANISMS (flagellates, bacterias)		CTD-rosette + preservation	Flow-Cytometry	Abundance + Biovolume	Biomass	D5C2* D4C2*
				Functional groups	Functional diversity	D1C6* D2C1,C2,C3* D4C1* D5C2*
PHYTOPLANKTON	Total / Size-fractionated	CTD-rosette + filtration	Spectrofluorometry	[Chl a]	Biomass	D5C2
			HPLC			
			Fluorometry		Productivity	D4C4
			HPLC	[Chl a]	Functional diversity	D1C6 D4C1 D5C2
				[Accesory pigments]		
	Pico- (< 2 µm) Nano- (2-20 µm)	CTD-rosette + preservation	Flow-Cytometry	Abundance + Biovolume	Biomass	D1C6 D4C2,C3 D5C3
				Functional groups	Functional diversity	D1C6* D4C1* D5C2*
	Nano- (2-20 µm)	CTD-rosette + inverse filtration + preservation	FlowCam	Abundance + Biovolume	Biomass	D4C2,C3 D5C3
	Micro- (> 20 µm)		FlowCam + ECOTAXA	Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1 D5C3
	Micro- (40-200 µm)	Bongo net + filtration + preservation	Utermöhl + Inverted microscope	Abundance	Biomass	D4C2,C3
				Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1
ZOOPLANKTON	Micro- (40-200 µm)	Bongo-type net + mesh filtration	Inverted microscope	Abundance	Biomass	D4C2
				Abundance + Community composit.	Diversity	D1C6 D2C1,C2,C3 D4C1
	Meso- (200-2000 µm)	WP2 net + mesh filtration	MOCNESS (multi-depth sampling)	ZooScan / FlowCam	Abundance + Area	Biomass	D4C2
	Macro- (>2000 µm)			ZooScan / FlowCam + ECOTAXA / classic taxonomy	Abundance + Community composit.	Diversity	D1C6 D2C1 D4C1
MARINE LITTER	Micro- (< 5mm)	"Manta" trawl net		superficial water column	abundance + composition	Spatial distribution	D10C2
		CTD-rosette				
		Box-Corer Megacores		seabed		
	Macro- (> 5 mm)	Shoreline and beach monitoring			abundance + composition		D10C1



