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CoAStal and marine waters integrated monitoring systems for ecosystems proteCtion AnD managemEnt

CASCADE

Project ID: 10255941

Priority Axis: Environment and cultural heritage

Specific objective: Improve the environmental quality conditions of the sea and coastal area by use of sustainable and innovative technologies and approaches

D3.2.1

ECOSYSTEM PILOT CHARACTERIZATION REPORT

PP in charge: PP10 – Institute of Oceanography and Fisheries

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Project acronym	**CASCADE**
Project ID number	**10255941**
Project title	**CoAStal and marine waters integrated monitoring systems for ecosystems protection AnD managemEnt**
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Activity number	**Activity 3.2**
Activity title	**Ecosystem characterization for each pilot**
Partner in charge	**PP10 – Institute of Oceanography and Fisheries**
Partners involved	**All PPs**

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Chapter 1 Introduction

The main objective of Work package (WP) 3 - Coastal Marine Environment characterization of (species and) ecosystems in the nearshore marine environment is to collect information and review existing monitoring activities in the pilot areas (P), such as institutional (e.g. MFSD, bathing waters) monitoring strategies, available species occurrences and characteristics, biodiversity and ecosystem data from research initiatives and data from previous and ongoing international projects, including Interreg IT-HR standard projects.

WP3 activities also aim to explore the lesser known aspects and collect new data on the coastaland marine environment by improving monitoring systems. The collected results will feed into the analysis of the chemical status of the pilot area and thus into the assessment of the usefulness of the operational implementation. In addition, an accurate characterization of the neglected benthic communities of the marine zoo and microplastics down to a few micrometers in all water column and sediment samples will be produced to address the lack of information on this topic.

The above WP includes four activities. In Activity 3.1.1, each project partner (PP) will provide existing observations and model results on the following pilots and with the contribution of the following partners:

P1: Grado and Marano Lagoon and Gulf of Trieste (IT); **PP:** ArpaFVG; UNIBO

P2: Transitional (e.g. Goro area and Bevano Mouth) and coastal areas in Emilia Romagna (IT); **PP:** ARPAe (it will sign a cooperation agreement among public bodies with CNR-ISMAR (PG/2019/0179584) for the EMR pilot activities); UNIBO; CMCC; Delta2000

P3: Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT); **PP:** Regione Puglia; CMCC; UNISALENTO

P4: Neretva river mouth (HR); **PP:** IOF, DNC; CMCC

P5: Coastal area in Veneto (IT); **PP:** IUAV with the support of CORILA;

P6: Miljašić Jaruga river mouth, Nin bay (HR); **PP:** City of Nin; IOF

P7: Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT); **PP:** UNIMOLISE

P8: Northern-eastern Adriatic in Croatia (HR); **PP:** IRB

P9: Cetina river mouth (HR); **PP:** Sea and Karst

P10: Torre del Cerrano, Pineto Abruzzo (IT); **PP:** UNIMOLISE

P11: Marche coastal area (IT); **PP:** Regione Marche.

The review of existing observations and models will allow to identify any gaps and/or critical aspects in order to design more efficient/optimal monitoring and management systems that will be implemented and tested in WP4-5.

In addition, activity 3.2.1 will use the data collected in act. 3.1.1 will be used to define the ecosystem health status in each pilot project. The output of this activity is the Ecosystem Pilot Characterization Report, a document prepared by the partners in the project CASCADE to collect and harmonise the existing data and information on biodiversity, ecosystems and environment in each pilot.

In order to develop optimal monitoring systems for transitional and coastal areas (Activity 3.3.1 Report on the development of optimal monitoring systems for the characterization of the coastal marine environment), the participating project partners will undertake targeted activities in the respective pilot areas. Local stakeholders will be involved in the design of each pilot. Activity 3.4.1. Model Development for Characterization of the Coastal Marine Environment will focus on the optimal development/updating of ocean, hydrological and biogeochemical models to be designed, developed and tested.

Finally, existing datasets and modelling products collected and provided by all partners in the different pilot projects will be harmonised to assess current strategies and plan optimal monitoring and modelling strategies.

The main expected outputs of WP 3 are:

1) critical review and analysis of existing monitoring activities in the pilot areas;
2) a detailed description of ecosystem and environmental characteristics for each pilot area;
3) optimal design of integrated monitoring and modelling tools (hydrological, oceanographic and ecosystem) for each pilot area.

These WP outputs contribute to the main project outcomes associated with programme output indicators 3.2O1 and 3.2O2. The results of WP 3 will be publicly available and disseminated through the WP 4 information systems and will be accessible to other organisations/regions/ countries outside the current partnership. Interaction with relevant stakeholders outside the current partnership will facilitate the transferability of WP3 results and their future use and exploitation.

Chapter 2 Geographical context

The Adriatic Sea extends approximately from 40° to 46° North for 800 km, it has a width ranging from 90 to 200 km and an area of 138 000 km². The Adriatic Basin (Figure 1) is divided into three sub-basins regarding bathymetry, where the northern sub-basin extends up to the line connecting Zadar and Ancona. This is the shallowest part of the Adriatic, considering that its depth does not exceed 50 m; it covers ~25% of the area and includes only 4% of the volume with a mean depth of -

43 metres (Vrdoljak et al., 2021). Some peculiar remarks include the stratification of water column, notable fluvial inputs and high productivity (Stachowitsch, 1991).

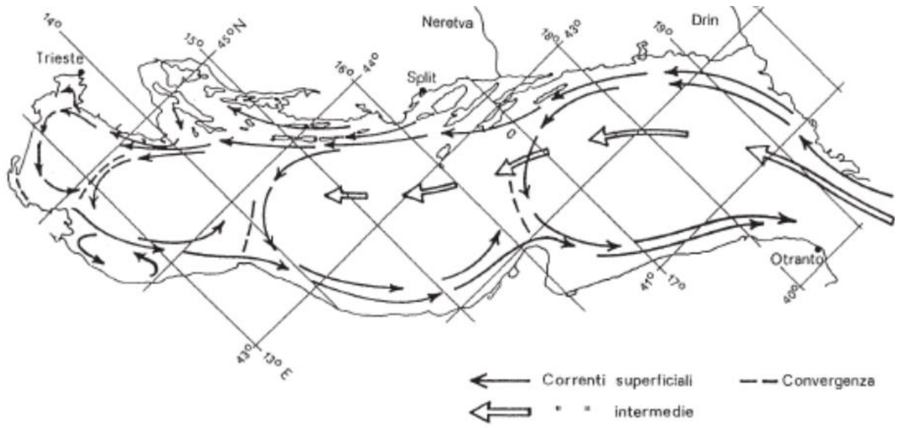


Figure 1. Adriatic Sea circulation.

In the northern Adriatic, several attempts have been made in order to determine both the interannual and seasonal variability of several environmental and biological parameters; however, most of these studies were based on data referring to a specific site (Solidoro et al., 2009). Even though the area was historically considered as one of the most productive of the Mediterranean Sea (Sournia, 1973), a high variability both in terms of space and time is now well-recognised. Hence, the northern Adriatic Sea is now divided into eutrophic, mesotrophic, and oligotrophic regions, with only western coastal waters considered as eutrophic. Nevertheless, Harding *et al.* 1999 identified an inverse trend towards oligotrophication of the basin during certain periods of the year, following the reduction of the phosphorus load in Po River water in the late 1980s (De Wit and Bendoricchio, 2001), the subsequent increase in N/P ratio (Degobbis et al., 2000) of the notoriously phosphorus-limited Northern Adriatic waters (Chiaudani and Vighi, 1982; Maestrini et al., 1997; Pojed and Kveder, 1977) and later a reduction in the values of ammonia and phosphate (Solidoro et al., 2009). These observations could be a result of both climatic factors and anthropogenic pressures (*i.e.*, cultural oligotrophication) as a consequence of both reduced outflows from rivers (higher salinity in coastal areas), a more sustained ingression of Levantine water (higher salinity in open waters) and a clear reduction in the concentration of phosphate and ammonia in coastal areas (Solidoro et al., 2009). The Adriatic Sea is characterized by a very strong outflow of fresh river water concentrated along the north-western Italian corner. Among all, Po River is the most important and one of the major driving forces of the near-coastal currents as well as of the circulation in the whole northern shallow basin. Therefore, in the shallow northern Adriatic Sea, algal blooms and discoloured waters are not exceptional Cabrini et al., 1992) due to an enhanced terrigenous input of inorganic and organic nutrients, and essential metals (Degobbis and Gilmartin, 1990).

Finally, the northern Adriatic Sea is densely urbanized and polluted (Lotze et al., 2006; Cozzi et al., 2014), and the areas around the Po River, the Venice Lagoon and the Gulf of Trieste bear the highest pressure (Solis-Weiss et al., 2007; Raccanelli et al., 2009). Indeed, it is considered a sensitive ecosystem mainly due to its intensive fisheries, industry and tourism.

The western coast of the Northern Adriatic, on the other hand, is low and sandy and has numerous rivers flowing into it (Tagliamento, Livenza, Piave, Sile, Brenta, Adige and Po) which are responsible for more than 20% of the fresh water supplies throughout the Mediterranean (Cozzi and Giani, 2011; Ludwig et al., 2009). From a geological point of view, the seabed of the area of the central western portion of the North Adriatic was an alluvial paleo valley. The current bottoms of the western coast of the Northern Adriatic are sedimentary, mainly muddy or sandy-muddy, in variable proportion based on local hydrodynamics, depth, distance from the coast and the influence of coastal terrigenous inputs of fluvial origin, and with a long and narrow strip of sand extending for about five miles off the western coasts, locally interrupted by the muddy sediments of lagoons and prodelta (Brambati et al., 1983). Two main currents dominate the area, sustaining a general cyclonic circulation. Riverine input generates the intense Western Adriatic Current (WAC), which flows south-eastward along the Italian coast, whereas the compensating Eastern Adriatic Current (EAC), which is saltier and warmer, flows north-westward along the Croatian coast (Cushman-Roisin et al., 2001). As for the hydroclimate, the Adriatic shows a strong seasonal thermal gradient, especially in the northern part, where the surface / volume ratio of the water is very high and the supply of relatively cold fresh waters (due to the presence of short distance on the plain) is high (ARPAV, 2010). Associated to water column stratification, hypoxia events frequently occurred in the bottom layer of the northern Adriatic Sea (NAd) from mid-summer to mid-autumn, with highly variable spatial extent and duration (Djakovac et al., 2015). More general biogeochemical characteristics of the area were described in the work by Solidoro et al. (2009), who analyzed a large set of oceanographic data concerning the state and scales of variability for temperature, salinity, nutrients, dissolved oxygen, and chlorophyll-a. Changes in the marine ecosystems of the northern Adriatic Sea, including changes in phytoplankton and zooplankton communities composition occurred over the last decades as a consequence of the reduced loads of nutrients from the rivers were reviewed by Giani et al. (2012), who also remarked that macrozoobenthic communities slowly recovered in the last two decades after the anoxia events of the 1970s and 1980s.

Chapter 3 Description of Pilot areas

3.1. P1 - Grado and Marano Lagoon and Gulf of Trieste (IT)

ARPA FVG and UNIBO are in charge of the pilot area P1. P1 includes both a coastal marine area and transitional waters represented, respectively, by the Gulf of Trieste and the Marano and Grado Lagoon. In these two regions, different activities will be performed in order to reach the mentioned project aims.

The Gulf of Trieste

The Gulf of Trieste is an epicontinental semi-enclosed shelf basin situated in the northern part of the Adriatic Sea and extended from Cape Savudrija (Croatia) to Grado (Italy).

It covers an area of approximately 500 km2 with a maximum water depth of 25 m (average depth 17 m) and it is characterized by a very low bathymetric gradient. Despite its limited size (about 20 km x 20 km; average depth 17 m; maximum depth 26 m), it plays an important role in the hydrographic properties of the Adriatic Sea (Cardin and Celio, 1997). The tidal range is approximately 90 cm while in the rest of the Mediterranean area the mean tidal amplitude is about 20-30 cm.

Seasonal variations of water temperature range from 8 to 24°C at the surface and from 8 to 20°C in the bottom layer. The salinity is typically marine, ranging between 33 and 38.5 (Ogorelec et al., 1991). The water circulation is mainly driven by the interplay of various forcing factors: the general circulation of the Adriatic Sea, winds (particularly the dominant Bora, E-NE direction), strong freshwater inputs and buoyancy fluxes (Salvi et al., 2020). Generally, the water enters the gulf in the southeast and continues to the northwest, following the general cyclonic circulation pattern of the Adriatic Sea but it can be variable, and even opposite currents can sometimes be observed in the upper and deeper layers modifying the vertical structure of the water column. The circulation, indeed, can be subdivided in three layers, the surface layer (0–5 m deep), which is mainly wind driven, and the intermediate (5–10 m deep) and the lower (10 m bottom) layers which follow a general cyclonic circulation (Stravisi, 1983). Residual currents are in the range 1–3 cm/s, but total currents as high as 30 cm s^{-1} are found in the upper layer, because of the effects of tides and wind stress (Mosetti and Purga, 1990).

As observed for hydrology and biogeochemistry, also the productivity is mostly influenced by river loads and wastewater discharges. The dynamics of nutrients, organic matter and production processes is determined in this area by riverine inputs (Malej and Malačič, 1995), emission from WWTP (Mozetič et al., 2008), atmospheric deposition and benthic fluxes. The trophic state of the Gulf of Trieste generally ranges from mesotrophic to oligotrophic conditions, with episodic eutrophication events: these phenomena occur jointly with the typical summer thermal stratification that also contribute to increase the nitrogen and phosphorous and organic carbon and organic nitrogen loads in the Gulf (Mozetič et al., 2008),). Indeed, while in winter the Gulf of Trieste is characterized by considerable vertical homogeneity, during the rest of the year the water column is well stratified. During persistent periods of weak circulation, the Gulf of Trieste has been affected by hypoxia, anoxia and mucilage “phenomena” and dinoflagellate and jellyfish blooms (Turka et al., 2007). Moreover, the freshwater inputs and the variability of the circulation pattern, which influence the distribution of the nutrients, have a significant impact also on plankton community structure and their productivity, but also on events of hypoxia/anoxia (Malej and Malačič, 1995), which have characterized the area in the past.

The rocky substratum of the northern coast of the Gulf of Trieste consists mainly of limestone, while the southern part is composed of flysch layers with soft marl and solid sandstone. Although the eulittoral zone is mainly composed of allochthonous substrata, the seabed in the upper-infralittoral belt is mostly a flat rock formation covered with a variable density of pebbles or decimetric rocks (sensu Ballesteros et al., 2007; Orlando-Bonaca et al, 2013).

The marine environment is affected to anthropogenic pressures such as maritime traffic, fishery, aquaculture, tourism and presence of several urban and industrial settlements along the coast, which include the largest cities of Trieste and Koper. In this context, anthropogenic loads and biogeochemical role of urea in the Gulf of Trieste have been studied (Cozzi et al., 1997). The only province of Trieste has a very high density of inhabitants (1090 inh. km^2), similar to those of other Italian metropolises, and a massive urbanization (82%) of the coastal belt included in the territory of the city (D'Ascola, 2013) which are both important factors impacting the neighboring marine environment. The coastal morphology of the Italian sector of the Gulf of Trieste has been heavily modified by human activity, and these modifications become more evident while approaching the city of Trieste (Brambati and Catani, 1988). In detail, from Miramare to Punta Sottile the coastal urbanization, tourism, the presence of important harbors (Trieste and Monfalcone) and other human pressures generated pollution (i.e., aromatic hydrocarbon, tributyltin and heavy metals) and the original littoral morphology is not easily found. Overall, these changes caused further stress on the sensitive local communities. The habitats, indeed, are strongly modified by the wide tidal range and the unceasing changes of the coastal morphology, which now appears completely modified compared to the past. Thus, the Gulf of Trieste is extremely sensitive to ecological changes (Battelli and Dolenc-Orbanić, 2009) and impoverishes its conservation status.

Vertical facies

The layers chosen for this study include the rocky-mesolittoral (or intertidal) and the underlying upper-infralittoral (or submerged) zone. Thus, studies on this biocenosis had been carried on during the 1960s but subsequently abandoned and now they are indeed still mostly unknown and undiscovered.

The midlittoral, eulittoral or intertidal zone includes the area included between the maximum of high tide and minimum of low tide lines (exposed to the air at low tide and underwater at high tide). Temperature is one of the most important abiotic determinants of organismal distribution and physiological performance in the rocky intertidal zone (Orton, 1929). Intertidal organisms are continuously exposed to rapid fluctuations of temperatures, light, salinity and water availability both in space and in time and are adapted to an environment of harsh extremes: recent studies have shown that exposure to high temperatures can have significant physiological consequences to these organisms (Hofmann and Somero, 1995) which can have significant ecological consequences. Competitive dominance of one species of barnacle over another varies with substratum angle (Wethey, 1984) and high, presumably as an indirect effect of thermal or desiccation stresses on the relative physiological performance of each species. This competition helps to identify the upper and the lower midlittoral, which is also often characterized by the typical blue mussel belt where mussels prevails on any other organisms.

The infralittoral zone is part of the sublittorral area, it starts immediately below the eulittoral zone and it is permanently covered with seawater (except during extremely low tides). Infralittoral rock zone includes habitats of bedrocks, gravels and cobblestones and typically supports erect seaweed high-biodiversity communities, since environmental conditions are less extreme than in the mediolittoral zone. As exeption, some turbid-water areas may be dominated by animal communities. It can be further subdivided into the upper and lower infralittoral (Hiscock, 1985).

Marano and Grado Lagoon

The Marano and Grado Lagoon (Figure 2) covers approximately 160 km^2 between the Tagliamento and Isonzo River deltas and is formally subdivided in six basins: Lignano, Sant'Andrea, Buso, Morgo, Grado and Primero. The western sector of the lagoon has few areas above the sea level and several channels connecting the plain spring rivers flowing into its internal edge towards the sea, whereas the eastern sector is shallow (<1 m, on average) and has a complex network of tidal flats, tidal channels and subtidal zones. The connection with the open sea occurs through several inlets, the most important being: Grado (390 m wide), Porto Buso (430 m) and Lignano (310 m) (Mancero-Mosquera I, 2013). Tides are semi-diurnal, with a mean range of 0.65 m and spring and neap ranges of 1.05 m and 0.22 m, respectively (Dorigo L., 1965).

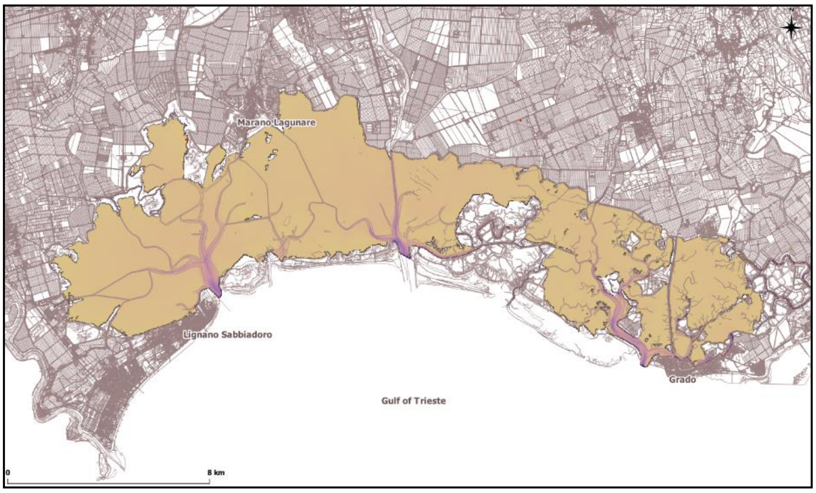


Figure 2. Marano and Grado Lagoon, the batimetry map realized by FVG Region, highlights the main canals.

Several rivers and a complex system of drainage pumps are responsible for the freshwater inputs and the Stella River (western sector) is the main contributor. However, some areas, especially in the inner part of the lagoon, suffer for scarce water renewal (Ferrarin et al., 2010). Tide and wind represent the main forcing factors for water circulation and renewal. Tide generates an important daily salinity gradient (more than 10 psu) in the western part of the lagoon and close to the mouths of the Aussa-Corno and Natissa. The Lignano, Buso and Grado inlets are responsible for about 35, 30 and 22% of the total water discharge between the lagoon and the sea. When the wind action occurs, the lagoon circulation changes radically (especially in case of E-NE Bora) and the tidal exerts a minor role.

Since 1971, the system is protected by the Ramsar Convention and, following the implementation of the Habitats Directive (92/43/EC), it was also identified in the state-sponsored ‘’Natura 2000’’ survey as a Site of Community Importance (SCIs – IT3320037). The lagoon hosts economic, touristic and industrial activities, thus experienced some remarkable impacts. For example, the Torviscosa industrial site was responsible for heavy contamination of soil, sediment and ground waters and part of the lagoon has been declared a “polluted site of national interest” (SIN; Ministerial Decree 468/01) (Ramieri et al., 2011). Contaminants such as potentially toxic elements (Hg) and polycyclic aromatic hydrocarbons (PAH) commonly exceed the set threshold guidelines in sediments. In addition, large amounts of anthropogenic nutrients, mainly in form of nitrogen, are released from inland due to intense agricultural activities and urban wastewaters and, in the past, abnormal growth of nitrophilous macroalgae and extensive red microplanktonic algae were observed, often leading to dystrophic crisis.

Marano and Grado Lagoon has been included in the pilot area P1 due to the typical characteristics of this environment. First, the remarkable biomasses and species richness need to be considered and preserved. Secondly, the lagoon provides ecological services significant to all the Gulf and Northern Adriatic that have to be monitored in order to protect this fragile transitional ecosystem.

3.2. P2 - Sacca di Goro (IT)

The Sacca di Goro is a shallow-water lagoon of the southern Po River Delta approximately triangular in shape with a surface area of 26 km^2, an average depth of 1.5 m, and it is connected to the sea by a large sea mouth. The lagoon at north is surrounded by embankments with exception of the west-southern border. The main freshwater inputs are the Po di Volano River (approximately 350.106 m^3 /yr), the Canal Bianco and Giralda, which have approximately the same discharge rates (20-55 x 106 m^3 /yr). Freshwater inlets are also located along the Po di Goro River and are regulated by sluices plus a large channel with unregulated input close to the lighthouse. There are no direct estimates of the freshwater input from the Po di Goro, which is usually assumed to be equivalent to that of the Po di Volano. The fresh water or hydraulic residence time oscillates monthly between 2.5 and 122 days with a mean value of 24.5 days, whereas the water exchange time ranges from 2 to 4 days. The tidal amplitude is ca 80 cm. The bottom of the lagoon is flat and the sediment is alluvial mud with high clay and silt content in the northern and central zones. Sand is more abundant near the southern shoreline, whilst sandy mud occurs in the eastern area. The climate of the region is Mediterranean with some continental influence (wet Mediterranean).

In terms of hydrodynamics, Sacca di Goro is strongly influenced by the semi-diurnal tides following what is observed in the Northern Adriatic. An important variation in terms of flood and ebb currents reflects the monthly differences in tidal amplitudes (between syzygy and quadrature) that can reach up to 80 cm. Moreover, the fresh water or hydraulic residence time oscillates monthly between 2.5

and 122 days with a mean value of 24.5 days, whereas the water exchange time ranges from 2 to 4 days. It is important to stress that besides the strong fluvial and the tidal influences, wind also plays a major role altering the lagoon's circulation pattern. While Sirocco (southerly winds) tends to fill the lagoon up, Bora conditions (NE winds) normally increase the discharge.