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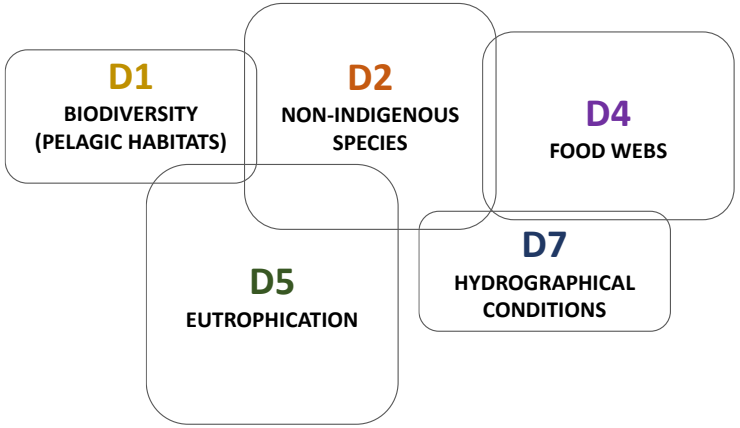
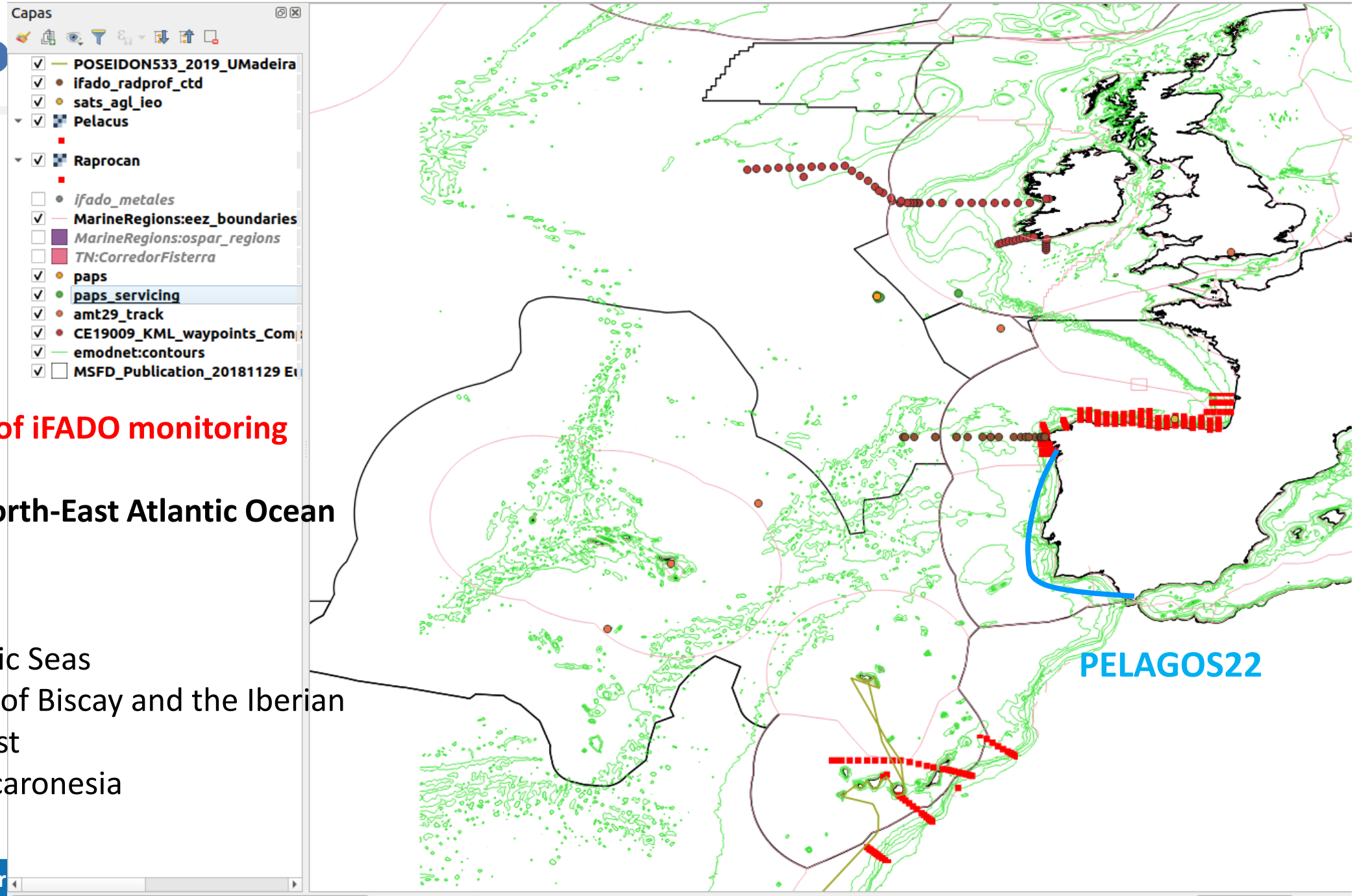
IMPLEMENTATION OF SOPS IN 22 IFADO CRUISES + 2 sampling sites

TRANSNATIONAL COOPERATION





Spatial coverage of iFADO monitoring

MSFD region = **North-East Atlantic Ocean**

=> Subregions:

- Celtic Seas
- Bay of Biscay and the Iberian Coast
- Macaronesia

- Review of current state of the knowledge and main gaps on iFADO target's descriptors, with a special focus on offshore and deep waters

- Contribution of iFADO to transnational harmonized in situ monitoring

Towards a harmonised assessment of offshore and deep pelagic ecosystems status in the North Atlantic Ocean. Transboundary cooperation through iFADO project.

Abstract

The Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC)

1. Introduction

Pelagic habitats provide very important ecosystem functioning and services as they play an essential role in regulating temperature on land, producing oxygen and food to support the marine food chain. The pelagic realm, the largest ecosystem on Earth, includes a continuum of water masses transport and mixing, depending on the interaction of multiple environmental and anthropogenic drivers acting on different spatial and temporal scales, to which biota respond (Stenseth et al., 2006; Bode et al., 2019). Planktonic organisms vary over a wide range of scales, from centimetres to basin-scales and from hours to decades (Haury *et al.*, 1978). Characterizing these patterns of variability and understanding their causes and consequences require long-term high-resolution monitoring.

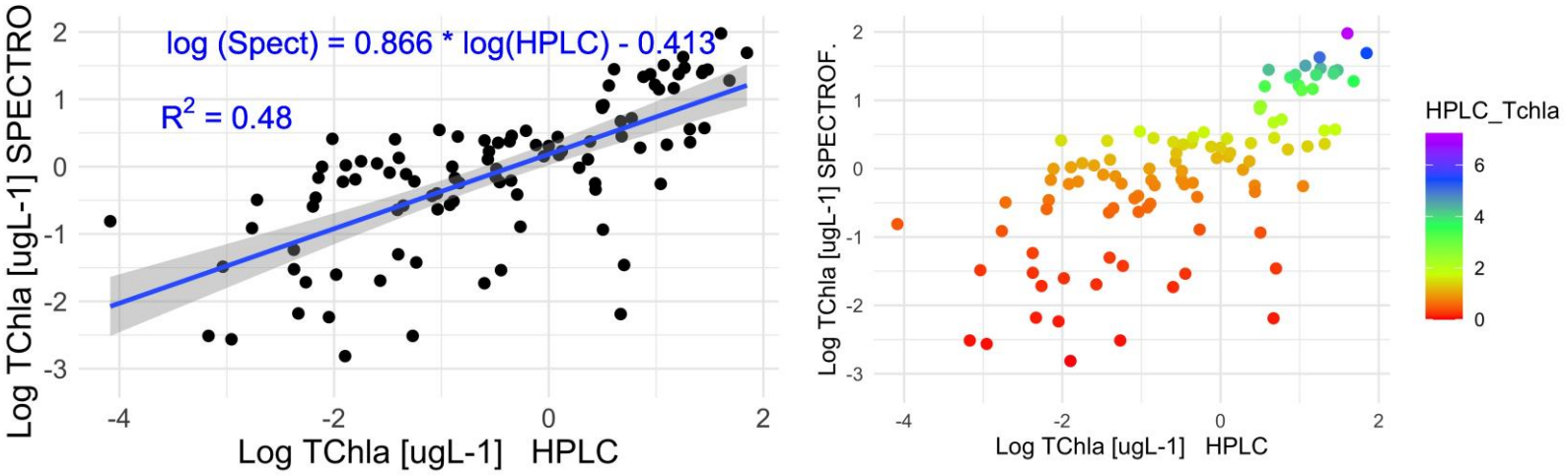
Furthermore, the deep ocean (>200m depth) represents the largest and least explored biome of Earth (<0.0001% of ocean surface) (Danovaro et al., 2020). In addition to their role in global biogeochemical and ecological processes, deep-sea ecosystems provide important goods (including biomass, bioactive molecules, oil, gas, and minerals) and services (climate regulation, nutrient regeneration and supply to the photic zone, and food) which turn them into essential for the sustainable functioning of the biosphere and for human wellbeing.

The extremely high costs derived from research surveys means that exploring the vast ocean with the necessary spatial and temporal resolution to characterise key oceanographic and biogeochemical processes is unaffordable. It has been repeatedly stated in the literature a general need for marine data, especially for the deep sea and open-ocean environments (Morato et al., 2016; Danovaro et al., 2020). Satellite and operational model (e.g., Copernicus Marine Services) data have been suggested to be key