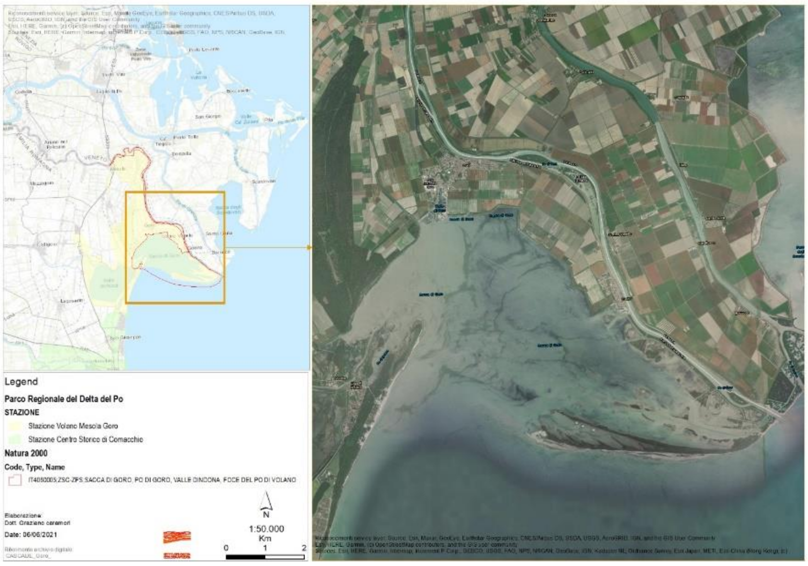


Figure 3. Satellite view of Sacca di Goro, Ferrara, Emilia-Romagna.

The lagoon of Sacca di Goro is a Natura 2000 site both SPA and SAC (code IT4060005) furthermore is inside the Parco del Delta del Po of Emilia-Romagna Region, the eastern area named Valle di Gorino is a Ramsar site of about 1,500 ha and the outer bank is Natural State Reserve.

From an environmental point of view the lagoon represent a typical coastal lagoon left by the great reclamation carried out in the last 150 years. The ecological peculiarities of this environment allow the establishment and presence of important plant and animal communities. In the area of the Valle di Gorino and the border between the Sacca and the Po di Goro, the most widespread marsh formation is the common reed *Phragmites australis*. In the deeper areas of the Sacca, there is a submerged vegetation dominated by algae. The submerged vegetation is poor in species, but it reaches enormous amounts of biomass, and in these communities a large number of planktonic and benthic animals live. In more sheltered waters, there were once submerged seagrass meadows (*Ruppia cirrhosa*) disappeared as in most of the delta. In the area between Goro and Gorino, near the embankment of the former Valle Vallazza, *Gracilaria verrucosa* (red algae) is common. The outer bank delimiting the border with open sea is in continuous morphological evolution, due to the contribution of sediments transported by the Po river. The outer bank tends to gradually extend towards west, consequently reducing exchanges with the open sea. The mixture of freshwater and seawater are somehow balanced and the Sacca di Goro is a typical brackish lagoon of the category transitional water. This feature allows an uncommon biodiversity richness in terms of habitats and species.

3.3. P2 - Lago delle Nazioni (IT)

Lago delle Nazioni (Ferrara, Emilia-Romagna), is a semi-artificial brackish lake located between Valle Nuova, the state-owned pine forest and the beaches of Volano and Lido delle Nazioni (Figure 4). It has an area of about 100 hectares to which, in order to delimit the exact naturalistic sector, approximately 70 hectares of the contiguous wild breeding of bulls and Camargue-Delta horses must be added. It is the biggest lake of the Ferrara district. The valley, which originated from repeated inflow episodes of marine waters, has changed shape several times following the growth of the coast, and was in direct contact with the sea until a few decades ago through “Bocca del Bianco”. Currently, the water exchange is ensured by a channel regulated by a siphon and a dewatering pump connected to the terminal section of the mouth of the Po di Volano. The external part of the lake is dominated by common reed, meanwhile the western site is surrounded by agricultural crops and mainly halophilic and halotolerant vegetation. In areas with almost desalted sandy soil, occasionally flooded by stagnant waters, a rare phytocoenosis is established, also interesting for the presence of orchids. During summer, the lake is extensively used for tourism thanks to the presence of the village hotel at the Northern shore and sailing and rowing sports; indeed, in the Southern area there is the sailing and kayaking school Lega Navale Italiana. Of recent establishment, there is the experimental breeding of clam made by the company Naturedulis s.r.l, whose headquarter is based in Goro, Ferrara.

Figure 4. Satellite view of Lago delle Nazioni, Ferrara, Emilia-Romagna.

3.4. P2 - The Pialassa Baiona lagoon (IT)

North of the Ravenna canal port, there is a brackish coastal lagoon, the Pialassa Baiona, covering an area of about 10 km^2. Discontinuous artificial embankments (

Figure 5) further divide the Pialassa Baiona into several shallow water ponds (average depth 1 m), connected to each other and to the sea by channels (depth ranging from 1 m, in the inner zones, to 8 m towards the sea). This arrangement of embankments, canals and ponds was initially created to promote the water circulation and the passage of small boats (Airoldi et al., 2016).

The Pialassa Baiona lagoon receives water inputs from five main channels that drain a watershed of 264 km^2, including urban (9%) and agricultural (87%) areas. The southern channel collects also the wastewater coming from urban and industrial sewage treatment plants and from two thermal power plants. Nutrient inputs are particularly high in the southern areas (Ponti et al., 2005). Complete water turnover in the lagoon has been estimated to take on average 3 days. The mean salinity is about 28 psu and varies from 0 psu, in areas close to the pinewood forests, to 37 psu in areas with low water exchanges during the summer. Salinity of the seawater in nearby coastal areas is low (mean value 30 psu), due to the fresh water input form the Po River (Ponti et al., 2005.).

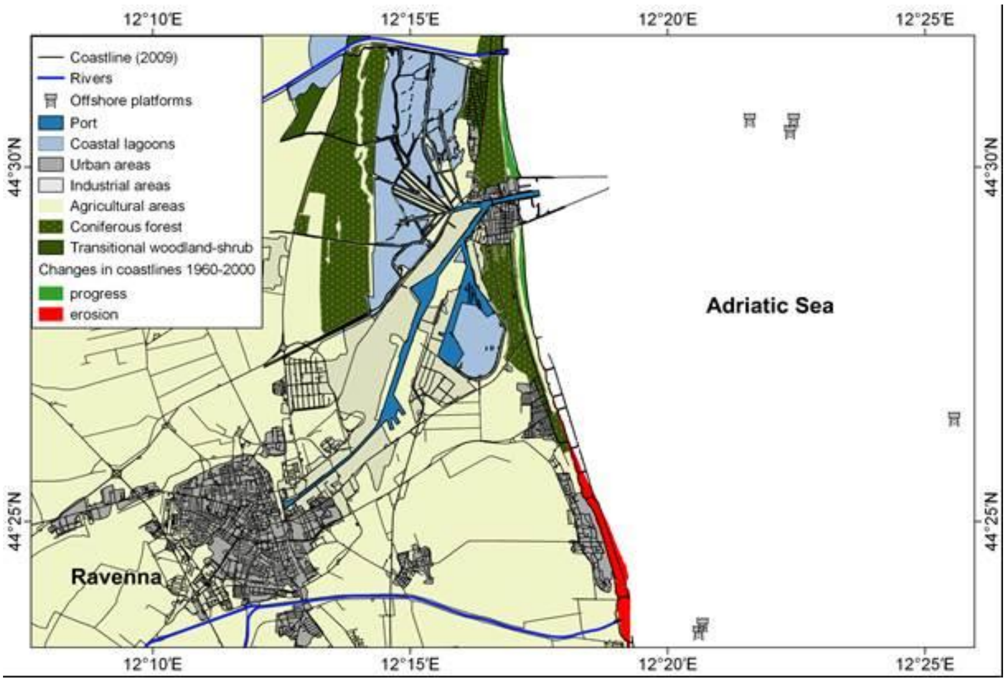


Figure 5. Port of Ravenna and surrounding areas. Land cover modified from CORINE, 2006; coastal erosion data from the national geoportal of the Italian Environmental Ministry; road and hydrographic networks from Emilia-Romagna Region geoportal; offshore platforms from the nautical map of the Hydrographic Institute (Mercator projection, datum WGS84).

In the Pialassa Baiona, bottoms are prevailingly sedimentary, ranging from mud to fine sand. The lagoon, ponds, and channels as well as a variety of man-made structures provide a variety of different habitats. Macrobenthic invertebrates are the best-studied component (Ponti & Abbiati, 2004; Ponti et al., 2011), including spionid and capitellid polychaetes, tubificid oligochaetes, amphipods, bivalves and gastropods. Species diversity is relatively high, even if hypoxic events occurring in the summer lead to a transitory reduction in the number of species, especially amphipods (Ponti & Abbiati, 2004). Saltmarshes are colonised by halophile vegetation of *Salicornia* spp., *Arthrocnemum* spp., and *Limonium* spp. There are reedbeds of *Phragmites australis*, marginal areas with *Juncus maritimus* and *J. acutus* wet meadows (Corbetta, 1990; Corticelli et al., 1999). A pinewood stretches all along the western edge of the lagoon. Submerged vegetation is far less studied (Ponti & Airoldi, 2009). The only seagrass species that have been reported to occur in the lagoon belong to the genus *Ruppia*. Blooms of phytoplankton and seaweeds (*Ulva* spp., *Gracilaria* spp.) were frequently observed, sometimes leading to dystrophic crises during the summer (Ponti & Abbiati, 2004), but virtually no information is available on their distribution, biology or ecology (Ponti & Airoldi, 2009).

Ravenna harbour and the connected coastal lagoons (Pialassa Baiona and Pialassa Piomboni) receive several civil and industrial wastewaters carrying nutrients, different types of pollutants and cooling water from a power station and several industrial plants. Although nowadays discharges comply with the current laws, their accumulation still raises concerns, especially in confined areas of the lagoons. Further, legal constraints in the past were more permissive, or even absent, and during 1958 to 1976, Pialassa Baiona was heavily impacted by industrial pollution that has led to persistent accumulation in the sediment of numerous pollutants. The pollution gradient decreases from south to north, in relation to the distance from the southern industrial area (Trombini et al., 2003).

By applying the single-box/single-layer LOICZ model, the annual nutrients budget of the Pialassa Baiona was calculated in 2000 (Ponti et al., 2005). Water turnover time in the lagoon was estimated at 3 days. Annual mean variation of both dissolved inorganic phosphorus (ΔDIP) and nitrogen (ΔDIN) were negative, indicating that the system acts as sink of both DIP and DIN. Stoichiometric calculations assumed nutrient rations in both Redfield proportions typical for phytoplankton (C : N : P = 106 : 16 : 1) and in proportions appropriate for macroalgae (C:N:P=335:35:1). The latter seemed more appropriate for the Pialassa Baiona, which was affected by seasonal blooms of macroalgae. Overall, the lagoon was considered as “autotrophic”, with a net ecosystem metabolism (production-respiration) varying from 1.0 to 3.0 mmol C $m^{-2}d^{-1}$ considering Redfield or macroalgal ratios, respectively. Denitrification dominated over nitrogen fixation, considering both phytoplankton and macroalgae nutrient ratios. Since 2000, part of the wastewaters has been diverted from the lagoon towards the harbour channel, reducing the pollution load, especially at low tide.

The Pialassa Baiona lagoon provides a wide range of habitats and conditions that over time have facilitated the establishment of populations of non-native species. The Manila clam (*Ruditapes philippinarum*) represents one of the most successful examples. Its introduction in the Venice lagoon, for experimental aquaculture, dates back to 1983 (Cesari & Pellizzato, 1985). It subsequently rapidly expanded outside the farming areas, and colonized most of the nearby coastal lagoons, replacing the native *Ruditapes decussatus* (Ambrogi, 2000) and likely facilitating the introductions of the invasive seaweed *Gracilaria vermiculophylla*. Today, the naturalized populations are commercially exploited wherever possible, including the Pialassa Baiona lagoon (Ponti et al., 2017). Other non-native species recently introduced in Pialassa Baiona include the Asian date mussel (*Arcuatula senhousia*) and the sphaeromatid isopod *Paracerceis sculpta*. The latter, native to the northeast Pacific, has been found for the first time Pialassa Baiona in 2012. Field data suggest that *P. sculpta* has become an invasive species, being more competitive than the native sphaeromatids (Vincenzi et al., 2013). Its initial spatial distribution, close to the lagoon-port connection, supports the hypothesis of maritime traffic as the main spreading vector. The enlargement of the Ravenna port in 2011, with a new cruise passenger terminal, and the increased connection with the Venice lagoon, where *P. sculpta* was initially found in 1981, may have facilitated this spreading. The latest record, probably from the Venice lagoon and by similar vectors, is the canopy forming alga *Sargassum muticum*, which was recorded for the first time between the port and the lagoon in spring 2015 (Andrea Desiderato, pers. com.).

3.5. P2 - Bevano river mouth (IT)

A few kilometres south of the city of Ravenna, there is the mouth of the Bevano river, one of the best-preserved transitional coastal areas of the Emilia-Romagna coast. Consisting of a complex mosaic of different habitats, all of particular naturalistic value, the Bevano river mouth presents a high environmental interest, because it is the last example of meandering estuary of the upper Adriatic and, to date, is protected by numerous landscape and naturalistic constraints: Ramsar Area, Site of Community Importance (SIC), Special Protection Area (SPA), Ravenna Coastal Dune Nature Reserve and part of the Po Delta Regional Park Station. The Bevano river originates at an altitude of 180 m above sea level, in the locality of Trebbo, in the hills of Bertinoro and, after a path of 34 km flows into the Adriatic between the localities of Lido di Dante and Lido di Classe (Montanari and Marasmi, 2013). The site includes about 5 km of coastal dunes, behind which are the state-owned pine forests of the Ramazzotti and Savio sections, planted starting in 1881. Almost all types of northern Adriatic halophilous vegetation are present in this area. The inner basins with shallower waters dry up during the summer, giving rise to mudflats where the annual halophilous communities typical of these environments settle. The terminal stretch of the river is characterized by a variety of sandy and muddy bottoms (Figure 6) that are home to natural Pacific oyster (*Magallana gigas*) beds which house a high marine biodiversity and protect the riverbanks (Figure 6). The seabed is also rich in estuarine shrimps (*Upogebia pusilla*) and Manila clams (*Ruditapes philippinarum*) (Abbiati et al., 2019).

The seabed in front of the mouth is sandy and home to rich benthic communities and natural populations of clams (*Chamelea gallina*), a species of high commercial importance. For this reason, it is believed that this coastal marine area would deserve strict protection, as already occurs in the land side (Figure 7) (Abbiati et al., 2019.).

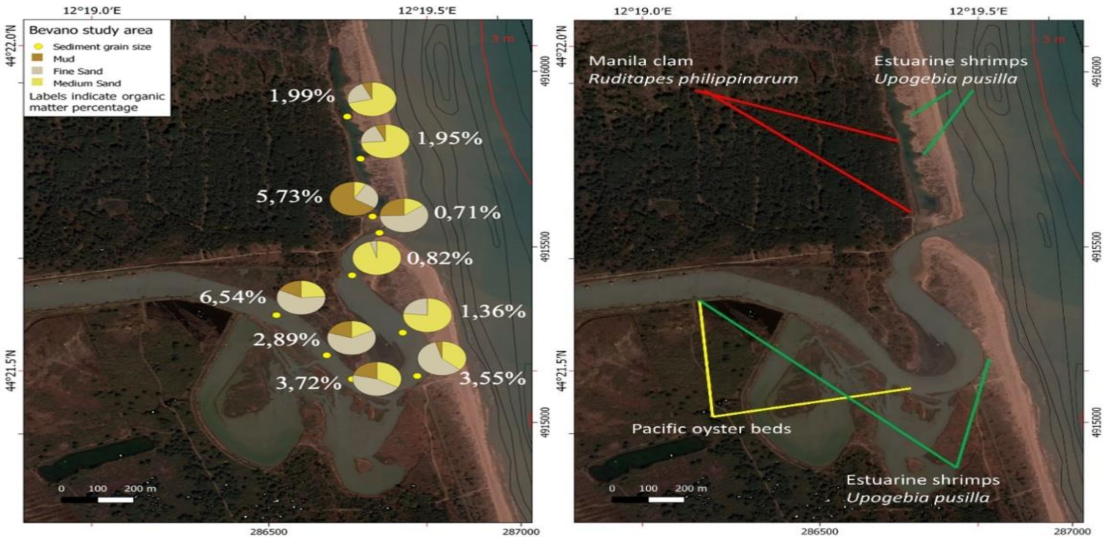


Figure 6. Bevano river mouth: on the left bottom sediment grain size and organic matter; on the right main benthic assemblages (UTM33 WGS84, base map Google Earth April 2, 2018).

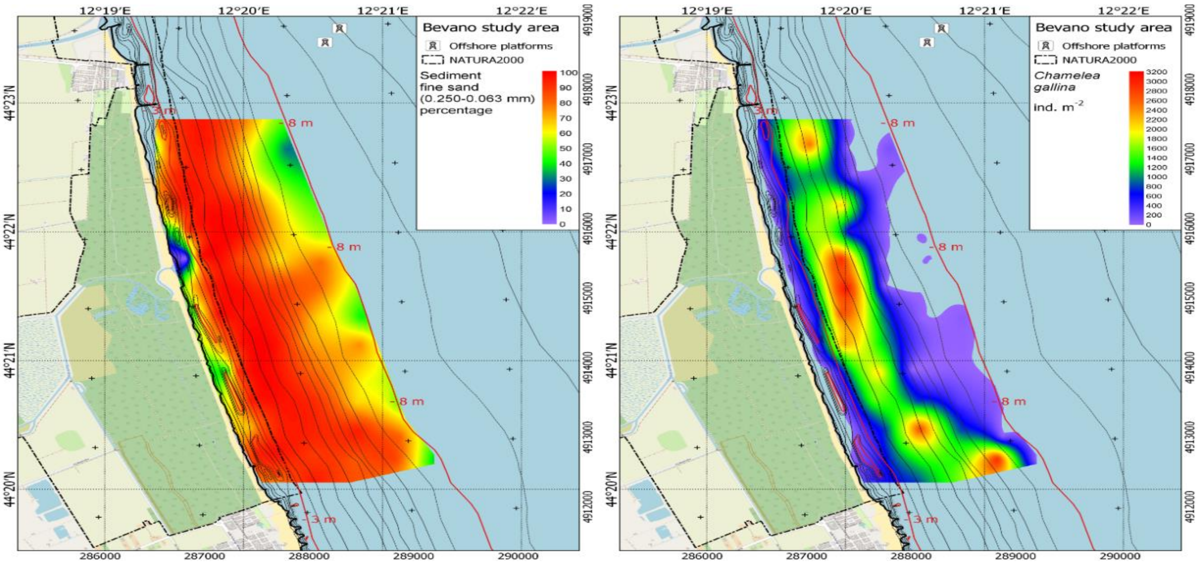


Figure 7. Bevano marine coastal area: on the left fine sand distribution; on the right the distribution of the clam Chamelea gallina (UTM33 WGS84, base map Google Maps).

3.6. P3 - Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT)

Marine Protected Area of Torre Guaceto - Canale Reale (BR)

The coastline is characterized, along the western sector, by a series of small subrectangular coves with pocket beaches. At East, the coast is mainly sandy, with reduced rocky formations and low rocks emerging right, characterized by a regular, sinuous coastline. The eastern coastal sector is incised by ten little valleys, some continuing underwater. The marine bottoms are always characterized by the presence of two submarine cliffs, running parallel to the coast line, with a medium slope, whose physiognomy is articulated and presents a convexity. Important organisms are present and Posidonia oceanica meadows are one of the most characteristic habitats of the sandy infralittoral.

Salina Punta della Contessa (BR)

In Salina Punta della Contessa area, next to the Salina Vecchia, there is the outlet of a drain, Foggia di Rau, that is dry during most part of the year. The pollutants, mainly run-off from agricultural operations and industrial discharge, can enter the aquatic environment as surface and groundwater and could also reach the sea following leaching through the dunes, in front of the saltwork.

Coastal marine area of Melendugno (LE)

In the area north of San Foca there is no important industrial activity, so run-off from agricultural operations represents the main source of pollutants. This area does not show any important water drain. In situ surveys showed the presence of different input of freshwater directly in the sea distributed along the shoreline. In this area there is the presence of TransAdriaticPipeline (TAP) and of a naturalistic important coralline reef.

3.6.1. Hydrodynamic oceanographic features of Apulia region

The three pilots described in previous section are here investigated in terms of oceanographic features, considering the circulation, temperature and salinity fields.

The dataset used in this section is the one provided by CMEMS (marine.copernicus.eu) in terms of physical reanalysis products for the Mediterranenan Sea (Escudier et al., 2020). The products are generated by a numerical system composed of a hydrodynamic model, supplied by the Nucleous for European Modelling of the Ocean (NEMO) and a variational data assimilation scheme (OceanVAR) for temperature and salinity vertical profiles and satellite Sea Level Anomaly along track data. The model horizontal grid resolution is 1/24˚ (ca. 4-5 km) and the unevenly spaced vertical levels are 141. The reference multi-year period ranges from 1987 to 2019. The reanalysis system is forced by hourly ECMWF ERA5 atmospheric forcing fields and assimilates reprocessed data. For the validation of the Mediterranean Sea Reanalysis system please refer to:

https://catalogue.marine.copernicus.eu/documents/QUID/CMEMS-MED-QUID-006-004.pdf.

In particular, here are shown the seasonal average maps of surface circulation (Figure 8), temperature (Figure 9) and salinity (Figure 10) for the Southern Adriatic Sea where the three Apulian pilots are located. The maps show (i) the role of the southward-oriented WACC (Western Adriatic Coastal Circulation) circulation along the coastal waters of Apulia region with highest intensity in Autumn period; (ii) the variability of temperature patterns when approaching to the Apulian coastline (well evident form Autumn to Spring); (iii) the fresher waters driven by the WACC affecting the overall coastal waters.

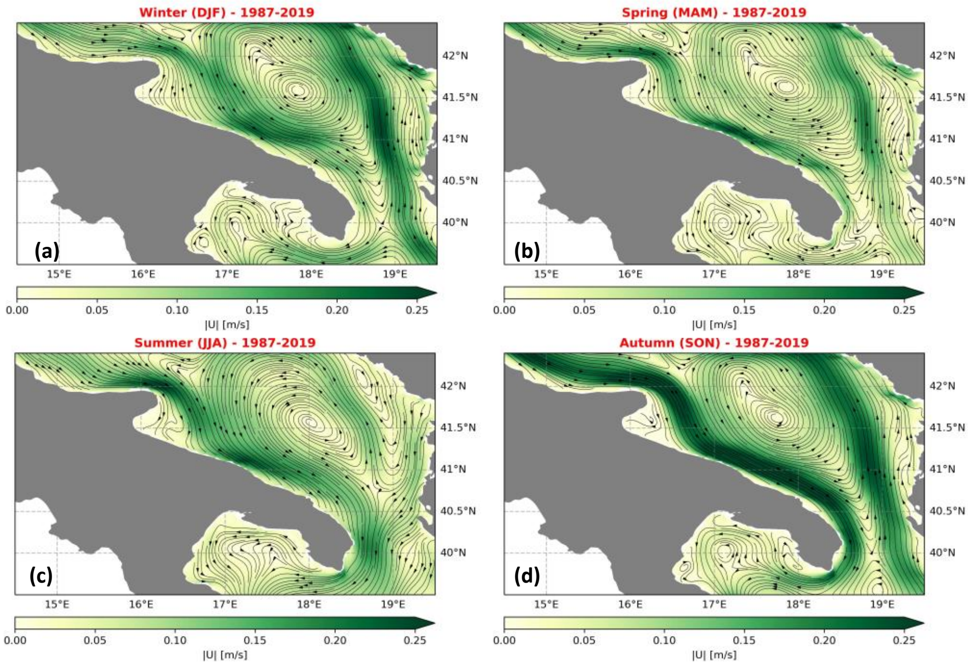


Figure 8. 1987-2019 seasonal average maps of surface circulation from CMEMS Mediterranean Sea Reanalysis Products. (a) Winter (December-January-February), (b) Spring (March-April-May), (c) Summer (June-July-August) and (d) Autumn (September-October-November) circulation.

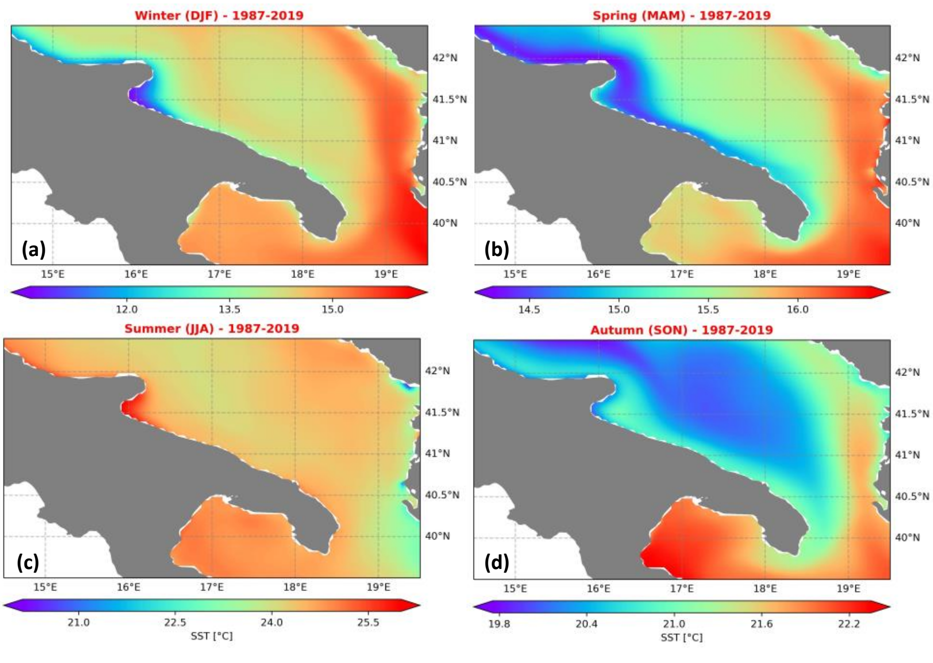


Figure 9. 1987-2019 seasonal average maps of surface temperature from CMEMS Mediterranean Sea Reanalysis Products. (a) Winter (December-January-February), (b) Spring (March-April-May), (c) Summer (June-July-August) and (d) Autumn (September-October-November) temperature.

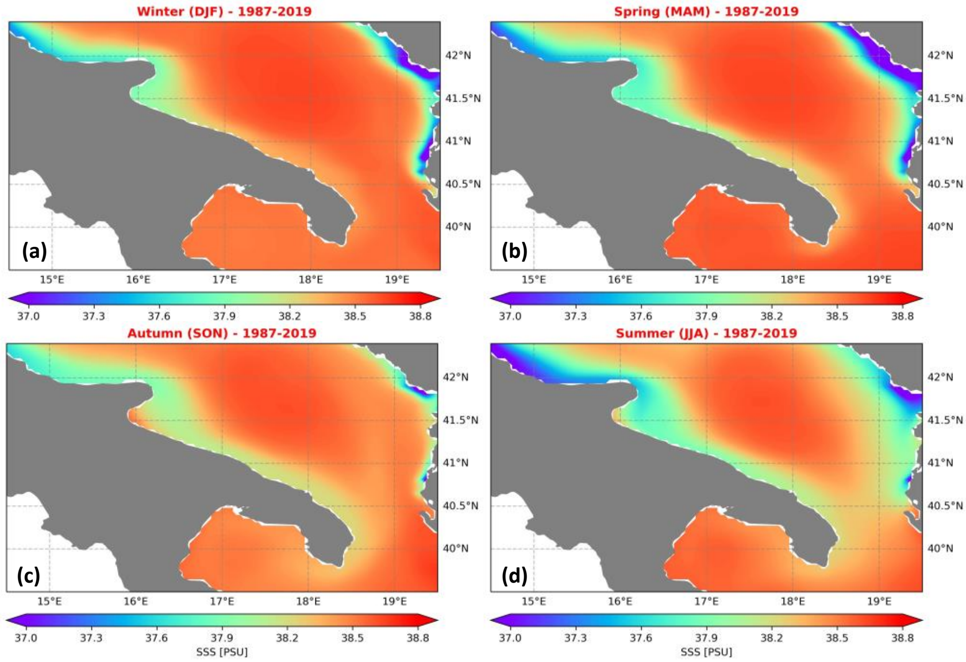


Figure 10. 1987-2019 seasonal average maps of surface salinity from CMEMS Mediterranean Sea Reanalysis Products. (a) Winter (December-January-February), (b) Spring (March-April-May), (c) Summer (June-July-August) and (d) Autumn (September-October-November) salinity.

3.7. P4 - Neretva River (HR)

Naron is the Roman name for Neretva, the largest river of the eastern part of the Adriatic basin which stretches for 225 km in Bosnia and Herzegovina and for the last 20 km in Croatia. It covers 20% of the total water basin of Bosnia and Herzegovina.

By its characteristics, Neretva is a typical mountain river with strong hydropower potential. This bioecological complex is divided between two countries: the delta-shaped river mouth, with the lakes of Modro oko, Desne, and Kuti belongs to Croatia, while the Nature Park of Hutovo blato belongs to Bosnia and Herzegovina.

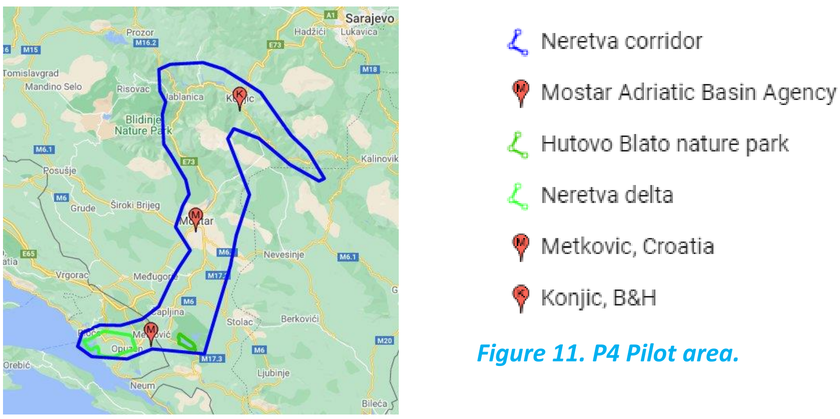


Figure 11. P4 Pilot area.

In the past, Neretva delta was well known because of the abundance and diversity of bird and fish fauna, as well as by the activities of hunting and fishing, partaken by almost all inhabitants of the region. Dense marshlands were overgrown with hydrophilic vegetation, which provided excellent conditions for fish spawning and bird nesting. This explains why this region was in the past a home to various species of herons, cormorants, ducks, and other water-birds, as well as colonies of the today regionally extinct species of Dalmatian pelicans.

Today, Neretva is the only river in this region with a delta at its mouth. The karst system slows the flows of the Neretva River significantly. Moreover, entire project area, especially its sensitive ecosystems of karst and wetlands. Sensitive karst system is endangered by different activities such as: negative human impact on freshwater ecosystems and global climate changes.

In many ways, the Neretva delta is different from other parts of coastal Croatia. Its unique landscape is a result of digging and depositing of the marsh soil. Furthermore, global climate changes have had and continue to have a strong influence on Neretva River and its flora and fauna. Constant oscillation of water levels, a lack of water during summer, increased water temperature, etc. have led to disruption in the reproductive cycle of shellfish and fish. Unsuccessful spawning and increased fry mortality due to the impacts of climate change have reduced overall recruitment.

In Croatia the Delta area is recognized as very significant for protection of nature and biodiversity, so that in last few years there are activities undertaken by Government to protect the Neretva delta and its population as a future Nature Park from further devastation.

3.8. P5 - Teqnue coralligenous (IT)

The marine area of the Tegnùe di Chioggia includes a very representative type of Mediterranean marine environment present in the Northern Adriatic Sea. It is characterized by calcareous bioconstructions formed over the last 3-4000 years and aggregates a great variety of species of flora and fauna, representing a unique hotspot of biodiversity in the context of the surrounding, sandy-silty bottom of the Northern Adriatic Sea. The Natura 2000 SCI is located at a distance between 3 and 8 NM from the coast and is divided in four areas (a bigger area of nearly 22 km^2 and three smaller areas of around 1,4 km^2 each) with a total extension of 26.55 km^2. The depth of the site varies between 18 and 23 meters.

The marine area of "Tegnùe di Chioggia" was proposed as SCI under the European Habitat Directive in 2010 and consequently declared as a Special Area of Conservation (SAC) in 2018. Previously, from the year 2002, it has been declared a Biological Protection Zone (BPZ) under the Italian Ministry of Agricultural, Food and Forestry Policies (Ministero delle politiche agricole, alimentari e forestali), with the aim of prohibiting any type of fishing activity.

Since its declaration as BPZ several projects and research studies have been implemented, focusing on the Tegnùe outcrops belonging to the SCI, and on other hard bottom outcrops located in the Gulf of Venice, in order to improve ecosystem knowledge and support the conservation and sustainable management of the area. Here we provide a list of some remarkable project focused in this area:

- 2001-2003 geomorphological survey of the seabed facing Chioggia. These studies were aimed at mapping all the rocky outcrops present within the BPZ and in the surrounding seabed.
- 2003-2005 Three-year project for the elaboration of an integrated management proposal of the Biological Protection Area of the Tegnùe di Chioggia and for a first management experimentation. Associazione Tegnùe di Chioggia. Project funded by the Veneto Region. Partners: CIRSA of the University of Bologna, ICRAM STS Chioggia, Hydrobiological Station of the University of Padua.
- Side Scan Sonar, bathymetric, ROV, video and photographic surveys and sampling of mobile and solid substrate as part of the" INTERREG III A / Phare CBC Italy - Slovenia Community Initiative Program;
- Le Tegnùe: study of some areas of particular environmental interest for the purpose of enhancing local fishery resources and naturalistic protection - VI National Three-year Plan for Fishing and Aquaculture (Law no.41 of 17.02.82). MEASURE 3 Research applied to fishing and aquaculture (studies and research on marine ecosystems);
- LEADER PLUS - Interventions for the protection and enhancement of a marine repopulation oasis called Tegnùa di Porto Falconera
- "Outcrops of Porto Falconeria, Malamocco, D'Ancona; Cavallino Lontana, Venice, Cavallino Vicina, Sorse and Chioggia ", A.R.P.A.V. - Upper Adriatic Observatory - Veneto Regional Center.
- 2006-2007 Tegnùe Integrated Project (P.IN.TE.) for the integrated study on the ecology of the organogenic rock outcrops (tegnùe) present in the stretch of coast in front of the Chioggia coast. Lead by CNR-ISMAR Venice, University of Padua, ICRAM of Chioggia, CNR-ISMAR of Venice, sponsored by the "Tegnùe di Chioggia" non-profit association.
- qualitative and quantitative analysis of fish fauna. University of Padova 2005
- 2006-2007 "Monitoring and management of the biological protection area of the Tegnùe di Chioggia" Extension of the three-year project for the elaboration of an integrated management proposal of the biological protection area of the Tegnùe of Chioggia and for a first management experimentation. Tegnùe Association of Chioggia. Project funded by the Veneto Region. Partners: CIRSA of the University of Bologna, ICRAM STS Chioggia, Hydrobiological Station of the University of Padua.
- 2005-2008 Research program "Evaluation of the effects of the Biological Protection Area of Chioggia on demersal and benthic populations and on the possibility of repopulation of species of commercial interest", Central Institute for Scientific and Technological Research Applied to the Sea (ICRAM) and Interdepartmental Research Center for Environmental Sciences (CIRSA) of the University of Bologna, funded by the Ministry of Agricultural and

Forestry Policies, Department of Market Policies, Directorate General for Fisheries and Aquaculture (MiPAF, 2003).

- 2019 – 2021. ECOlogical observing System in the Adriatic Sea: oceanographic observations for biodiversity (ECOSS) Project with the overall objective to establish the ECOlogical observing system in the Adriatic Sea (ECOAdS), shared between Italy and Croatia, able to integrate ecological and oceanographic research and monitoring with Natura 2000 conservation strategies. Coordinated among the National Research Council, Institute of Marine Sciences – CNR ISMAR – Lead Partner (Venice and Bologna), National Institute of Oceanography and Experimental Geophysics – OGS (Trieste), Regional Agency for Prevention, Environment and Energy in Emilia Romagna (Bologna), Institute of Oceanography and Fisheries (Split), Po Delta Veneto Regional Park (Rovigo), Blue World Institute of Marine Research and Conservation (Lošinj), Public Institution for the Management of Protected Natural Areas of Dubrovnik Neretva County (Dubrovnik), Public Institution for the Management of Protected Areas in the County of Split and Dalmatia "Sea and Karst (Split), Shoreline (Trieste), Department Of Environmental Sciences, Informatics And Statistics, Ca Foscari University Of Venice (Venezia).
- LIFE - GHOST project. 2013-2016 GHOST project, co-funded by the LIFE + Biodiversity instrument of the European Union, to promote concrete measures to preserve and improve the ecological status of rocky habitats (Tegnùe) in the northern Adriatic Sea. Developed in partnership by the National Research Council - Institute of Marine Sciences (CNR/ISMAR), University IUAV of Venice and Laguna Project s.n.c.
- Research in the sector of benthic populations coordinated by the Interdepartmental Research Center for Environmental Sciences of the University of Bologna, together with ISPRA STS Chioggia - Chioggia Hydrobiological Station, University of Padua, ISMAR Venice, CNR, CIRSA Ravenna - University of Bologna.
- Evaluation of the impact of fishing on the benthic and demersal populations of the Tegnùe of Chioggia. Research in this sector is coordinated and developed by the Central Institute for Scientific and Technological Research Applied to the Sea (ICRAM) of Chioggia.

3.8.1. The tegnùe of the Adriatic Sea

In a very large area of the north-western Adriatic, between 15 and 40 meters deep from the Isonzo mouth to the northern area of the mouth of the Po (Caressa et al., 2002), there are rock formations known in the Veneto with the name of tegnùe (term with which the fishermen of Chioggia indicate a grab that holds and / or damages the fishing nets (ARPAV, 2010.), trezze and grebeni in Friuli Venezia Giulia, scagni along the Istrian coast. With extensions ranging from a few to several hundred square meters (Mizzan, 1994.), elevations up to 2-3 meters from the seabed and very diversified morphologies (some outcrops are characterized by large horizontal surfaces, whereas others are composed of scattered conglomerates of small rocks (Falace et al., 2015.), they represent the main natural hard substrates in the Adriatic.

These are bio-concretioned rocky calcareous build-ups formed in the last 3-4000 years, often superimposed on older substrates, which host a rich and diverse flora and fauna (ISPRA, 2010.); their origin appears complex and, in some cases, can be traced back to an initial carbonate cementation of sandy sediments mixed with shells and exoskeletons of echinoderms and crustaceans which constitute the more or less thick base layer (Ponti e Mastrototaro, 2006; Bertasi, 2007.).

The presence of these natural rocky outcrops surrounded by sandy/muddy sediments of detrital type, of varied and complex morphology, together with the naturally eutrophic and not excessively deep waters, locally creates areas rich in micro-environments and ecological gradients that favour an increase in the specific diversity of both benthic and pelagic populations (Ponti et al., 2011; Falace et al., 2015). The Tegnùe are in fact “biotopes of exceptional ecological value, for environmental and fishery purposes, as well as for natural repopulation, reproduction, nursery and refuge for numerous valuable fish species” (ARPAV, 2010; Melli et al., 2017).

The rocky outcrops located along the coast of the Veneto Region, in particular from the mouth of the river Brenta to the city of Grado, are unevenly distributed, but seem to be positioned along parallel bands at 3–5 miles, 10-12 miles and 20 miles off the coast, at depths ranging from 8 to 40m (Mizzan, 1994). The dimensions can be very different, ranging from a few square meters to several thousand square meters in the larger ones, with elevations from the seabed ranging from a few decimetres in the low and flat formations, sometimes called "lastrure", to a few meters in the higher ones, often located at greater depth (ARPAV, 2010). The most extensive, highest from the bottom and best known Tegnùe are present between 3 and 5 nautical miles in front of Chioggia (Figure 12): here the rocks extend seamlessly for hundreds of meters, taking on complex shapes often meandering which recall the course of deltaic mouths and natural lagoon channels. (ISPRA, 2010.)

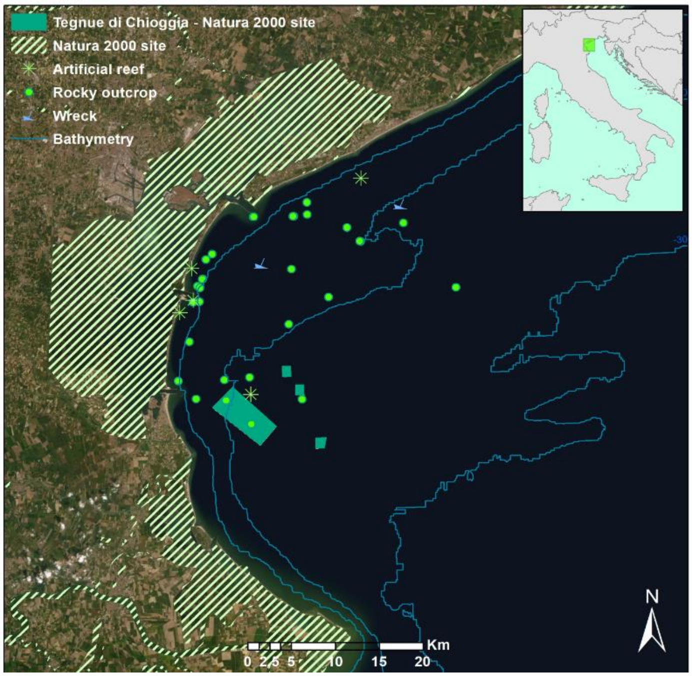


Figure 12. Overview of the subtidal rocky outcrops and hard structures surrounding the Tegnue di Chioggia area (Modified from Nesto et al. 2020).

3.8.2. Geology and Morphology

From the granulometric point of view, the analyses (ARPAV, 2010) show that the incoherent bottoms surrounding the outcrops in the areas facing the Venetian coast are generally characterized by sandy sediments with very variable percentages of fine fraction (<62.5 µm). In particular, based on the average percentage of sand the areas surrounding the Tegnùe can be divided into four types of inconsistent substrate:

- 100-75% - substrate consisting mainly of medium-fine, slightly silty sand;
- 75-50% - substrate consisting mainly of silty or silty sand;
- 25-50% - substrate consisting on average of fine sand with silt and clay;
- 25-0% - substrate consisting mainly of clayey silt or clay.

The spatial distribution of the four groups of incoherent substrates thus assorted shows a progressive decrease in the percentage of sand proceeding from the open sea towards the coast, confirming what is already known in the literature about the general granulometric characteristics of the coastal sediments of the North Adriatic (ARPAV, 2010.).

From a lithological point of view, the tegnùe were made up of carbonate rocks of sedimentary or organogenic origin. By definition, carbonate rocks are composed of at least 50% of carbonate minerals (calcite, aragonite, dolomite) which can have different origins (detrital, organogenic and chemical) (ARPAV, 2010.).

The mineralogical analysis confirms the carbonate constitution of the hard substrate and highlight two groups of tegnùe characterized by different mineralogical phases:

1. group where dolomite predominates, an indication of a detrital origin of the rock. This mineral, in fact, characterizes the sediments transported to the North Adriatic by the Veneto-Friuli rivers (especially Piave and Tagliamento) in recent times and by currents;
2. group where calcite rich in magnesium and aragonite are the main mineral constituents. Aragonite and calcite rich in magnesium reflect the presence in the rock of fragments of shells of molluscs or structures generated by building organisms. (ARPAV, 2010.)

The results of the chemical analysis of the major elements reflect and confirm this mineralogical composition; the groups differ in the percentages of CaO, MgO and SiO_2; in this context, the three oxides are indicative respectively of the presence of calcium carbonate ($CaCO_3$ - calcite, aragonite), double carbonate of calcium and magnesium ($CaMg(CO_3)_2$ - dolomite) and of the silicate fraction (quartz, feldspar, mica, etc). (ARPAV, 2010).

From the integration of the results of the mineralogical and chemical analyses it emerges that the tegnùe are characterized by rocky outcrops with different lithology: they can be made up of clastic carbonate sedimentary rocks originating from the cementation of sediments rich in dolomite accompanied by a non-negligible silicate component, or by sedimentary rocks, with carbonate fraction mainly of organic origin (ARPAV, 2010).

The numerous geological studies have in fact allowed a typification of the outcrops under the morphological and structural profile (Caressa et al., 2002.; Giovanardi et al., 2003.):

- Clastic sedimentary rocks formed by carbonate cementation of sediments (sands) or organogenic detritus (essentially mollusc thanatocoenosis), probably linked to phenomena of sea level variation in recent geological times, and commonly referred to as "beachrocks". They often have the appearance of sub-horizontal slabs, emerging from the bottom for very variable thicknesses, with usually very slight inclination, which causes them to be easily submerged by sediments, as well as, on the other hand, when the erosive action of the currents prevails. They can allow the emergence of new structures or the excavation of depressions and cavities along the perimeter areas (ARPAV, 2010).
- Sedimentary rocks of chemical deposit, the genesis of which would be linked to the emergence of methane gas from the bottom and its reaction with sea water. A phenomenon that caused the initiation of a process of precipitation of carbonates followed by cementation of the sediments. Subsequent erosive actions would cause the structures to emerge (ARPAV, 2010.; Casellato et al., 2005.; Donda et al., 2014.).
- Organogenic rocks, or structures produced by the action of constructive organisms, plants and animals, whose calcareous skeleton by stratifying can form structures of moderate thickness. Such a generative process leads to extremely varied and irregular morphologies,

with formations very rich in porosity, micro and macro cavities due to the different speed and irregularity of growth of the various building organisms (ARPAV, 2010).

Another hypothesis for the genesis of the tegnùe, advanced for the dendriform outcrops in front of Chioggia, is that the concretions have grown on sandy-gravelly cores consisting of wrecks of natural embankments and center-channel bars or meanders in a flood or delta environment after having been definitively drowned and submerged between 6000 and 2000 years ago (Giovanardi et al., 2003., ISPRA, 2010.). To confirm this, recently Tosi et al. (2017.) proposed a model for the genesis of Holocene coralligenous buildups occurring in the northwestern Adriatic Sea offshore Venice at 17–24 m depth. These morphologies are inferred to origin from Pleistocene fluvial systems reactivated as tidal channels during the post- Last Glacial Maximum transgression, when the area was a lagoon protected by a sandy barrier. The lithification of the sandy fossil channellevee systems was likely due to the interaction between marine and less saline fluids related to onshore freshwater discharge at sea through a sealed water table. The carbonate-cemented sandy layers served as basis for subsequent coralligenous upper growth.

The different typologies often coexist: organic formations can develop on sedimentary structures creating extremely different and irregular morphologies rich in micro- and macro-cavities (Mizzan, 1992.; Nesto et al., 2020.).

The proportion of the vegetable fraction over the animal fraction in the bioconstructions is very variable and essentially depends from sunlight intensity, which in turn varies depending from depth and turbidity of the water. If on the one hand the high turbidity due to the large amount of suspended material reduces the brightness of the seabed, on the other it constitutes an inexhaustible source of nourishment for filter feeders and detritivores, which in fact constitute the dominant element, even quantitatively, in these communities (Mizzan, 2000.; Casellato et al., 2005.; ARPAV, 2010.)

A georeferenced morphological map, elaborated by ICRAM on the basis of acoustic surveys carried out in 2001 with Side Scan Sonar (SSS) and Multibeam, was able to distinguish the areas characterized by rocky outcrops from those basically detrital and bioclastic and sand-muddy; moreover, the global view of the seabed of the entire area 1 of the SIC Tegnùe di Chioggia (Figure 13) finally threw light on the various hypotheses of origins of the Tegnùe di Chioggia (Giovanardi et al., 2003). The map was further detailed, distinguishing the outcrops by types (for example, grouped-isolated) and classifying them in greater detail by type and extent on the surface (Boscolo et al., 2005.; Franceschini e Giovanardi, 2005.). The four areas that make up the SICZTB of Chioggia are from 3 to 9.5 nautical miles from the Porto Canale di Chioggia; the total area of the SICZTB is 26.2 km^2; almost all of the natural rocky outcrops in the area are within the SICZTB (Figure 13).