Methods intercalibration

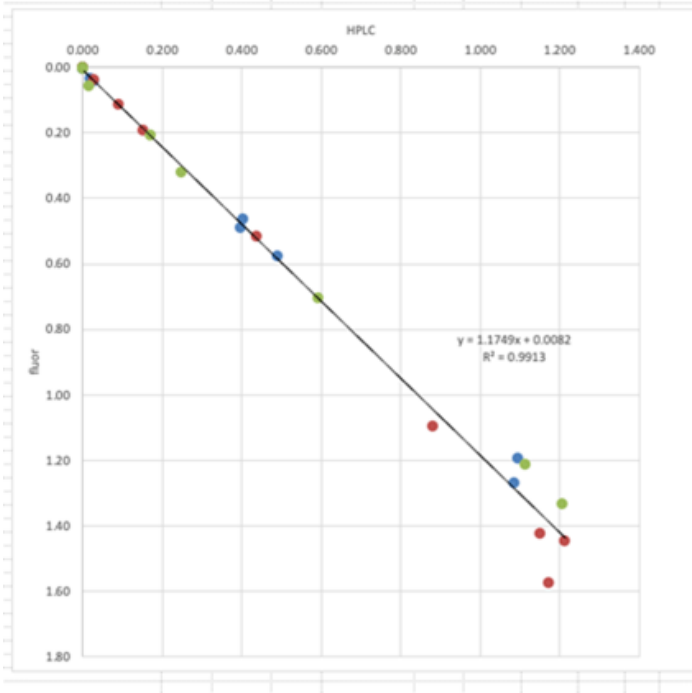
Chla concentration => HPLC *versus:*

Spectrofluorometry



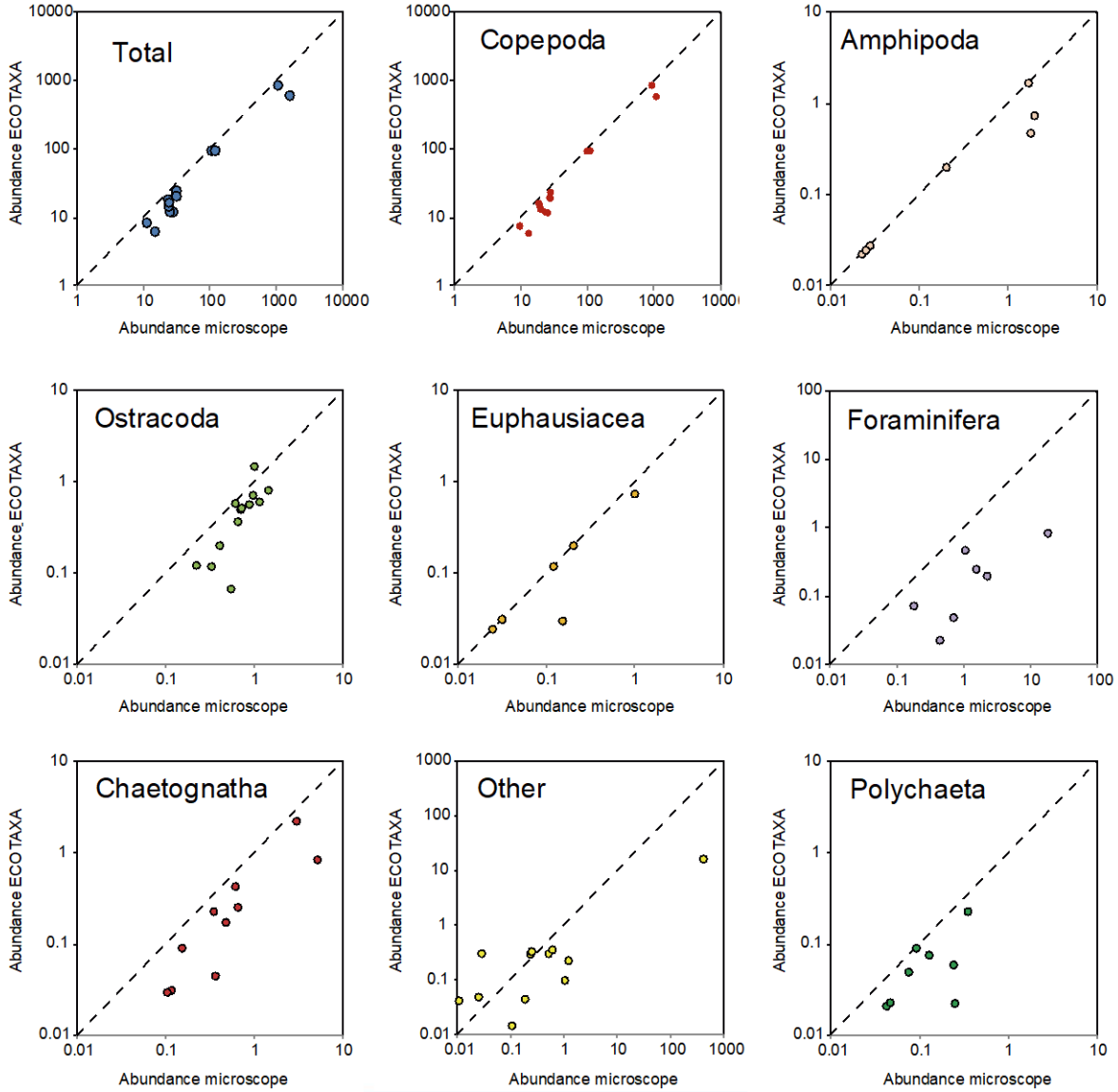
Spectrofluorometry slightly underestimates Chla concentration (~14%)