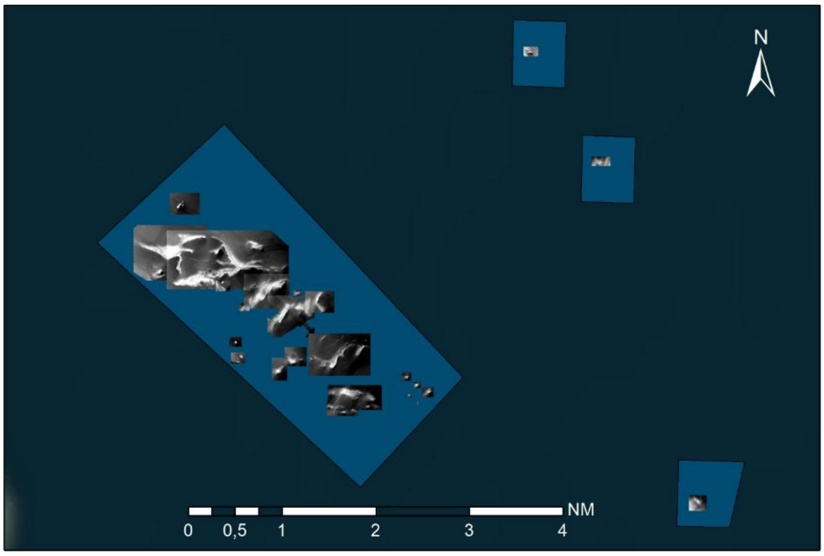


Figure 13. Georeferenced morphological map rocky outcrops "tegnue" within the SIC of Tegnùe di Chioggia (bathymetry data from Fortibuoni et al. 2020).

3.9. P6 – Miljašić jaruga (HR)

Characterization of the ecosystem of the coastal bay at the mouth of the river Miljašić in Nin

The area of town Nin is located in the north western part of Zadar County. It is a well-known area from antic times, with the historical center in the lagoon area of the Adriatic Sea. In 1346 Nin was turned into an islet (0.23 km²) and is still connected to the mainland by bridges (from the 18th century). The town is surrounded from the east side by the salt mines, from the north side with sandy beaches and forests and natural areas on the west side. The coastal area of Nin is characterized by shallow and sandy coast, canals, lagoons and cliffs and the fertile land of Ravni kotari. Hence, from the past up to now, growing of vines, olives, figs, but also fishing and saltworks are commercially very important in the Nin area (http://www.enciklopedija.hr/). The Nin salt mines are one of the oldest and still operational salinas on the Adriatic seashore of Croatia and they cover 55 ha. They are so-called solar salt works, where only solar energy is used to concentrate salt in the evaporation pans (Horváth et al., 2018). Although Nin is a small town with a population of 2.876 citizens in 2015 according to Croatian Bureau of Statistics, Nin stands out as an important tourist sea resource. Nin's most famous beach is the sandy Queen's Beach. This beach is named after the wife of King Tomislav, the first Croatian king. According to the legend, this sandy beach with the special healing mud was the favorite place of the Queen. Due to the area's shallow water and the high salt concentration, the coastal strip and the lagoons are home to many endemic and rare plants, birds, and various animals. Further from the coast, the landscape is changing from muddy and sandy shores to rocky grasslands (Breiner, 2017). The characteristic elements of the area: the shallow water, low, sandy and rocky shores, grasslands and high salt concentration results in a unique environment, a habitat for several rare and endangered species. Hence, the area of Nin and its surroundings is a unique landscape of rare plant and animal species with 8 NATURA 2000 habitats, 5 endemic, 4 critically endangered, 1 endangered and 5 sensitive plants. In the Nin lagoon specific rare habitats are formed: a low muddy and sandy coast with marshy areas containing the distinctive flora and fauna and sand dunes with rare plants. The important ecological value of the Nin habitats is well recognized, and the plant communities connected with sandy and muddy shores are strictly protected and listed as important habitats in the natural ecological network. The preservation of nature is carried out according to the criteria of the world's largest coordinated network protection areas, NATURA 2000 (https://www.nin.hr/en/natural-heritage/natura-habitats).

A significant factor influencing these muddy and sandy coastal habitats and one of the main challenges in the management of this area are the processes of coastal erosion, where wind and waves bring the sandy material from one part to the other parts of the coast or to the sea. Shape changes of the sea shores are especially visible on Ždrijac beach and Queen's beach. According to some previous studies it is estimated that the coastline, due to erosion processes, moved up to 10-15 m in some places during the last 10-15 years. As a consequence of these processes, eroded beaches are rehabilitated by refueling, ie. by filling sand taken from the sea in a nearby area, while also deepening the waterway (Natura Jadera, 2021.). Furthermore, the area of Nin could also be endangered by the potential consequences of climate change and sea-level rise, since its noticeable coastal erosion problems (Baric et al., 2008.).

Figure 14. The area of Nin bay

Figure 15. Sandy beaches in area of Nin bay

Characterization of the ecosystem at the mouth of the river Miljašić in Nin

This area is characterized by specific conditions such as constant input of fresh water at the river mouth, a small sandy beach 150 meters long on the east side, a sandy plateau in front of the river mouth containing seagrass *Posidonia oceanica* and *Cymodocea nodosa*, and a shallow underwater wall overgrown with various algae approx. 200 meters from the river mouth on the west side.

Figure 16. Miljašić jaruga channel

The most interesting part is the large rocks which act as a defensive wall for the dock on the right bank of the channel. Rocks and stones are overgrown with various species of algae, sponges, tunicates, cnidaria, etc. Most of these rocks are located in the tidal zone, and next to it is a shallow sandy bay with meadows of seagrass *Cymodocea nodosa*. On the east side of the bay is a small rocky cape, and further on is a stone waterfront that served the needs of the former brickyard. We should also mention the underwater wall that starts approx. 200 meters west of the river mouth and extends 2 kilometres west in the direction of Queen's Beach.

Figure 17. Supralittoral and mediolittoral zone of the rocks on the right side of the channel

Figure 18. Inner side of the channel

For biological monitoring, we used the method of direct visual census with the use of equipment for autonomous diving (SCUBA), and photo and video documentation were taken. An inspection within the final part of the channel at the exit to the sea confirms the presence of species typical of seaport habitats (F.5.1. Anthropogenic coastal habitats; F.5.1.2. Constructed shores, G.1.1.2.1 Seaport waters, G.1.1.2.3 Water areas of coastal settlements, harbors, and mandrakes). Anthropogenic impact on mediolittoral biocenoses within the channel and direct access to the sea is visible (G.2.5. Anthropogenic habitats in the mediolittoral; G.2.5.2.1. Mediolittoral facies of concreted and built shores (ports, harbors, shipyards) and other human structures in the sea (eg. gas platforms). The mediolittoral zone is dominated by the presence of marine gastropods such as *Patella rustica* and *Chthamalus stellatus*. The mediolittoral zone on both sides of the canal consists of fine sand and is characteristic of the entire coastal area around the town of Nin (G.2.5.1. Facies of tourist beaches and healing muds). The concreted bottom at the end of the channel descends vertically to a depth of about 1.5 m in the infralittoral zone.

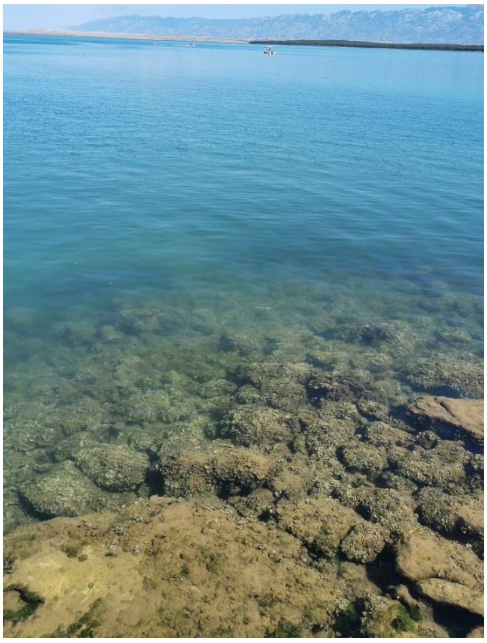


Figure 19. Shallow infralittoral communities

In the infralittoral zone, in the final part of the channel, the bottom of the muddy type is covered with a thick layer of green algae of the genus Ulva spp (G.3.1.1.8 Association with species of the genera Ulva and Enteromorpha). The depth is about 2 m in the middle of the channel. This indicates eutrophication and anthropogenic impact of the channel, given that these algae are characteristic in shallow polluted seas (Turk, 2011). Within the channel, below the very edge of the paved canal, there are communities typical of ports and paved shores, dominated by sea cucumbers of the genus Holothuria spp. and gastropod *Hexaplex trunculus* (G.3.8. Anthropogenic habitats in the infralittoral; G.3.8.2. G.3.8.2.1 Communities of infralittoral of concreted and built shores (ports, harbors, shipyards) and other human structures at sea (eg. gas platforms).
Green algae of the genera Codium and Ulothrix, brown algae *Zanardinia typus*, *Padina pavonica* and *Dictyota dichotoma* appear on the rocky base along the edges of the channel. On the outer part on the side of the channel (towards the north side) on the muddy and sandy bottom there is a meadow of sea grass *Cymodocea nodosa* (G.3.2. Infralittoral fine sands with more or less mud; G.3.2.3. Biocenosis of silted sands of protected shores; G.3.2.3.4 Association with *Cymodocea nodosa*). The benthic fauna is dominated by sea cucumbers (Holothuria) and the gastropod *Hexaplex trunculus*. Also, the population of the bivalve *Pinna nobilis* was observed, but a more detailed examination did not show any live individuals, but only dead specimens in the sediment. This is due to the occurrence of mass mortality in the Mediterranean and the Adriatic during 2019 and 2020 (Saric et al. 2020). By moving away from the channel on both sides, the bottom changes from muddy to sandy (G.3.2.

Infralittoral fine sands with more or less mud; G.3.2.2. Biocenosis of fine uniform sands), and is occasionally interrupted by smaller and larger stones overgrown with brown algae of the genus Cystoseira spp. The rocks and larger rocks are dominated by bivalves *Ostrea edulis* and *Arca noae*. The gastropod *Haxaplex trunculus* also occasionally appears on the sandy bottom. Among the sponges, the species *Crambe crambe* and *Aplysina aerophoba* were observed and common. Cnidarians such as *Condylactis aurantiaca* and *Anemonia viridis* are present also. The presence of the seagrass *Posidonia oceanica* was not observed in the closer vicinity of the channel. On both sides of the channel there are sandy shores that are used as tourist beaches during the summer season (F.2. Sandy sea shore; F.2.2. Supralittoral sands; F.2.2.1. Biocenosis of supralittoral sands; F.2.2.1.1. Facies of sands without vegetation). On several places these are flooded with leaves of seagrass *Cymodocea nodosa* (F.2.2.1.5. Facies of flooded remains of seagrass). We didn't notice any invasive species of flora and fauna during the monitoring on the research site. However, due to increased abundance of some invasive species in the Adriatic Sea in recent years (e.g. Blue crab *Callinectes sapidus*, Bluefish *Pomatomus saltatrix* or algae such as *Caluerpa taxifolia* and *Caulerpa cylindracea),* the monitoring of their presence should be more investigated in future.

Figure 20. Seagrass Cymodocea nodosa with dead specimens of bivalve Pinna nobilis

Figure 21. The edge of the sea grass Cymodoeca nodosa and sandy sea bottom 30 m away from the channel

Figure 22. The seabed in the middle of the channel covered with green algae Ulva spp.

NKS code and name (I level)	NKS code and name (II level	NKS code and name (III level	NKS code and name (IV level	NKS code and name (V level)	Remark
F. Sea shore	F.2. Sandy sea shore	F.2.2. Supralittoral sands	F.2.2.1. Biocenosis of supralittoral sands	F.2.2.1.1. Sandy facies without vegetation	Present on both sides
	F.5. Anthropogenic sea shore habitats	F.5.1. Anthropogenic sea shore habitats	F.5.1.2. Coastal communities on hard substrate under anthropogenic impact	F.5.1.2.1. Built and constructed sea shore	On the north side of the channel
G. Sea	G.1. Pelagial	G.1.1. Pelagic communities	G.1.1.2. Pelagic communities under anthropogenic impact	G.1.1.2.1. Sea harbour aquatorium	
				G.1.1.2.3. Aquatorium of inhabited coasts and ports	
	G.2. Midlittoral	G.2.5. Anthropogenic habitats in midlittoral	G.2.5.1. Midlittoral communities under anthropogenic impact (mudd and gravel)	G.2.5.1.1. Facies of touristic beaches and curative mudds	Present on both sides
			G.2.5.2. Midlittoral communities under anthropogenic impact on hard surface	G.2.5.2.1. Midlittoral facies of constructed shores (harbours, ports, shipyards) and other constructions on sea (eg. gas platforms)	Within the channel

	G.3. Infralittoral	G.3.1. Infralittoral sand, mudd, gravel and rocks in euryhaline and euthermic environment	G.3.1.1. Euryhaline and euthermic biocenosis	G.3.1.1.8. Associations with species from genus Ulva and Enteromorpha	Within the channel
		G.3.2. Infralittoral small sands with more or less mudd	G.3.2.2. Biocenosis of uniform sands	G.3.2.2.1. Association with species *Cymodocea nodosa*	On both sides of the channel
		G.3.3. Infralittoral large sands with more or less sand	G.3.3.1. Biocenosis of large sands and small gravel influenced by the waves	G.3.3.1.1. Association with rhodolites	Closer to the channel
		G.3.6. Infralittoral hard substrate and rocks	G.3.6.1. Biocenosis of infralittoral algae	G.3.6.1.2. Association with species *Cystoseira amentacea* (var. *amentacea*, var. *stricta*, var. *spicata*)	On both sides of the channel
				G.3.6.1.10. Association with species *Cystoseira crinita*	On both sides of the channel
				G.3.6.1.15. Association with species *Cystoseira compressa*	On both sides of the channel
		G.3.8. Anthropogenic habitats in infralittoral	G.3.8.2. Anthropogenic infralittoral communities on hard substrate	G.3.8.2.1. Infralittoral communities of constructed shores (harbours, ports, shipyards) and other constructions on sea (eg. gas platforms)	Within the channel

Table 1. List of the determined habitats according to the National classification standard (NKS, 88/2014)

3.10. P7 - Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT)

Pilot area 7 comprises two Special Areas of Conservation (SACs; Habitats Directive 92/43/EEC: Biferno river mouth-Campomarino Coast - IT7222216, and Saccione mouth Bonifica Ramitelli - IT7222217) included in the municipalities of Campomarino and Termoli, as well as a 15 km long sandy seashore (Figure 23).

Figure 23. P7 Pilot Area is part of the Long Term Ecological Research network (LTER - https://deims.org/088fe3af-c5bb-4cc8-b479-fe1ea6d5be80) and includes two N2k sites: Biferno river mouth-Campomarino Coast (IT7222216), and Saccione mouth Bonifica Ramitelli (IT7222217).

The Pilot Area includes well-preserved coastal dune and halopsamophile ecosystems that are rapresentative of the Central Adriatic coast. In fact, coastal dunes are among the most valuable ecosystems due to the presence of a high specialized fauna and flora. The area is part of Nature 2000 (Biferno river mouth-Campomarino Coast (IT7222216), and Saccione mouth Bonifica Ramitelli (IT7222217)) and LTER network (https://deims.org/088fe3af-c5bb-4cc8-b479-fe1ea6d5be80) and hosts several species and habitats of European conservation concern (Habitats Directive 92/43/EEC). Major habitats are: Habitat 1210 (Annual vegetation of drift lines), Habitat 2110 (Embryonic shifting dunes), Habitat 2120 (Shifting dunes along the shorelines with Ammophila arenaria, white dunes), Habitat 2230 (Malcolmietalia dune grasslands), Habitat 2260 (Cisto-Lavanduletalia dune sclerophyllous scrubs) and the habitat of priority concern 2250 (Coastal dunes with Juniperus spp of priority concern). On the Biferno mouth, we also found the following habitats: 1410 (Mediterranean salt meadows - Juncetalia maritimi); 1420 (Mediterranean and thermo-Atlantic halophilous scrubs -Sarcocornetiea fruticosi); the priority Habitats 1510* (Mediterranean salt steppes) and 3170* (Mediterranean temporary ponds) (Stanisci et al 2007; 2014).

Due to their unique position between land and sea, coastal dunes are dynamic systems guaranteeing essential benefits to society (MA, 2005). In fact, they provide important ecosystem services, such as coastal protection, erosion control, tourism attraction, recreation, education, research opportunities, as well as soil carbon stock (Drius et al. 2019). Coastal dunes host unique habitat assemblages due to a strong environmental sea-inland gradient, which supports a highly specialized flora and fauna sharing relatively few species with other terrestrial ecosystems. The unique dune plant diversity is not only valuable by itself (so-called ES "Existence value of biodiversity"), but also as it underpins the other ES provided by dunes, both directly (e.g. Protection from wind and aerosol, Erosion regulation, and Recreation & Tourism) and indirectly (e.g. Climate regulation).

Overall, the pilot area hosts one of the most threatened landscapes at national and European level. Specifically, these Mediterranean coastal ecosystems are extremely vulnerable and threatened by both erosion and increasing human pressure (e.g. urban expansion, trampling, beach litter accumulation, alien species invasion; Carranza et al. 2018; De Francesco et al. 2019, Prisco et al. 2021; Marzialetti et al. 2019). These threats lead several dune habitats to reduce and/or even disappear, implying negative consequences on biodiversity and human well-being, as e.g. a loss of coastal defense ability, a low perception of environmental quality by the tourists and a reduction of the ability of mitigate extreme climate events (Drius et al. 2019). Among the threats that impinge on coastal ecosystems in Pilot area 7, the well-known globally invasive plant *Acacia saligna*, an evergreen tree native to Western Australia, is particularly relevant. In fact, this species is able to invade several habitats of conservation value, altering biodiversity and ecosystem functioning (e.g. runoff, nutrient cycles, soil properties, aesthetic and recreational value). For these reasons, *A. saligna* has been listed among the invasive alien species of European Union concern (Regulation (EU) No. 1143, the so-called IAS Regulation).

3.11. P8 - The northern eastern Adriatic coastal area (HR)

The coastal area of the eastern northern Adriatic could be divided in two zones considering thermohaline properties: the western Istrian coast (WIC) and Rijeka Bay including the adjacent interisland area (RBIA). WIC waters are quite shallow (30-40 m) and under a significant inflow from the open northern Adriatic waters considerably influenced by atmospheric forcing.

Considering the long term research of thermohaline properties carried out in front of Rovinj (CRM data set from 1921), station RV001 (1 Nm west off Rovinj) could be used as an adequate reference for the yearly average of these properties (Figure 24; seasonal changes in sea temperature (t), salinity (s) and reduced density (γ) for some depths during the period 1972-2006).

Exactly this is the area where all the activities of pilot 8 will take place.

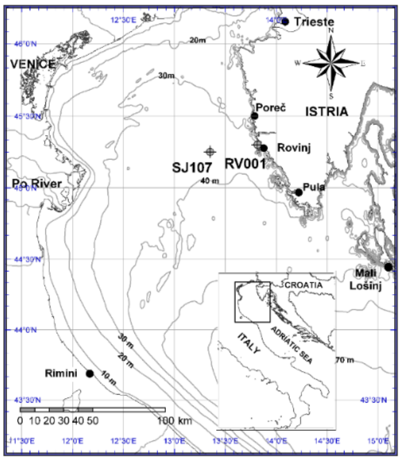


Figure 24. P8 Pilot Area is depicted in blu.

The northernmost part of the Adriatic Sea, north to the Rimini-Pula section is a shallow (up to 50 m), landlocked area. It receives freshwater inputs from the Po River, one of the Mediterranean's largest freshwater sources. Main winds in the region are dry bora, blowing from NE, and jugo, moist wind blowing from SE (Cushman-Roisin et al., 2001). The basic hydrographic conditions of the region, temperature, salinity and density, are influenced by air-sea interaction and Po River discharge (Supić and Ivančić, 2002). Although wind induced currents of the region are pronounced (Kuzmić et al., 2007) the geostrophic component presumably plays a major role in redistributing freshened waters and organic/inorganic substances across the NA (Supić et al., 2012). Processes in the ecosystem of the region depend on sea conditions and are thus dependent on changes in air-sea and hydrological (mainly Po River) forcing.

The NA has been monitored since 1920, when continuous bi-weekly measurements of temperature and salinity started in the coastal zone off Rovinj. With few data gaps the measurements continued until today and the dataset obtained is among one of the longest time series in the Mediterranean area, enabling a good insight into climatic changes of basic hydrographic conditions of the region. More comprehensive monitoring of various physical, chemical and biological data in the northern Adriatic started in 1972 and included six stations at section between the Po River delta and Rovinj, monitored seasonally or monthly. Based on that long-term monthly dataset the basic characteristics of the NA ecosystem were described in numerous publications. The pronounced variability in year-to-year cycles in it were observed and related to interannual changes in atmospheric, hydrologic and sea dynamic conditions.

Considering the long term research of thermohaline properties carried out in front of Rovinj (CRM data set from 1921), station RV001 (1 Nm west off Rovinj) could be used as an adequate reference for the yearly average of these properties (Figure 25; seasonal changes in sea temperature (t), salinity (s) and reduced density (γ) for some depths during the period 1972-2006).

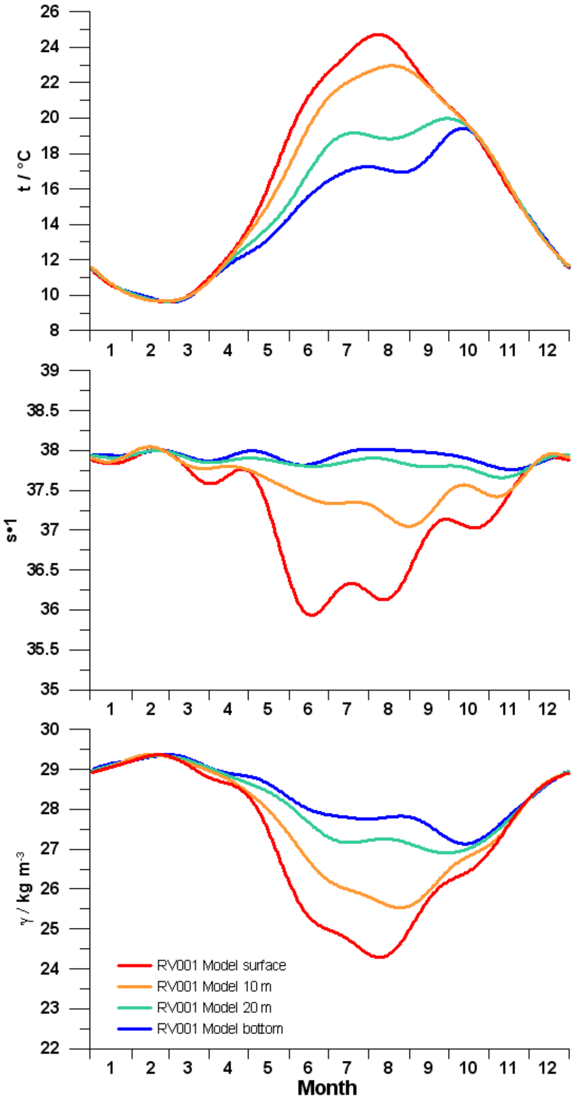


Figure 25. Average seasonal variation of sea temperature (t), salinity (s) and density (γ) for distinct layers in the period 1972-2006. A function with three harmonics was fit to the data.