Fluorometry



Fluorescence slightly overestimates Chla concentration (~17%)

Methods intercalibration



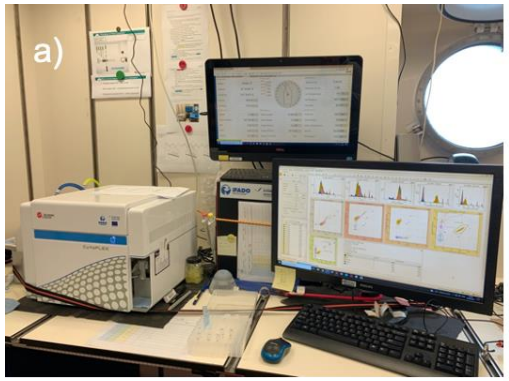
Zooscan + Ecotaxa *versus* microscopy

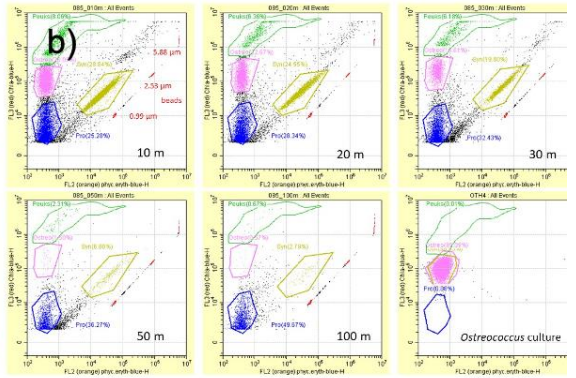
- **good correlation between the abundances assessed by both techniques for abundant or relatively large organisms** (e.g., copepods, amphipods and euphausiids).

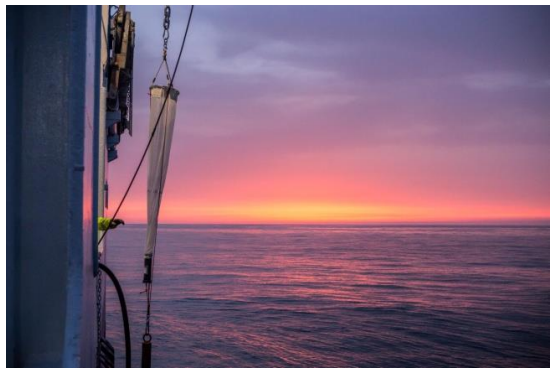
- rare, in low abundance, organisms, or those producing low contrast images (e.g., foraminifera or chaetognaths) are underestimated by semi-automatic methods.

In situ monitoring during iFADO time

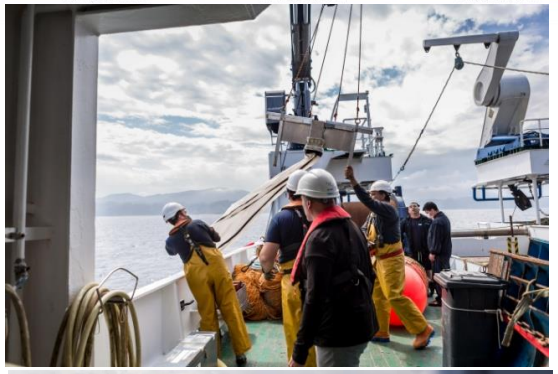















iFADO

INNOVATION IN THE FRAMEWORK OF THE ATLANTIC DEEP OCEAN

THANK YOU SO MUCH

Marine Institute
Foras na Mara

National Oceanography Centre

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