Seasonal average temperature values on RV001 vary between 10-24°C, salinity 35.5-38.2, and reduced density 24.3-29.5 kg m^3. Long-term changes of thermohaline parameters are significant and complex, because of seasonal variability of freshwater inflows, atmospheric conditions and intrusions of warmer and high saline waters in the northern Adriatic from southern parts. Stratification of the water column starts in March and persists until October. However, stratification does not appear simultaneously for both thermohaline properties. In fact, at the beginning of the warmer season, the water column remains homogenous in temperature due to vertical mixing originated by strong winds and still lower warm atmosphere, but salinity values start to change as a result of spring-freshwater inputs. In the surface layer the first minima of salinity values are detected at the beginning of June, and the second one at the beginning of August. On yearly scale, oscillations of salinity are greater on the surface (up to 3), than in the bottom layers (up to 1), and dominantly correlated with the changes in reduced density values.

Water column in the region shows a pronounced seasonal cycle of basic hydrographic conditions, temperature, salinity and density. It is generally stratified during the heating period that lasts from March to August (Supić and Orlić, 1999). The interannual variability in it, apart from changes in air-sea fluxes, generally depends on advection of two water masses, the oligotrophic from central Adriatic and the eutrophic originating at the Po River delta area (Supić and Ivančić, 2002; Lyons et al., 2006; Djakovac et al., 2012). The central Adriatic waters arriving in the area are occasionally modelled by waters from the Mediterranean sea (e. g. Supić et al., 2004) which can be of very high salinity if originating from the Levantine basin (Levantine Intermediate Water, LIW) or by somewhat lower salinity water from the western Mediterranean (Civitarese et al., 2010). Po River affected waters are usually accumulated within several gyres which appear in open waters of the NA, are persistent for longer time periods and can be spots in which the accumulation of nutrients and organic materials (Orlić et al., 2013), large quantities of mucilaginous material (Supić et al., 2000) or bottom layer anoxia (Djakovac [6] et al., 2012) take place. Especially large quantities of Po River affected waters seem to be drawn into the NA when an anticyclonic gyre close to the coast, off Rovinj, appears. Its presence is indicated by the pronounced Istrian Coastal Countercurrent geostrophic current of southern direction (ICCC; Supić et al., 2000). Long-term changes in the intensity of the ICCC were found to be related to the intensity of large phytoplankton blooms (Kraus and Supić, 2011, Maric et al.2012). Over the past decade, and corresponding to the decrease in Po River discharge (Zanchettin et al., 2008), a significant reduction of phosphates and ammonia in northern Adriatic waters occurred (Solidoro et al., 2009). Concomitantly a decrease of phytoplankton biomass was observed in northern Adriatic waters (Socal et al., 2008 [11]; Mozetič et al., 2010 , Maric et al.2012) as well as a general shift toward smaller plankton size classes (Bernardi-Aubry et al., 2006; Pugnetti et al., 2008; Mozetič et al., 2010). The oligotrophication of the area was related to changes in geostrophic circulation according to which advection of central Adriatic waters prevailed over the appearance of the ICCC and to changes in the Po River discharge, which became reduced with respect to previous periods (Djakovac et al., 2012; Marić et al., 2012). Long-term changes in the appearance of the ICCC seem to be related to complex air-sea interaction processes and are to a certain extent predictable several months in advance (Supić et al., 2012).

The results accomplished so far showed that productivity level in the northern Adriatic goes through marked spatial and seasonal changes. The most prominent shift from oligotrophic to eutrophic conditions was observed during the stratification period changing the food web structure. During the eutrophic conditions classical food web took place, whereas the microbial food web became very important during oligotrophic conditions (Fuks et al., 201). After 2003 heterotrophic bacteria abundance showed a substantial decrease and change in their growth characteristics as a response to changed hydrographical conditions, reduced substrate supply and quality changes (Ivančić et al., 2010). The overall system-specific environmental conditions reflected on the dynamics and diversity of phytoplankton (Godrijan et al., 2012.; 2013.) as well as picoplankton community structure in the northeast Adriatic coastal zone (Šilović et al., 2012.).

3.12. P9 - Cetina river mouth (HR)

Cetina estuary is a site of Natura 2000 network (code: HR3000126) proclaimed as such in 2013. The site is located in south of Croatia, in front of the town Omiš and includes the mouth of river Cetina. The river Cetina is historically, economically and ecologically one of the most important rivers in the Adriatic basin. The length of its course is 104 km, while the area of the entire basin to the mouth of the Adriatic Sea is about 4,145 km^2. The coastal belt that is under the hydrological influence (groundwater) of the river Cetina stretches from Vrulja in the southeast to the river Jadro in the northwest. It is a coastline about 30 km long. In the central part of this area is the mouth of the river Cetina protected as Natura 2000 site.

Cetina estuary is a 100% marine area of 6.67 km^2. Lithostratigraphic unit represented around this area are cretaceous rudist limestone and eocene flysch sediments. Area is characterized by a brackish lagoon, coastal marine area with sandy and muddy bottoms. The coast is relatively flat with no pronounced bays. The seabed in the area under consideration can be described in the same way. Around the mouth and west of it, the seabed is shallow and relatively gently stretches from the coast towards the middle of the Brač Channel. Significant amounts of water are constantly flowing into this channel through the river Cetina, and with them organic and inorganic suspensions and various solutes. As such this is an important site for estuaries and for *Petromyzon marinus* reproduction. Its' geographical coverage is from 4808485 on south, to 4811749 on the north, 511486 on west and 517597 on east (measures in HTRS96). On the north, the site is bordering with another Natura 2000 site River Cetina – canyon part (site code: HR2000929). Area is characterized by wooded canyon, maquis and garrigue, dry and wet grasslands, springs, river and cliffs with its endemic species. The site includes part of significant landscape Canyon of Cetina - protected area on the national level. Conservation of all mentioned sites natural values is under management of Public Institution Sea and Karst.

The entire considered coastal belt has undergone significant changes due to intensive use/ settlement. Larger changes occurred in those areas that were more easily accessible and favourable for settlement, and these are flattened parts west of the mouth. By far the biggest changes have occurred in the area of the estuary where the town of Omiš developed. With the construction of the hydropower plants, the regime of the river flow changed significantly, so the banks of the river at the very mouth were arranged accordingly. Since the mouth of the river has been substantially altered, from a natural delta with shallow backwaters overgrown with sedges into a funnel-shaped regular bed, the conditions for the interaction between the river and the sea have completely changed. Today, town of Omiš is a touristic destination not only because of its large sandy and pebble beaches, but also because of tourist activities on the river Cetina (rafting, canyoning, etc.)

Figure 26. Map of Natura 2000 site Cetina estuary.

According to Water Framework Directive (WFD 2000/60/EC) this area has 3 water bodies of transitional water that as such were defined in 2012. Transitional water areas are "transitional waters" of inland waters near estuaries that are partly saline due to the proximity of coastal waters. The transitional type of surface water occurs between freshwater and coastal water, where the boundary with freshwater in the upper part of the watercourse is defined by the occurrence of salinity greater than 0.5 PSU, and in the estuary by a link between opposite estuaries or the appearance of a pronounced horizontal salinity gradient. Starting from the one with the hight influence of the fresh water, P1_2-CEP encompassing estuarian part of the mouth with total surface of 0.17 km2, then P2_2-CE with a surface of 2.18 km2 and the largest one which is partly included in Natura 2000 site P2_3-CE with its surface of 13.5 km2. Transitional water of river Cetina makes 10% of the total area of all transitional waters of the Republic of Croatia and is among those that have the largest number of water bodies and the greatest diversity of types, and thus the associated ecosystems.

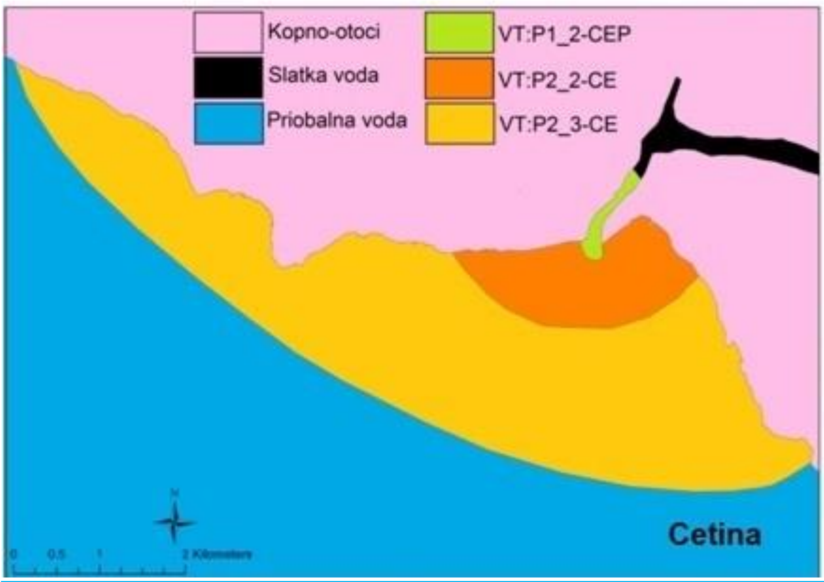


Figure 27. Cetina river transitional water bodies.

3.13. P10 - Torre del Cerrano, Pineto Abruzzo (IT)

The Pilot area 10 is the Torre Cerrano Marine Protected area (AMP). Among the coastal biodiversity facets Torre Cerrano is known for (https://www.torredelcerrano.it/), we focused on coastal dunes hosting valuable relicts of well-preserved natural ecosystems.

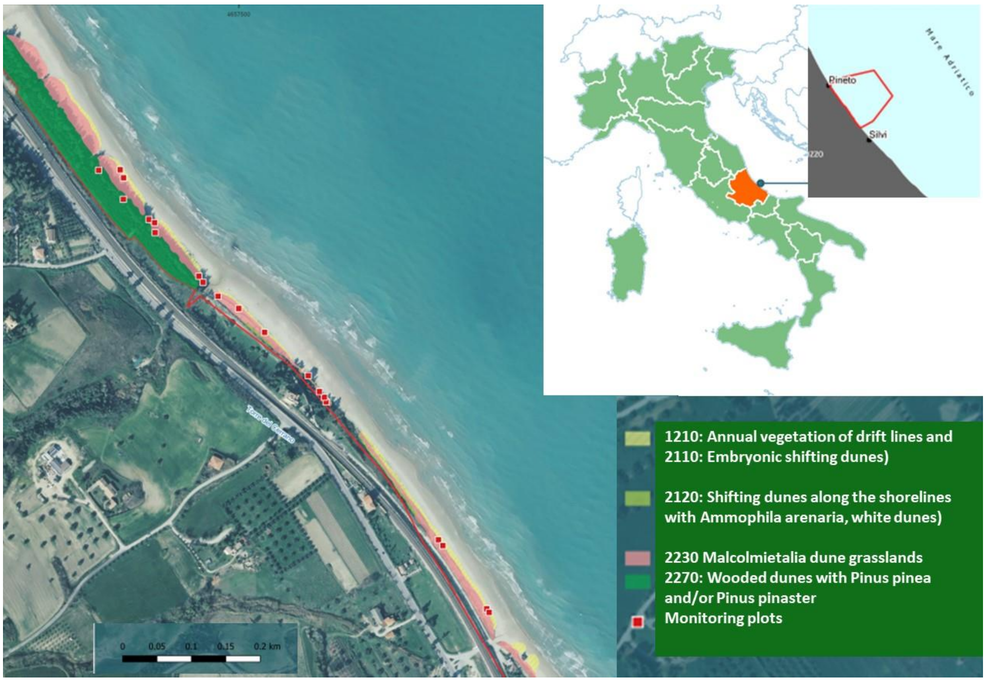


Figure 28. P10 Pilot area is a Marine Protected area (https://www.torredelcerrano.it/area-marina-protetta.html) along with coastal dune habitats (Habitats Directive 92/43/EEC). Some monitoring plots are also reported.

The Pilot area 10 includes well preserved coastal dune ecosystems rapresentative of the Central Adriatic coast along a 6 km long seashore. It hosts several species and habitats of European conservation concern (Habitats Directive 92/43/EEC). Major dune habitats are: Habitat 1210: Annual vegetation of drift lines; 2110: Embryonic shifting dunes; 2120: Shifting dunes along the shorelines with Ammophila arenaria, white dunes; 2230 Malcolmietalia dune grasslands; 1410: Mediterranean salt meadows - Juncetalia maritimi; 2270: Wooded dunes with Pinus pinea and/or Pinus pinaster (Carranza et al 2008; Stanisci et al 2014).

Along the Adriatic coasts (as well as on most of the coastal areas in the world), unregulated urbanization, intensive farming and increasing road infrastructures coupled with massive beach tourism have led to pollution, biological invasions, over-exploitation of the natural resources and to a decline in quantity and quality of coastal habitats. Usually, tourists, tour operators and local admimnistrators see the beaches exclusively as a place for vacation and leisure sports; thus, sand beaches are often cleaned mechanically and dunes severely trampled. In fact, human trampling associated with recreational activities is the most common disturbance on sand dune habitats, especially during summer, whose extremely negative effects include substrate erosion, decreasing of plant community's diversity and wildlife dismissal from no longer suitable habitats (Prisco et al 2021).

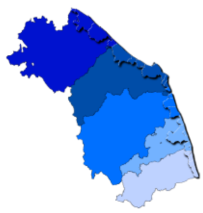
In this example, we measure changes in vegetation assembly after the establishment of wooden boardwalks (protecting dunes form human trampling) across different coastal dune habitats.

3.14. P11 - Marche coastal area (IT)*

The regional planning framework in the coastal area

Last December 2019, MARCHE REGION approved the REGIONAL ICZM Plan, which is the official planning and programming act that regulates the use and management of the entire coastal area of MR territory. It complies with:

- EU Floods Directive 2007/60/EU,
- National Guidelines for the defense of the coast from erosion and the effects of climate change (MATTM and ISPRA, 2017)
- PROTOCOL on Integrated Coastal Zone Management in the Mediterranean connected with the sustainability strategies towards resources ecosystem and coastal resilience.



ICZM Plan is the new planning and programming tool for coastal defense interventions in Marche region, an important aspect of which is the environmental characterization based on three issues: quality of the rear reef sediments, bathing and biocoenosis, analyzed by ARPA Marche (Regional Environmental Protection Agency) and revised and integrated with the scientific support of CNR IRBIM of Ancona and the Universities of Camerino, Urbino and Ancona.

<u>Marche coastal area</u>

MARCHE REGION is in charge of the *pilot area P11*. P11 includes the entire coastal marine area of the Marche region. The P11 Pilot site characterization is ongoing and it is part of the monitoring programme of the regional ICZM plan ed the pilot action is is composed by two main tasks:

- "Analysis of biocenosis emerged and submerged along the entire coastal strip"
- "Service of coastal engineering of technical-scientific deepening and verification with consequent proposal of re-perimeter of coastal areas subject to marine flooding of five pilot sites of the Marche region already perimeter in accordance with EU Directive 2007/60/EC and Legislative Decree no. 49 of 23/2/2010 and included in the ICZM Plan"

The Marche coast is one of the most characteristic aspects of the Region. It extends for 180 km from the promontory of Gabicce Mare to the mouth of the Tronto river, awarded with numerous European Blue flags that sanction the excellent quality of bathing water and the care taken for the protection of the marine environment.

The coast, consisting of an alternation of beautiful pebble, rocky and sandy beaches, responds to all the needs of visitors of our land and confirms an ancient tradition of hospitality. On the northern coast, long and thin beaches alternate here and there interrupted by a promontory, by small coves or by the mouth of a stream. The famous seaside resorts of Gabicce Mare, Fano, Pesaro characterized by wide sandy beaches offer a quiet seaside life even to the inexperienced and small bathers.

**based on the research “Coastal studies n.30/2021 – The Marche Region coastal line and the new ICZM Plan ISSN 1129-8588 which is under approval of Marche Regional Government”*

From Ancona, capital of Marche Region, you can see Monte Conero, a promontory of extraordinary beauty overlooking the blue of the Adriatic Sea.

From here begins the most beautiful stretch of the Marche coast: the "riviera del Conero", full of white bays sometimes reachable only by boat or through paths, cut out in the green of the Mediterranean scrub. From the same southern gates of Ancona, there are suggestive tourist resorts: the Portonovo Oasis, the award-winning Sirolo overlooking the sea, Numana with its equipped and functional tourist port and Marcelli the most modern with tourist villages, residences and adequate accommodation facilities.

To the south of the Conero, the coast offers wide and flat beaches up to an area full of pine forests in Porto Recanati, Porto Potenza Picena and Civitanova Marche. Furthermore, we cannot forget the "verde Riviera Picena", which extends between Porto Sant'Elpidio, Lido di Fermo, Porto San Giorgio and Pedaso, and the exotic "Riviera delle Palme" between Cupramarittima, Grottammare and San Benedetto del Tronto, with its 7000 palm trees that also grow on the very fine and white beach that slopes into the sea characterized by shallow waters.

Chapter 4 Assessments related to sea water, sediments and marine organisms

The Water Framework Directive 2000/60/EC (WFD), which was transposed in Italy and Croatia, establishes a framework for the “Community action in the field of water policy". The most innovative approach of the WFD is to consider aquatic ecosystems as a whole together with the related terrestrial ecosystems and wetlands in order to protect and improve the water quality. Primary importance is given to characteristic animal and plant organisms responsible for the self-purifying processes of the water themselves and, as a consequence, the idea of biomonitoring was introduced. In fact, by means of the continuous and selective biomonitoring of an area, it becomes possible to investigate the effects on living organisms and on biological parameters and, then, detect and evaluate various pollution phenomena caused by the introduction into the environment of different contaminants (current and/or past), and anomalies of chemical-physical parameters such as temperature, pH, salinity, etc. In this context, new legal approaches, which are based on management and monitoring systems of water resources, were introduced. WFD has as ultimate aims to assess water quality, guarantee protection and sustainability and achieve the environmental quality objectives.

4.1. P1 - Gulf of Trieste and Marano and Grado Lagoon

4.1.1. Chemical data in the Gulf Of Trieste (2006-2019)

From 2009 to 2019 an operational monitoring has been implemented in all marine-coastal and lagoon water bodies (Figure 29). It includes the analysis of more than 130.000 data, collected and analysed by ArpaFVG and Friuli Venezia Giulia Region in the Gulf of Trieste and Marano and Grado Lagoon shallow waters (ArpaFVG and SNPA, Monitoraggio delle acque di transizione e marino costiere della Regione Friuli Venezia Giulia (D.Lgs. 152/2006)). The whole dataset includes hydromorphological, physical-chemical and chemical parameters (*i.e.*, nutrients, priority hazardous substances) that were selected on the basis of the common legislation, usage in the regional area and toxicity.

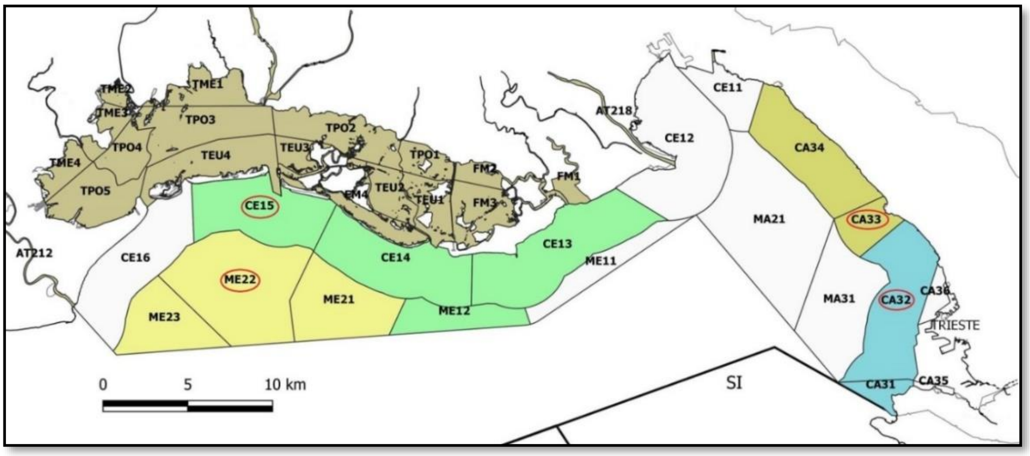


Figure 29. Regional water bodies of transitional and marine-coastal waters. The four groups for coastal marine waters are highlighted in different colours. (ArpaFVG and SNPA, Monitoraggio delle acque di transizione e marino costiere della Regione Friuli Venezia Giulia (D.Lgs. 152/2006)).

Due to the number of parameters considered, in this report we provide only a short description of the methods employed and of substances investigated. More information together with the whole dataset can be found in ArpaFVG website and at the following link:

https://www.dati.friuliveneziagiulia.it/Ambiente/Acqua-Acque-di-classificazione-Superficiali-marino/qcsf-bwk5/data

4.1.1.1. Sample collection and preparation of chemical data

Surface water samples were collected in marine and transitional coastal waters: about 2 liters' borosilicate glass vial with "Schott Duran" blue PP cap associated with HD polyethylene plastic rod metal-free and Niskin bottles were used to collect and subsample for nutrients and priority hazardous substances for the subsequent specific analysis.

4.1.1.2. Method description for the determination of chemical data

Macronutrients are represented by inorganic salts of nitrogen (nitrate, nitrite, ammonium), phosphorus (phosphate) and silicon (silicate). The analysis is carried out by colorimetric method by means of a segmented continuous flow system (Autoanalyzer model QuAAtro, Seal Analytical GmbH) equipped with an autosampler and specific monochromatic filters. The reagents used are reported in the following methods:

- UNI EN ISO 13395: 2000 (nitrite)
- EPA 353.4 rev 2.0 1997 (nitrite nitrogen)
- EPA349.0 1997 (ammonium)
- UNI EN ISO 11905-1: 2001 + UNI EN ISO 13395: 2000 (total nitrogen)
- EPA 365.5 version 1.4 1997 (phosphate)
- EPA 365.1 Revision 2.0 1993 (total phosphorus)
- EPA 366.0 rev. 1.0 1997 (silicate)

Analytical grade reagents are prepared following the provided guidelines by the Seal Analytical methods. Nutrients concentration is calculated through a calibration curve by analysing 5 standard solutions (which have increasing concentration similar to the values). Check quality controls analyses are performed by the use of certified control materials.

The following list describes the methods used to determine the priority hazard substances considered in this report.

- GC-HRMS: Gas Chromatography/High-Resolution Mass Spectrometry
- GC-MS: Gas Chromatography-Mass Spectrometry
- GC-MS/MS: Tandem Gas Chromatography-Mass Spectrometry
- LLE: Liquid-Liquid Extraction
- LLE+Deriv.: Liquid-Liquid Extraction + Derivation
- P&T: Purge and Trap
- SPE: Solid-Phase Extraction
- UHPLC: Ultra High-Performance Liquid Chromatography

4.1.1.3. Research results of chemical data

Priority substances

The main results obtained for samples collected in the period 2017-2019, both in coastal and transitional waters, are briefly described (mainly as classification of the water bodies investigated). Following the processing of the results obtained in the 2009-2016 period, the sampling frequency was set to every three months.

The areas most influenced by industrial activities (CA32, CA35 and CA36) showed the higher levels of priority substances together with two outermost water bodies (ME11 and CE15) belonging to the Trezzo-Punta area Sdobba and Porto Buso-Sant'Andrea.

In detail, Benzo(a)Pyrene, Tributyltin and others three other substances (Fluoranthene, Lead and Heptachlor + Heptachlor epoxide an organochlorine insecticide) exceeded the thresholds set by the WFD. Thus, the status of 'not good' was assigned to the relative water bodies. Similar results were found in transitional water bodies (Table 2).

Water body	Chemical status 2017-2019
CA32	NOT GOOD (TBT, B(a)P)
CA33	GOOD
CA35	NOT GOOD (B(a)P)
CA36	NOT GOOD (B(a)P)
CE11	GOOD
CE12	GOOD
CE15	NOT GOOD (Pb, TBT)
CE16	GOOD
MA21	GOOD
MA31	GOOD
ME11	NOT GOOD (TBT, B(a)P, Fluo, Ept)
ME22	GOOD

Table 2. Chemical status of coastal marine water bodies monitored in the period 2017-2019. TBT = Tributyltin, B(a)P = Benzo(a)Pyrene, Pb = Lead, Fluo = fluoranthene, Hept = heptachlor + heptachlorepoxide). (ArpaFVG and SNPA, Monitoraggio delle acque di transizione e marino costiere della Regione Friuli Venezia Giulia (D.Lgs. 152/2006).

Water body	Chemical status 2017-2019
TEU1	NOT GOOD (TBT)
TEU2	GOOD
TEU3	NOT GOOD(B(a)P)
TEU4	GOOD
TME1	NOT GOOD(B(a)P)
TME2	GOOD
TME3	GOOD
TME4	NOT GOOD (TBT)
TPO1	GOOD
TPO2	GOOD
TPO3	GOOD
TPO4	NOT GOOD (B(a)P,TBT)
TPO5	NOT GOOD (TBT, Cyp)
FM1	GOOD
FM2	NOT GOOD (B(a)P, Cyp)
FM3	NOT GOOD(B(a)P)
FM4	NOT GOOD(B(a)P)
AT212	GOOD
AT218	GOOD

Table 3. Chemical status of transitional waters in the three-year period 2017-2019. TBT = Tributyltin, B(a)P = Benzo(a)Pyrene, Cyp = Cypermethrin I, II, III, IV.

Nutrients

In transitional waters, reactive phosphorus was always characterized by values lower than the limits set in table 4.4.2/a - Annex 1 to Part Three of Legislative Decree 152/06 for water bodies with salinity greater than 30 psu. For the mesohaline and polyhaline water bodies the class limits are not defined, however the reactive phosphorus values were in any case very low so the classification for this parameter can be considered as good. Taking into consideration the DIN, Legislative Decree 152/06 reports two class limits in relation to salinity (> or < compared to 30 psu). For water bodies with salinity <30 psu the limit is 30 µM (420 µg/L), while for those with salinity> 30 psu it is 18 µM (253 µg/L). In this case, several water bodies, both euhaline, polyhaline and mesohaline, exceed the established limits, thus which the final classification was sufficient.

In coastal waters, the classification proposed by the application of the WFD/2000/60/CE is conducted by calculating the Trophic Index (TRIX), which was introduced by Vollenweider *et al.* (1998) This index combines the contribution of nutrients in form of DIN (Dissolved Inorganic Nitrogen as sum of N-NH_4^+, N-NO_2^- and N-NO_3^-) and TP (Total Phosphorus) expressed as µg/L, Chl *a* (µg/L) and Dissolved Oxygen (DO as absolute deviation from % saturation) through the following equation:

$TRIX = [\log_{10}(Chl\ a * DO * DIN * TP) + k]/m$

with *k* and *m* as scale coefficients, introduced to fix the lower limit and to define the extension of the trophic scale from 0 to 10 (Acquavita et al., 2021).

In detail, the index includes both the nutritional factors that contribute to the increase in algal biomass and the effects of the increase in biomass itself. This allows to evaluate the risk of dystrophies in marine-coastal water bodies affected by conspicuous fluvial inputs, and to report significant deviations from the trophic conditions typical of naturally low trophic level areas. (Acquavita et al., 2015). The calculation (annual average) for the period 2017-2019 is reported in Table 4, and showed a good quality status for all marine-coastal water bodies.

Water body	TRIX	STATUS 2017-2019
CA32	3,3	GOOD
CA33	3,5	GOOD
CA35	3,3	GOOD
CA36	3,2	GOOD
MA31	3,4	GOOD
CE11	4	GOOD
CE12	4	GOOD
CE15	3,9	GOOD
CE16	4	GOOD
ME11	3,6	GOOD
MA21	3,9	GOOD
MA22	3,8	GOOD

Table 4. TRIX index values and ecological status of the Gulf of Trieste between 2017 and 2019 (ArpaFVG and SNPA, Monitoraggio delle acque di transizione e marino costiere della Regione Friuli Venezia Giulia (D.Lgs. 152/2006)).

4.1.2. Phytoplankton data in the Gulf Of Trieste (2009-2019)

The dataset contains phytoplankton abundance data collected in the Gulf of Trieste and Marano and Grado Lagoon.

4.1.2.1. Sample collection and preparation of phytoplankton data

Samples were collected following the technical methods of the Directive WFD/2000/60/CE, D.Lgs 152/06, D.M 131/08, D.M. 56/09 and D.M.260/10.

Water samples were collected in the surface layer of marine and transitional waters. 2000 ml borosilicate glass vial (with "Schott Duran" blue PP cap associated with HD polyethylene plastic rod without any metal details), Niskin bottles and 20-micron phytoplankton net were used. The samples collected were fixed using 1.8-2.0 ml of Lugol solution and stored in dark and cold until the final determination that was conducted in one or two weeks.

4.1.2.2. Method description for determination of phytoplankton data

Phytoplankton analyses were carried out by qualitative and quantitative measurements of the microalgal community, in order to obtain a complete assessment of the ecological status of water bodies. Water surface samples were collected and the analysis were performed using an inverted optical microscope. Floristic lists were produced with the relative cellular abundances, thus providing useful information about the composition and structure of the community, and allowing, in some cases, to detect the formation and the following evolution of any algal blooms in progress.

4.1.2.3. Research results

In the period 2017-2019, a total of 216 phytoplankton samples were collected in 12 water bodies. The overall average abundance is 1,171,480 cells/L. The years 2017 and 2018 are very similar (997,372 and 972,294 cells respectively), while in 2019 there is a significant increase with an average value of 1,543,777 cells/L (Table 5). The phytoplankton community shows the typical seasonal temporal evolution with maximum in spring-summer and minimum in winter (ArpaFVG and SNPA, Monitoraggio delle acque di transizione e marino costiere della Regione Friuli Venezia Giulia (D.Lgs. 152/2006)).

In particular, 2017 was characterized by the absence of blooms, the maximum abundance (2,544,695 cells/L) occurs in July in CA35 due to the presence of indeterminate nanoflagellates and small diatoms. In 2018, nanoflagellates still prevailed, bringing the peak of cellular abundance to 5,849,408 cells/L in May (MA211). In the same month a bloom of *Chaetoceros simplex* (1.262.089 cells/L), a small diatom, was detected in CE122. Then, in 2019, the increase of cellular abundance was due to the blooming of *Chaetoceros socialis*, a small diatom, with a maximum peak detected in CE12 (4,754,100 cells/L) and the constant presence of nanoflagellates, which in May significantly increased the annual abundance values. Overall, the water bodies CE16, CE15, ME22 and ME11, that are close to the Lagoon of Marano and Grado, showed rather constant and more contained abundances over the three years, while the areas affected by the flows of Isonzo River (CE12, MA21 and MA31) reported the higher abundances such as the coastal water bodies CA35, CA32. (ArpaFVG and SNPA, Monitoraggio delle acque di transizione e marino costiere della Regione Friuli Venezia Giulia (D.Lgs. 152/2006)).

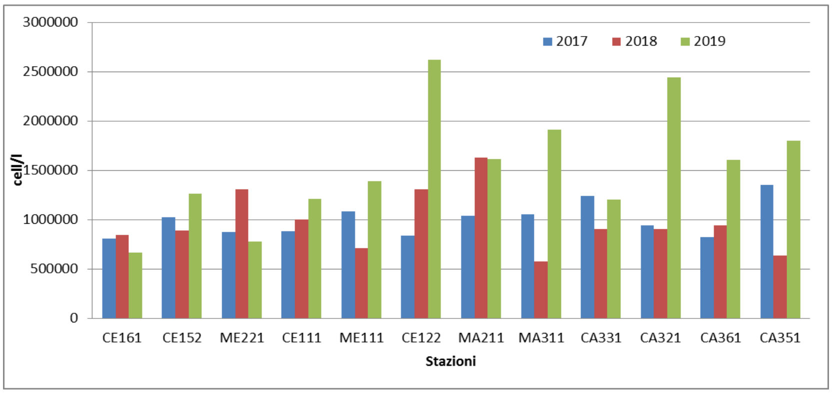


Table 5. Average values of phytoplankton abundances in the 12 water bodies in the three years of survey (ArpaFVG and SNPA, Monitoraggio delle acque di transizione e marino costiere della Regione Friuli Venezia Giulia (D.Lgs. 152/2006)).

4.1.3. Physical and chemical data collected in the Gulf of Trieste and Marano-Grado Lagoon (2009-2019)

The dataset contains physical and chemical data obtained by multiparametric probe vertical profiles collected in the Gulf of Trieste and Marano and Grado lagoon during monitoring monthly activities.

These data are used for many purposes as for example:

- ArpaFVG website's report
- Support for papers
- Support for Interreg-projects

4.1.3.1. Sample collection and preparation

The physical and chemical data considered are the result of high frequency vertical profiles along the water column in the points showed in Figure 30 and Figure 31.

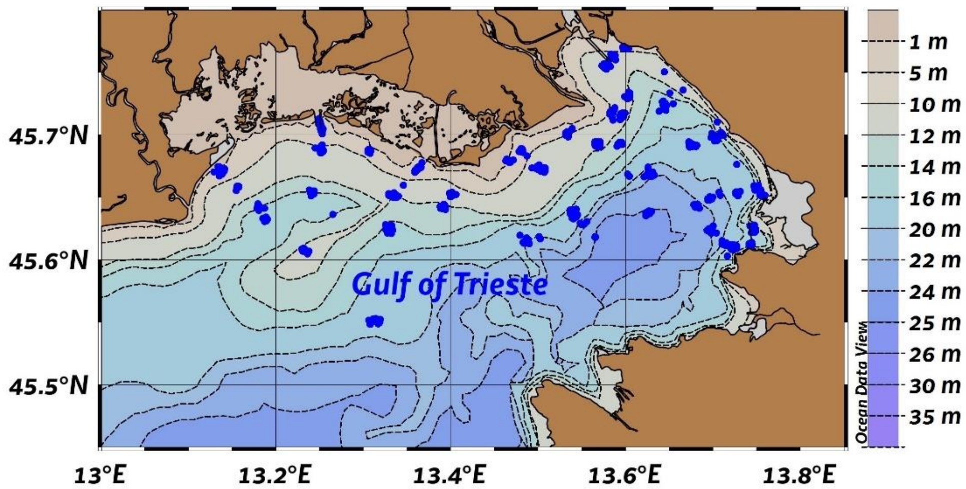


Figure 30. Vertical profiles measured in the Gulf of Trieste.

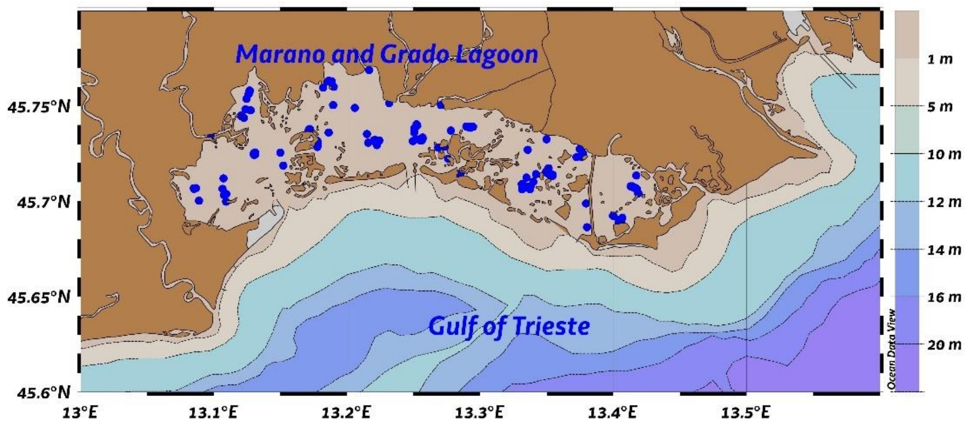


Figure 31. Vertical profiles measured in the Marano and Grado Lagoon.

During monitoring the following sampling protocol was used: the route to the stations and the subsequent correct boat positioning were determined by GPS; at the first station pressure (in air) and oxygen calibration (in air) were carried out according to the probe manufacturer protocols (Idronaut 316 multiparameter probe was equipped to measure the following parameters: pressure/depth, temperature, conductivity, dissolved oxygen, pH, chlorophyll *a*). Then the probe was positioned in the sea at about 2 m depth for hydration and stabilization of the sensors, after about 2 minutes the probe was positioned at the surface (0.30-0.50 m depth) and the data were collected at a low speed of about 0.3 m/s at a sampling frequency of 4-10 Hz. At the other stations the pressure and oxygen calibrations were repeated only in case of uncertainty about data reliability and additional profiles were performed only in case of bad data acquisition.

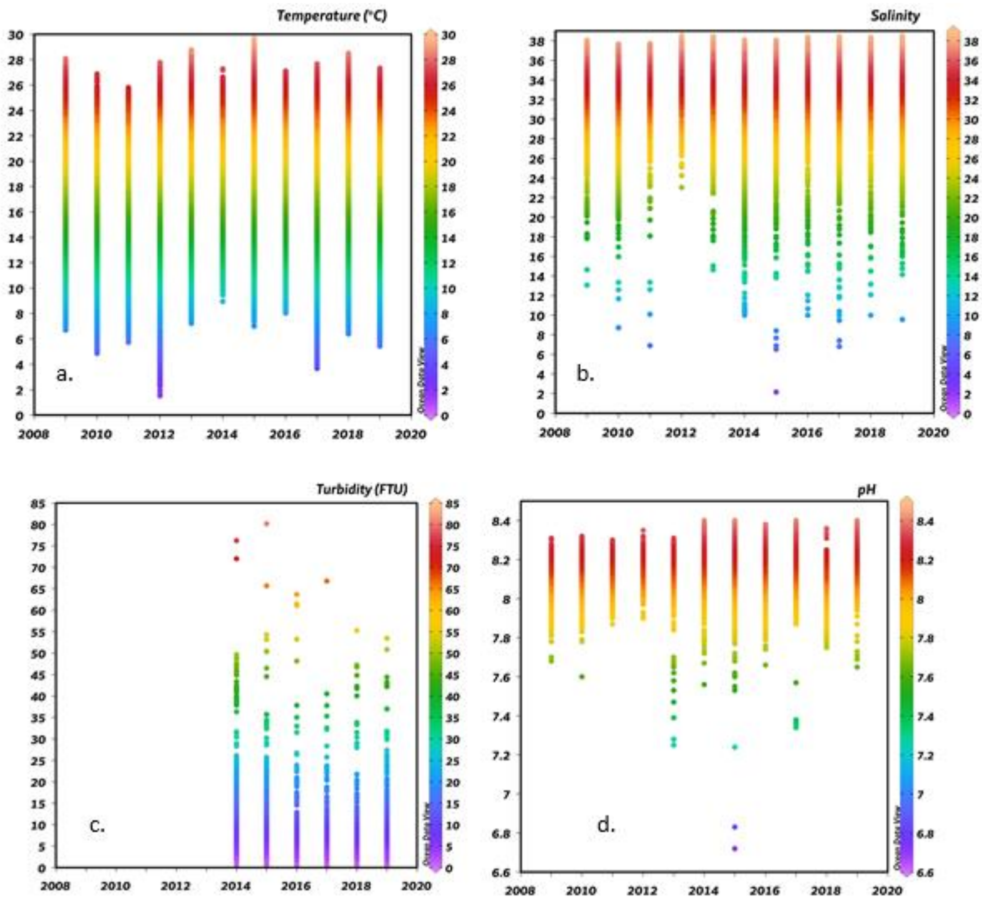
4.1.3.2. Determination of phytoplankton data of physical and chemical data

The quality check procedure started soon after data acquisition. First ASCII raw data were checked by visual inspection, in order to remove blatant outliers. Then data underwent a semiautomatic procedure performed by means of a computer program that linearly interpolated possible data gaps and then computed mean values at 0.25 m depth intervals.

4.1.3.3. Research results of physical and chemical data

Due to the huge amount of data obtained for priority substances and nutrients, only the overall trends will be considered. The following graphs show the temporal trends of the measured parameters over the ten years' period between 2009 and 2019. The following grids display the maximum and the minimum for total sampling considered in the mentioned period.

The Gulf of Trieste



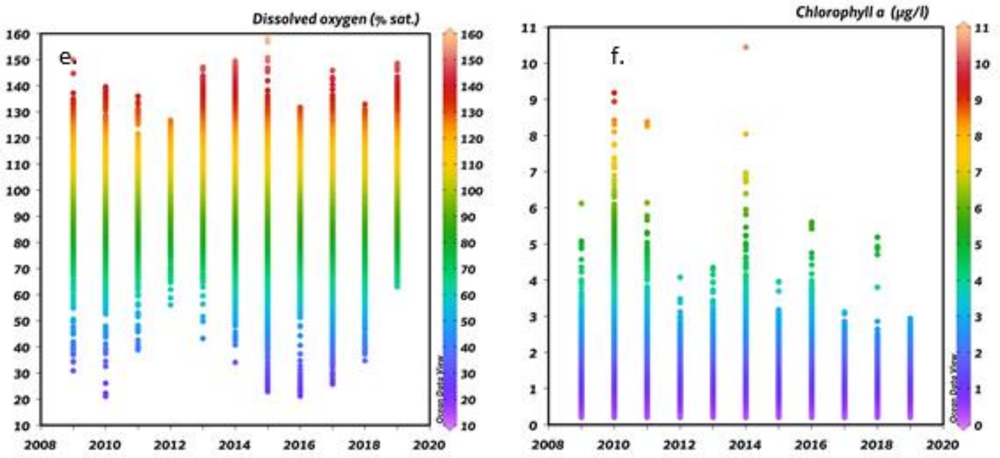
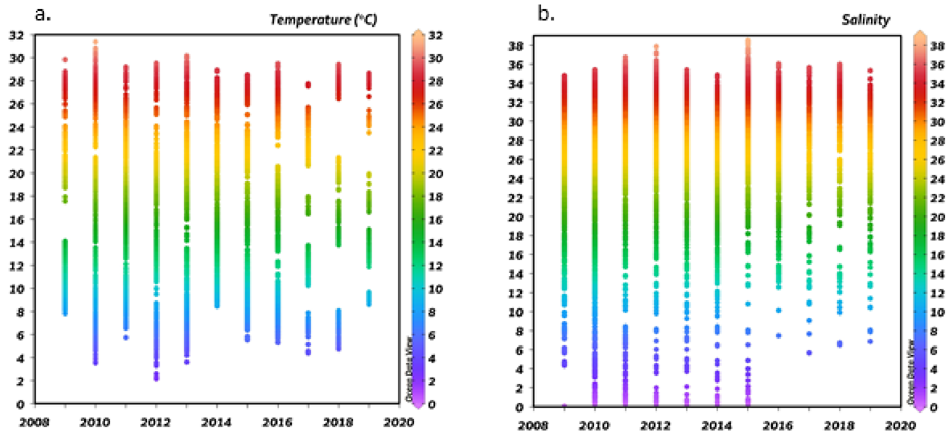


Figure 32. Graphs of physical and chemical data collected in the Gulf of Trieste (a) temperature; (b) salinity; (c) turbidity; (d) pH; (e) dissolved oxygen; (f) chlorophyll-a.

<u>The Marano and Grado Lagoon</u>



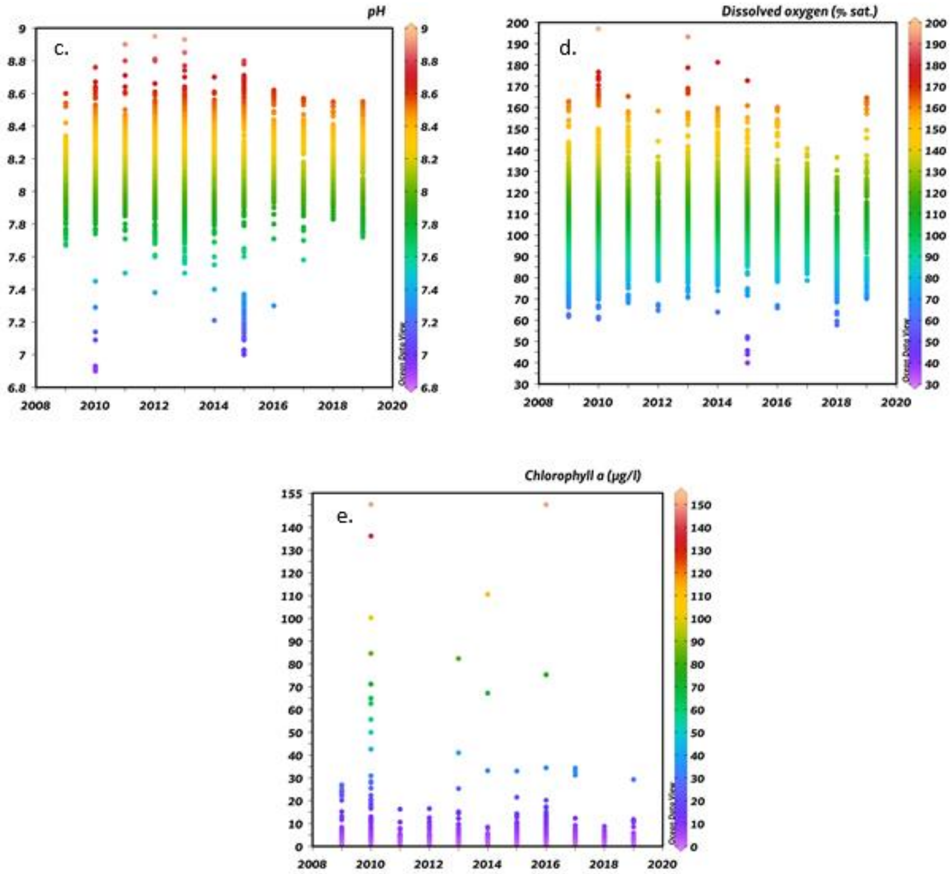


Figure 33. Graphs of physical and chemical data collected in the Marano-Grado Lagoon (a) temperature; (b) salinity; (c) pH; (d) dissolved oxygen; (e) chlorophyll-a.

4.1.4. Continuous physical and chemical data by several multiparametric probes - Marano and Grado lagoon (2013-2020)

Dissolved oxygen (DO) is a key element for the life of marine organisms, its level being regulated through the close connection of several processes. DO has been introduced as a chemico-physical supporting element in the current Water Framework Directive (WFD/2000/60/CE). In this work, ARPA FVG deployed a network of multiparameter probes to acquire DO data together with ancillary parameters in the Marano and Grado Lagoon (Mediterranean Sea, Italy). This experimental set-up continuously acquired data (*i.e.*, every 30 minutes) without the need for fieldwork and subsequent laboratory determination, and was conducted from 2013 to 2020.

4.1.4.1. Sample collection and preparation of 'Continuous physical and chemical data by several multiparametric probes - Marano and Grado lagoon (2013-2020)'

The dataset was acquired using a network of 5 multi-parametric probe units (SMATCH "Autonomous multi-parameters probe for the measurement of Pressure, Temperature, Salinity, Dissolved Oxygen and pH" Probe, NKE Instrumentation, Lorient, France). The probes were developed by customers in collaboration with the Ifremer (Institut français de recherche pour l'exploitation de la mer) for water quality control, environmental monitoring, monitoring in oyster culture zones and salinity and turbidity measurements for adjustment of hydrodynamical models. The probes are identified by the serial numbers 20137, 20138, 20139, 20140 and 20141, and are equipped with an integrated GPS-GPRS modem with a submersible antenna 1.1 m cable length to be mounted on a specific float that permit to transmit the measured parameters per email. The float avoids the sinking into the sediment of the lagoon. The probes were placed inside anchored buoys in order to protect them from breakage and keep them on the seafloor during low tide. One of the main advantages is that these probes are capable of functioning for several months without human intervention: in fact, they are equipped with an automatic protection system to counter bio-fouling using a local chlorination (© Ifremer). The chlorination is based on the electrolysis of seawater performed by means of a two-electrode system, a working electrode (chlorination grid) and a counter-electrode. Chlorine is periodically produced on the chlorination grid and spread close to the sensor. In order not to influence measurement, chlorination is automatically deactivated 30 seconds before the new measurement cycle.

Overall 18 sites have been investigated (Figure 34).

4.1.4.2. Method description for determination of 'Continuous physical and chemical data by several multiparametric probes - Marano and Grado lagoon (2013-2020)'

The dataset was cleaned of data related to malfunction or damaging of the instrumentation and also all the values that had been recorded during any period of time spent outside the water due to particularly intense low tides or maintenance activities carried out directly on the monitoring site.

Regarding dissolved oxygen data, all instrument readings outside the 0-150% saturation range (with ± 5% accuracy) have been eliminated according to technical specifications and calibration of the DO sensor. Values outside the 7-9 pH range have been removed because they are considered to be outliers or incorrect sensor readings, mainly due to instrumental drift correlated to long periods of deployment in the water.

4.1.4.3. Research results of 'Continuous physical and chemical data by several multiparametric probes - Marano and Grado lagoon (2013-2020)'

The analysis of the dataset obtained (a global collection of more than 220,000 data points) was considered at the entire lagoon basin scale; the sites name suggests their position: M-sites are located in the Marano lagoon while the G-sites are inside the Grado lagoon.

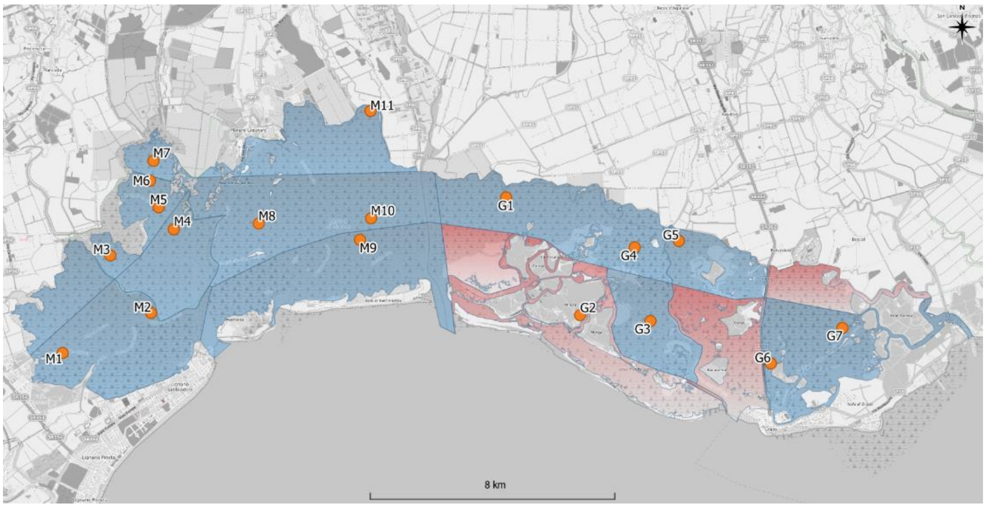


Figure 34. All sites investigated from 2013 to 2020. The demarcated areas visible within the lagoon indicate the water bodies into which it is divided. The blue indicates the water bodies investigated by means of the probes while the red indicates the areas where the instruments have never been positioned.

Water temperature showed a high degree of variability, ranging from 3.02 to 33.5°C (mean ± dev. std. = 20.5 ± 5.88) during December 2013 and late June 2019 at M1 and G7 sites, respectively (Figure 35).

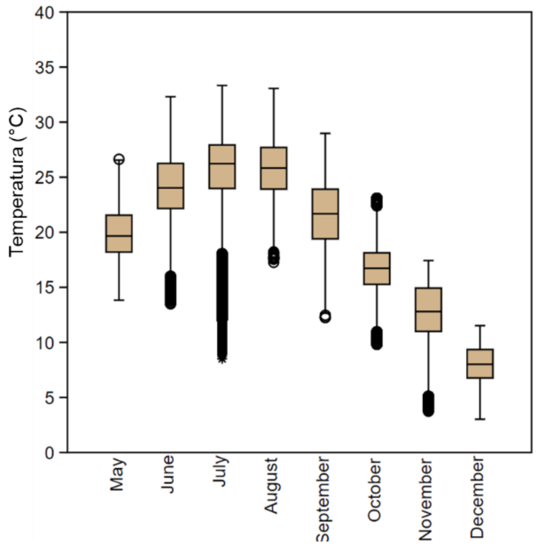


Figure 35. Water temperature values and their variability over the various months.

Salinity (PSU) ranged from 0.01 to 44.6 (mean ± dev. std. = 21.8 ± 9.44) and strongly depends on the amount of freshwater inputs, that in the westernmost sector is related to the rainfall amounts, and to a lesser extent to the active evaporation. Generally, the sites that belong to Marano basin showed lower values than Grado (17.0 ±8.54 and 29.3 ±4.63, respectively). Due to the effect of freshwater discharge, the variability of salinity was higher at Marano. Inside the Grado basin, only G5, which is under the effect of Natissa River freshwater discharge, showed comparable minimum values (Figure 36).

Figure 36. Interpolation of average salinity values (2013-2020 data) on the lagoon basin. The iteration between the river mouths and the lagoon mouths is highlighted on the map, as well as the different characteristics relating to the western portion (Marano Lagoon) and the eastern one (Grado Lagoon).

Considering the whole seasonal trend (from May to December), it can be observed a high variability: in fact, the late spring and autumn are characterized by the lowest values (18.1 ± 9.07 and 19.7 ± 7.81 in June and November, respectively), whereas in September the salinity reached an average of 24.6 ± 8.60.

The pH was on average 8.20 ± 0.28 and was quite similar among the whole basin. However, values close or lower than 8.00 were recorded in sites subjected to freshwater inputs (M6, M7, M3 and M4) and the sector of Grado exhibited values slightly higher than Marano (8.26 ± 0.29 and 8.17 ± 0.27, respectively).

The DO contents are expressed as dissolved oxygen concentration (mg/L) as well as derived oxygen percentage of saturation (%). DO concentration showed a high degree of variability ranging from <1.0 to 18.1 mg/L (mean ± dev. std = 7.13 ± 2.13 mg/L), with a significant difference among monitored sites. The highest average value was found at G3 in the open Grado Lagoon (8.32 ± 2.06 mg/L), whereas the lowest at G2 (3.63 ± 2.84 mg/L), which is a confined area within the commercially active Valle Noghera fish-farm (Figure 37). Here, the presence of several man-made embankments has heavily altered the pristine morphology, the hydrodynamics of the area, with water exchange limited and regulated by periodic opening of sluice gates.

Generally, DO decreased from late spring to late summer (8.57 ± 1.60 and 6.37 ± 1.83 mg/L in May and September, respectively) followed by an increase with the maximum reached in December (8.87 ± 1.63 mg/L) as a consequence the monthly difference is significant (Figure 38).

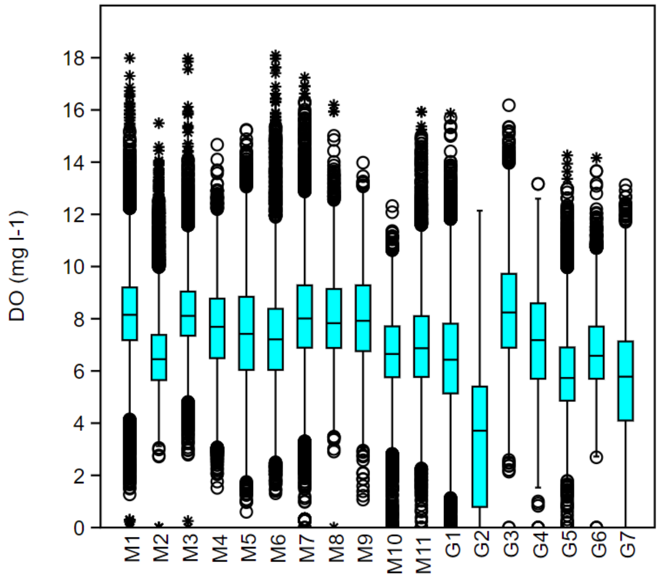


Figure 37. A comparison of the entire pool of values obtained on the different sites investigated.

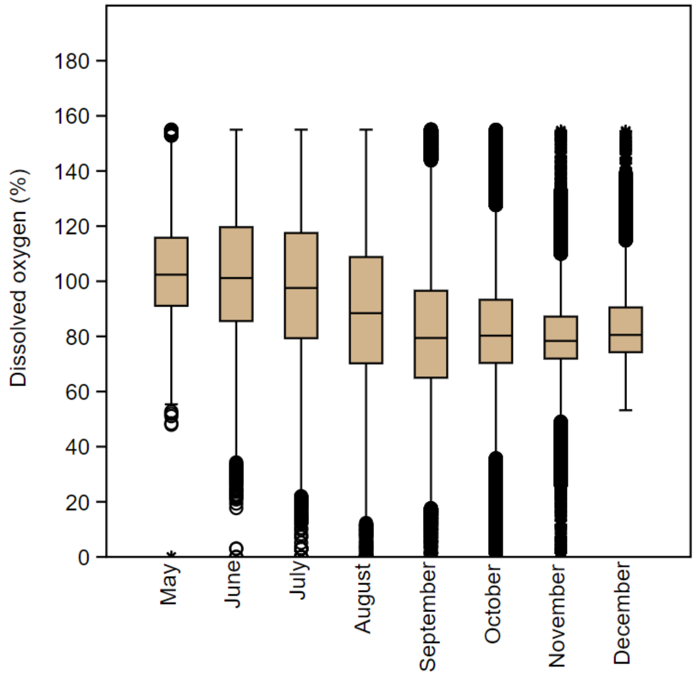


Figure 38. Dissolved oxygen values and their variability over the various months.

Taking into consideration the whole dataset, the oxygenation in the Marano basin is higher than at Grado (7.59 ± 1.86 and 6.40 ± 2.32 mg/L, respectively (Figure 39).

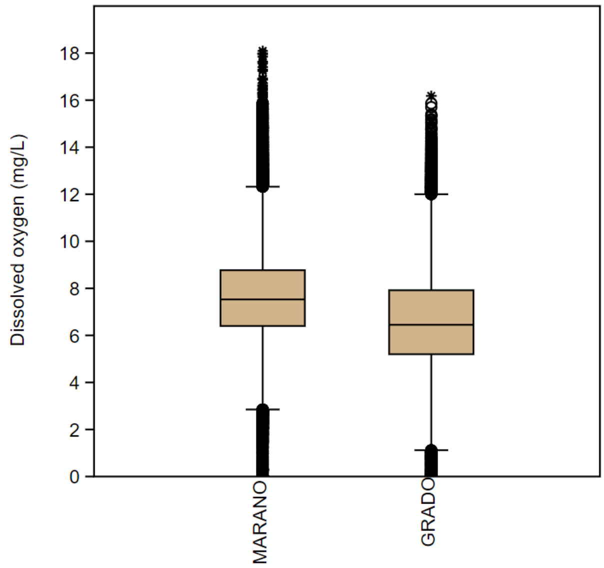


Figure 39. Dissolved oxygen values Marano vs Grado basin.

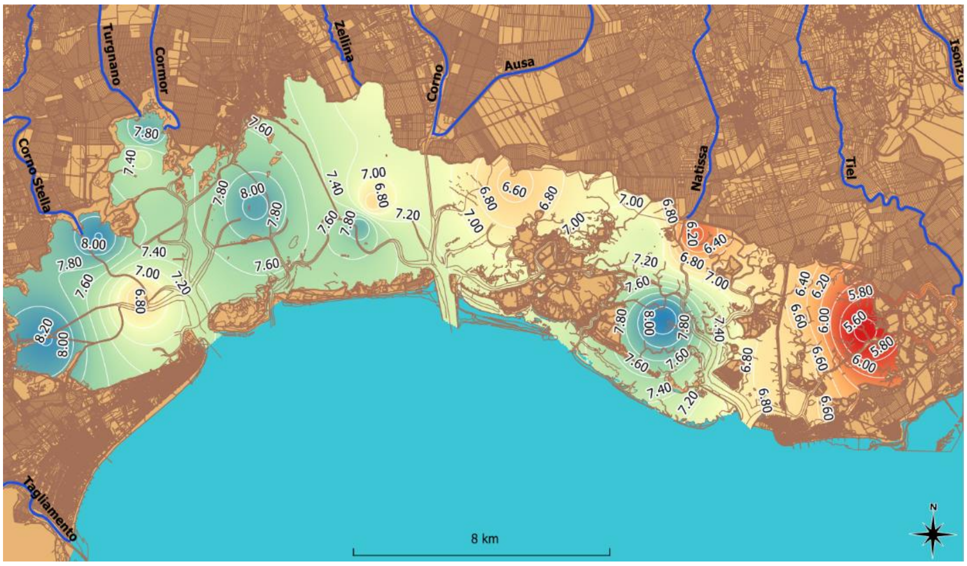


Figure 40. Interpolation of average DO values (2013-2020 data) on the lagoon basin.

The condition of hypoxia (DO <2 mg/L) was recorded in about 1.7% of the 210,796 observations and up to 80% was linked to the measurements conducted at G2 and G7 sites. As aforementioned G2 is an active fish-farm, whereas G7 is located in a confined area characterized by a strong modification of the hydrological regime occurred (Figure 40). If the measurements conducted in the fish-farm (G2) are excluded and are taken into consideration only those recorded in the open lagoon, hypoxia occurred mostly during summer, especially in August (N=330, 1.16% of total measures) and is absent in May and December. Values below the threshold of 5 mg/L, which represent a potential threat for sensible species of fish and invertebrate, were recorded in 13.9% of the measurements (n=29,224).

Actually, no prolonged periods of severe hypoxia or anoxia were registered and a sketch of daily trend shows clearly that after hypoxia levels are reached during late night/early morning there is a constant raise of DO which in the late afternoon reached hyperoxia level (Figure 41).

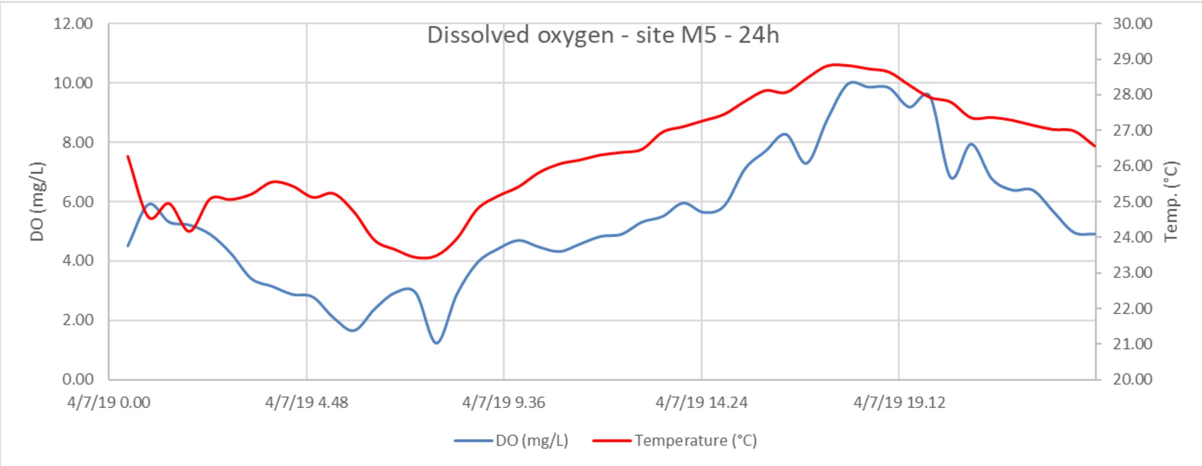


Figure 41. Daily trend of dissolved oxygen during a "hot" day of summer.

4.2. P2 - Sacca di Goro and Lago delle Nazioni

4.2.1. Water quality and phytoplankton organisms

The Transitional Waters include lagoons, saline lakes, river estuaries and deltas, are highly dynamic and heterogeneous systems widely distributed in the Italian Peninsula. Hazardous substances can endanger the balance of ecosystems consequently health of human marine organisms in the food chain. In addition, river ecosystems are under pressure from several different impacts. Due to their location between land and sea, coastal lagoons are characterized by large fluctuations in physical and chemical conditions (Kjerfve, 1994). These aquatic systems are subjected to strong anthropogenic pressures and freshwater inputs, which are rich in organic and mineral nutrients. Moreover, several of them support strong shellfish farming, as the Sacca di Goro. All these factors are responsible for important disruptions in ecosystem functioning characterized by eutrophic and dystrophic conditions (Cioffi et al., 1995; Harzallah & Chapelle, 2002; Pugnetti et al., 1992; Zaldıvar et al., 2003).

The monitoring program proposed by the European Water Framework Directive (Directive, 2000) is an important assessment of ecological status and the knowledge of their variability is a key point for the conservation and the management of these aquatic systems. Moreover, the deepen monitoring and the integrated management of the coastal ecosystem is an essential object to develop a predictive modeling of coastal water's quality.

4.2.1.1. In-situ physical parameters

Physical parameters are important tools to assess the ecological status of ecosystems. Variations in temperature, freshwater discharge, saltwater intrusion, bathymetry, circulation, and further biological production and respiration combine to produce strong estuarine dissolved oxygen gradients (Viaroli & Christian, 2004) and references within). Dissolved Oxygen (DO) refers to the amount of oxygen contained in water and define the living conditions for oxygen-requiring (aerobic) aquatic organisms. DO concentrations reflect an equilibrium between oxygen-producing processes (e.g., photosynthesis) and oxygen-consuming processes (e.g., aerobic respiration, nitrification, chemical oxidation), and the rates at which DO is added to and removed from the system by atmospheric exchange and hydrodynamic processes (Best et al., 2007). Oxygen usually ranges from 6 to 14 mg/L in the water, a shortage of DO – hypoxia (below 2-3 mg/L) or anoxia (0.5 mg/L) - can have dramatic impacts on marine life conditions and affected the ecosystems, including direct loss of habitat or habitat compression, altered trophic relationships, changes in migration patterns, and changes in biodiversity.

4.2.1.1.1. Sample collection

Data were collected at the water surface from different locations of the Sacca di Goro, reached on a monthly base with a fishing boat, and in the southern area of Lago delle Nazioni, from the land. The four sampling points are listed below and shown in the Figure 42:

- Porto Gorino (PG)
- Gorino (G)
- Foce Volano (FV)
- Bocca Mare (BM)
- Lago delle Nazioni (LN)

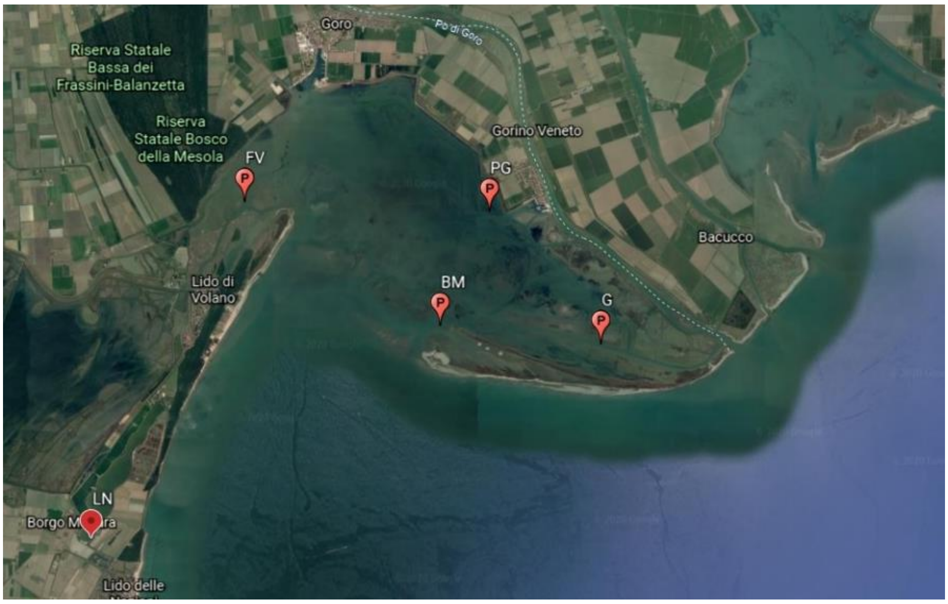


Figure 42. Location of the sampling stations in the Sacca di Goro and Lago delle Nazioni.

Sampling Points	Latitude (North, WGS84)	Longitude (Est, WGS84)
PG	44°49'04"	12°20'07"
G	44°47'35"	12°21'51"
FV	44°49'11"	12°16'23"
BM	44°47'47"	12°19'21"
LN	44°46’10”	12°14’30”

4.2.1.1.2. Methods

Temperature (°C) and Dissolved Oxygen (mg/L and saturation %) were measured in each station using a multiparameter water probe HQ30d (Hach-Lange GmbH). Salinity (psu) was determined with a refractometer Atago S-10 properly calibrated with distilled water. Depth (m) was determined using a tape measure equipped with a ballast.

4.2.1.1.3. Results

Temperature ranged from a minimal average value of 7.6°C in December to a maximal value of 29°C during summer (July 2020). Salinity reflects the strong influence of the freshwater inputs from the rivers in the external parts of the lagoon (Figure 43c). Foce Volano (FV) and Gorino (G) have the lowest values, with an average of 10 psu and a minimum peak of 0 psu in FV in May 2021. On the other side, Porto Gorino (PG) and Bocca Mare (BM), due to their central position and connection to the sea, show average values of 20 psu with maximum peak of 31 psu in BM, the most southern station. Ecologically critical oxygen concentration (2-3 mg/L) was found only at the station FV (Figure 43d) in the late spring period (May-June 2021), while a border intermediate condition (3-5 mg/L) was found during the autumn season (September-October 2020) in the same station. Oxygen depletion is more commonly observed as a consequence of the sinking and decay of blooms. Microbial respiration (e.g., nitrification) may deplete the water column and sediments of dissolved oxygen in the presence of organic matter (Best et al., 2007). Overall, FV represents the most sensitive site strongly influenced by the freshwater input of the Po di Volano, which could negatively affect the life of benthic organisms.

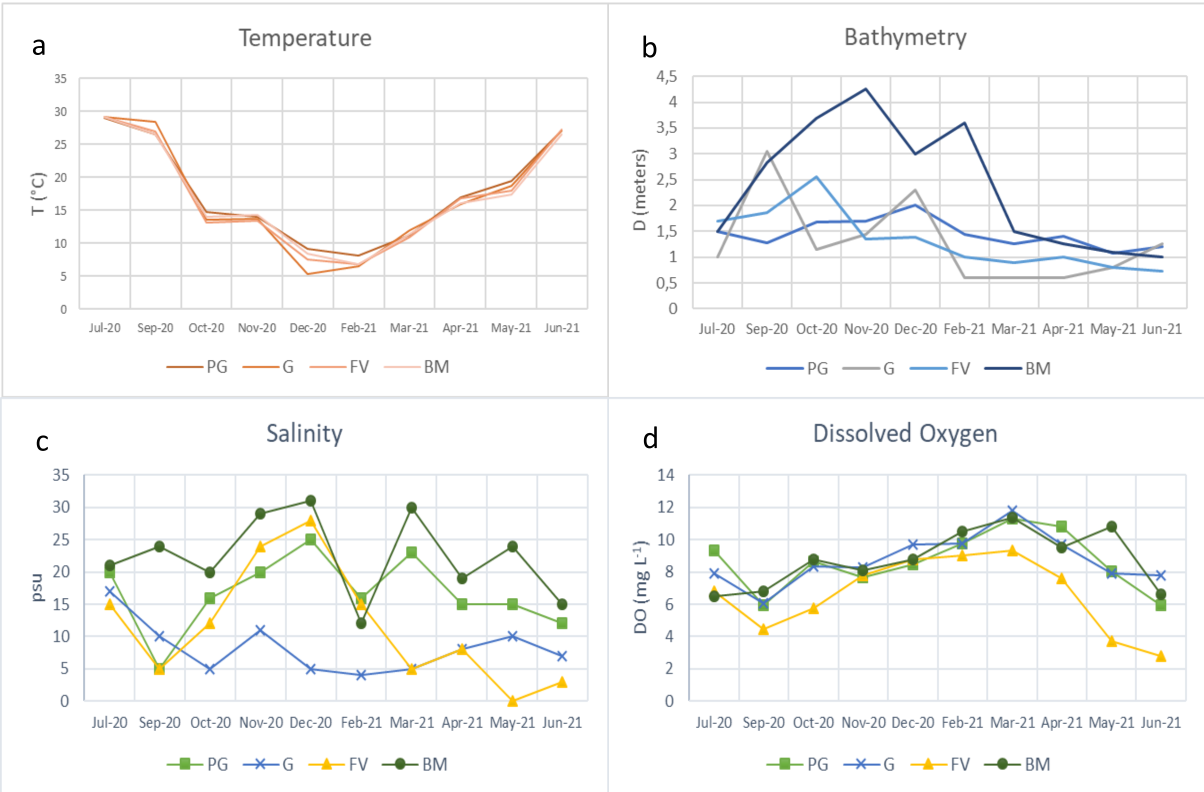


Figure 43. Temporal trend of (a) Temperature, (b) Bathymetry, (c) Salinity and (d) Dissolved Oxygen of the four stations of the Sacca di Goro.

Lago delle Nazioni, due to its poor turnovers, has a very low fluctuation of salinity, with a constant average of 22 psu during the whole year. Temperature ranged from a minimum of 6 to a maximum of 28°C. The dissolved oxygen ranged from 7 to 14 mg/L with an average concentration of 11.5 mg/L due to the frequent blooms occurring in this area.

4.2.1.2. Water column analysis

4.2.1.2.1. Sample collection and preparation

Samples were collected at water surface in the four stations of the Sacca di Goro and in one of the Lago delle Nazioni described in 2.1.1. Samples were collected in triplicate in polyethylene sample bottles of 1 L each, kept in a cool and dark box during transportation to the laboratory, based in Ravenna, and processed within few hours from the sampling.

4.2.1.2.2. Methods description for determination of water column's quality

Chlorophyll-a and phaeopigments.

400 ml of water were filtered on glass fiber filters (Whatman GF/F, 47 mm) and stored in the freezer until determination. The extraction of chlorophyll-*a* was performed with acetone 90% in plastic testing tubes, kept in a cool and dark place for 20 hours and spectrophotometrically analysed at 750 and 665 nm. Determination of phaeopigments was performed through acidification with HCl 0,66 M and red again at the same wavelength after 60 sec of reaction (Lorenzen, 1967).

Dissolved Inorganic Nitrogen (DIN) and Reactive Phosphorous (RP).

Filtered water of each sample was collected for nutrients analysis in PE bottles, pre-washed with HCl 10%. Nitrates and nitrites were determined in filtered seawater (Whatman GF/F, 47 mm) using the ion chromatograph 883 Basic IC Plus (Metrohm AG, Herisau, Switzerland) equipped with anion column Metrosep A Supp 4-250/4.0. For Lago delle Nazioni's samples, total nitrogen was determined with the colorimetric method by cadmium reduction (absorbance 543 nm). The total phosphate was determined with the colorimetric reaction of orthophosphate with molybdate ions (absorbance 885nm) (Parsons, 1972).

Particulate Organic Carbon and Nitrogen (POC and TPN).

Samples were filtered on glass fiber filters (Whatman GF/F, 25 mm), prior washed and combusted at 450°C, and kept in the freezer until the analysis. Filters were oven-dried (60°C), acidified with HCl 1M and dried again (60°C, over-night), individually prepared in aluminum capsules and introduced in the CHNS-O analyzer (Gordon Jr, 1969; Menzel & Vaccaro, 1964; Parsons, 1972).

4.2.1.2.3. Results

Chlorophyll-a and phaeopigments.

Chlorophyll a is a direct measure of the active phytoplankton biomass, and it is a good, accepted criteria used for trophic status classification of water bodies (OECD, 1982). The chl-*a* concentration in the station FV is the most variable, ranging from 0.5 ± 0.2 µg/L during the winter to 31.8 ± 0.5 µg/L in the late summer. The other stations (PG – G – BM) except for high values in summer (July 2020), did not exceed 8 µg/L along the year (Figure 44).

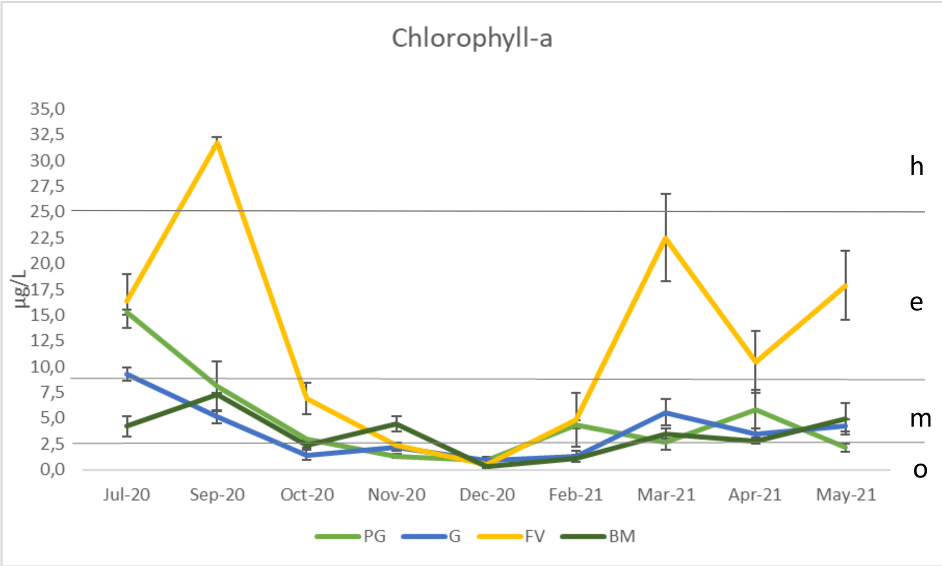


Figure 44. Concentration of chlorophyll-a in the 4 stations of Sacca di Goro. The letters represent the trophic state based on OECD criteria (o:oligotrophic; m:mesotrophic; e:eutrophic; h:hypereutrophic).

Overall, based on the OECD criteria (1982), the mean chlorophyll-*a* concentration determined in the Sacca di Goro ranged from a mesotrophic (means 2.5 – 8 µg/L) to eutrophic (means 8 – 25 µg/L) condition (Figure 45).

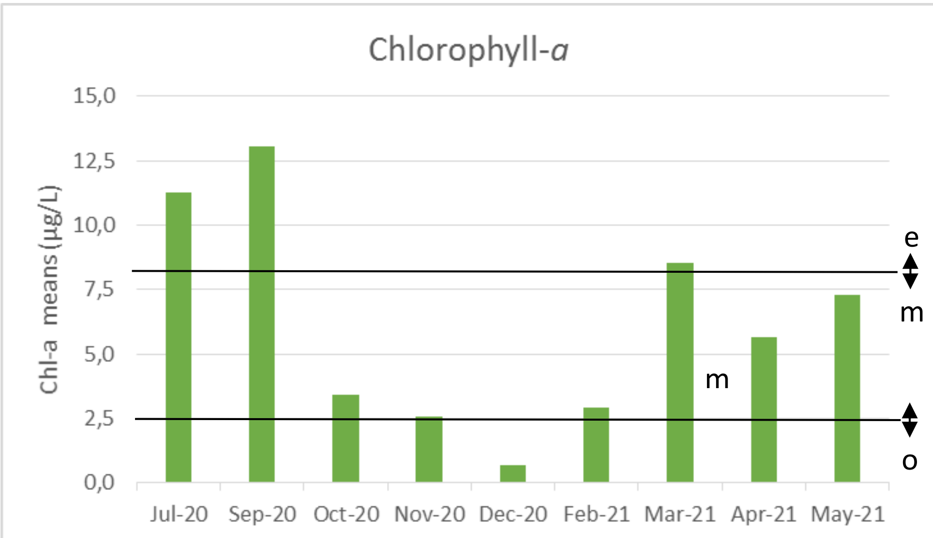


Figure 45. Means chlorophyll-a concentration in the Sacca di Goro (O:oligotrophic; m:mesotrophic; e:eutrophic).

Lago delle Nazioni showed a high chlorophyll concentration along the whole year, ranging from a minimum value of 14.8 ± 0.8 µg/L in July 2020 to a maximum of 67.3 ± 10.5 µg/L during the spring season (May 2021). Based on OECD critetia, the Lago delle Nazioni showed a constant hypereutrophic condition. The massive proliferation of phytoplankton can cause dystrophic event in this system.

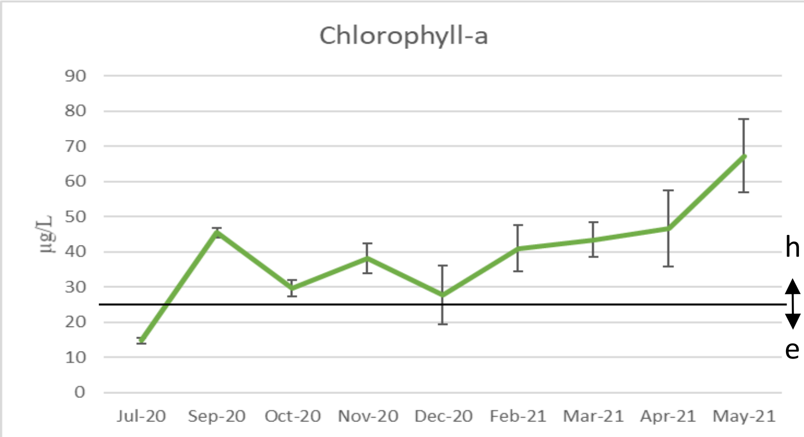


Figure 46. Concentration of chlorophyll-a in the Lago delle Nazioni (e:eutrophic; h:hypereutrophic).

Dissolved Inorganic Nitrogen (DIN) and Reactive Phosphorous (RP).

Total inorganic nitrogen (N-tot), calculated as the sum of the nitrites and the nitrates, is shown in the Figure 47. Along the year the concentration ranged from a minimum average value of 0.2 ± 0.07 mg/L in July 2020 to a maximum of 3.6 ± 0.1 mg/L in May 2021 in the station Foce Volano (FV), corresponding to critic level of oxygen concentration and low salinity (see 4.2.1.1.3). The Gorino (G) station often showed the highest N concentration, due to its enclosed position and freshwater inputs from the Po di Goro River. The other stations showed comparable patterns with fluctuation about 0.5 to 1.0 mg/L.

Soluble reactive phosphorous (RP) showed different concentrations among the stations and times. The maximum value was recorded in December 2020 in the Gorino station (G) reporting 53.9 ± 5.7 µg/L. Overall, mean values of RP ranged from <10 to 40 µg/L.

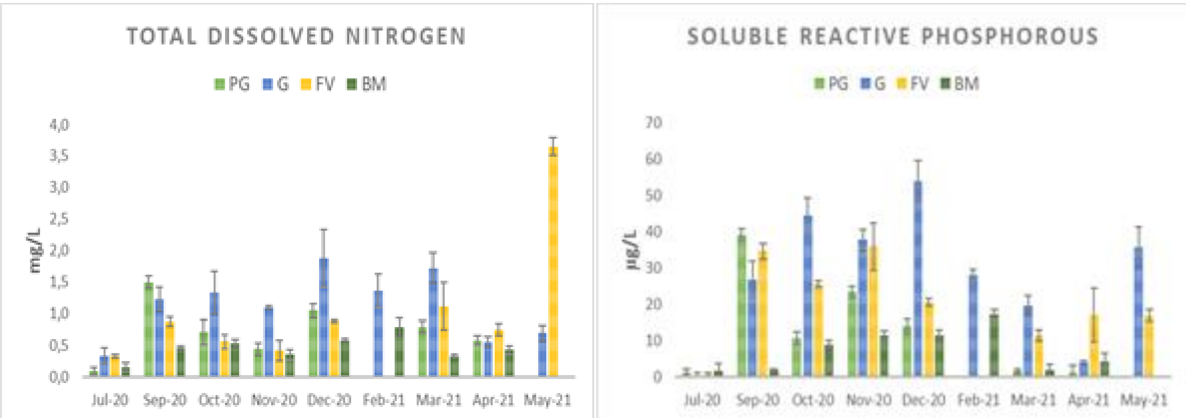


Figure 47. Total dissolved nitrogen (left) and soluble reactive phosphorous (right) concentration in the Sacca di Goro.

Lago delle Nazioni showed very low concentrations of both N and P: from 0 to 16.6 µg/L and 1.1 to 4.8 µg/L, respectively (Figure 48). Hypereutrophic systems usually have a very high primary production, due to frequent blooms (Karpowicz et al., 2020); as a result of algae uptake, there was a depletion of inorganic nutrients in the upper water layer, thus the concentrations were very low (<15 µg/L).

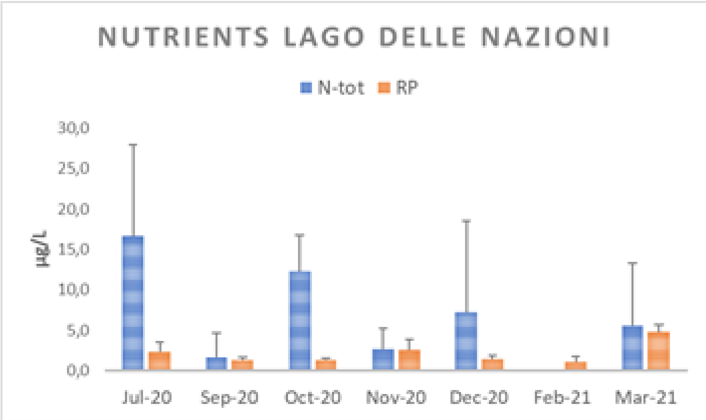


Figure 48. Total dissolved nitrogen (N-tot) and soluble reactive phosphorous (RP) concentration in the Lago delle Nazioni.

Particulate Organic Carbon (POC) and Total Particulate Nitrogen (TPN).

In the organic matter, the ratio of carbon, nitrogen, and phosphorus atoms is reported to be 106:16:1 (Redfield, 1934). Phytoplankton is an important link in the transformation of nutrients from inorganic to organic forms, thus reducing the amount of reactive nutrient forms (Karpowicz et al., 2020). The uptake of different forms of nutrients can show temporal and spatial changes due to their association with different phytoplankton groups (Yu et al., 2018). Hence, changes in phytoplankton taxonomic structure and growth rates can affect their nutrient demand and uptake and can influence their carbon: nutrient stoichiometry (Sterner & Elser, 2017). Therefore, the nutrient cycles can depend on phytoplankton community structures.

The concentration of organic carbon followed a temporal distribution with the highest values during the autumn and spring season and the lowest during winter; similar pattern is spatially distributed in the four sampling sites. FV showed the highest and variable values than other stations, ranging from 3.3 ± 0.3 mg C/L (September 2020) to 0.5 ± 0.2 mg C/L (December 2020). Likewise, concentration of organic nitrogen followed the same trends, resulting in a good removal and transformation of matter and subsequent incorporation trough the food web (Figure 49).

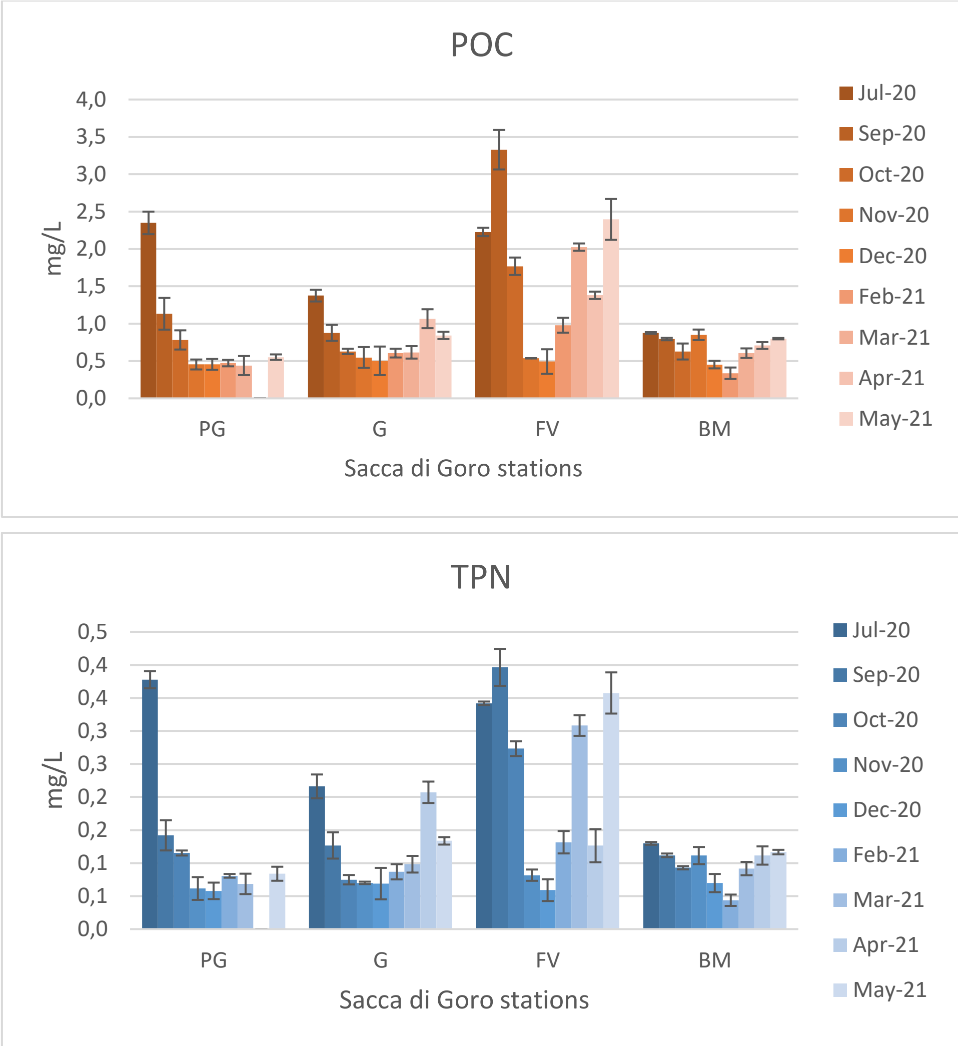


Figure 49. Temporal distribution of POC and TPN in the four stations of the Sacca di Goro.

Lago delle Nazioni showed a higher carbon content than the lagoon, ranging from 2.0 ± 0.01 mg C/L (December 2020) to 11.6 ± 0.5 mg C/L (May 2021). A similar pattern is showed for the nitrogen content that ranged from 0.1 ± 0.01 to 1.3 ± 0.04 mg N/L (Figure 50). The accumulation of organic matter in the water column is an effect of hypereutrophication of the system due mainly to the phytoplankton composition (see below).

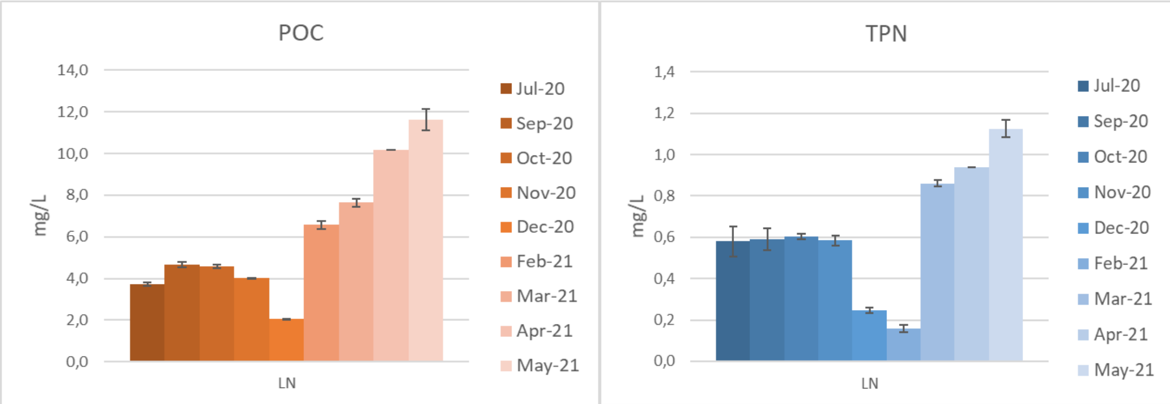


Figure 50. Temporal distribution of POC and TPN in the Lago delle Nazioni.

4.2.1.3. Phytoplankton abundances

Phytoplankton community is an important component in the functioning of aquatic ecosystems. Phytoplankton structure and dynamic are influenced by physical (e.g., light availability, stratification and mixing process), chemical (especially N and P), biological (e.g., predation) and hydrological factors, which show temporal and spatial changes (Becker et al., 2010; Lopes et al., 2005; Rangel et al., 2012). Due to its short life cycle and prompt response to changes in the environmental conditions, phytoplankton has been used in the water quality assessment (Crossetti & Bicudo, 2008; Padisak et al., 2006; Santana et al., 2017; Shen et al., 2014). To understand the interaction and association of species with the chemical and physical variables, Reynolds (2006; 2002) proposed a first model of functional groups for phytoplankton, arranging polyphyletic groups of species with similar morphological and physiological characteristics, response and tolerance to specific environmental conditions. This context is being continuously studied and developed worldwide, in order to deepen the knowledge of phytoplankton assemblages and their variation in a Changing World.

4.2.1.3.1. Sample collection and preparation

Samples were collected in triplicate as described in 4.2.1.2.1.

4.2.1.3.2. Methods description for determination of phytoplankton

Samples were fixed with Lugol's iodine solution (Lugol's) and kept in dark glass bottles until microscope analysis. Total abundances of cells were analyzed with the Utermhöl method (Utermöhl, 1931). A standard volume (5 – 10 – 25 ml) was chosen for each sample based on the chlorophyll-*a* concentration and other environmental parameters and let settle in the Utermhöl chambers from 5 to 24 hours. Cells count was performed with an inverted microscope Axiovert 100 (ZEISS Germany) at 32x magnitude and identification of phytoplankton organisms was carried out using taxonomic manuals, reaching genus and species level when possible.

4.2.1.3.3. Results

Sacca di Goro showed high variability in the phytoplankton composition (Figure 51). Any anomalous bloom events was registered during the sampling period, except for the proliferation of *Heterosigma akashiwo* (Y.Hada) in October 2020 (data not recorded).

One of the most variable site was Foce Volano (FV), reflecting the strong freshwater influence. Chlorophyceae reached a maximum relative abundance of 52% (November 2020) with an average of 20% through the year. Diatoms were always accounted with an average abundance of 31% and a peak during winter (62%, February 2021), as well as cryptophytes as one of the most common and abundant algal group (71%, December 2020). Total average abundances were 1.1 x 10^6 cells/L, with peaks in summer and spring. Bocca Mare (BM), due to its position alongside the Adriatic Sea, showed typically marine species, with a dominance of diatoms (49%) followed by cryptophytes (27%) and other algae (18%). Diatoms were represented mainly by *Chaetoceros* spp. and *Skeletonema* spp., the latter is a species common to proliferate during the winter. Total abundances ranged from a min value of 4.4 x 10^4 cells/L in (December 2020) to 3.0 x 10^6 cells/L in spring. Porto Gorino (PG), along the year, showed a dominance of diatoms (53%) followed by cryptophytes (20%). Along the sampling time, it has been recorded a peak of phytoplankton abundance in February of 6.6 x 10^6 cells/L due to the seasonal *Skeletonema* bloom. Gorino (G) showed a predominance of diatoms (48%) followed by cryptophytes (25%) and other algae (17%). The diatom group was widely represented by benthic Naviculales (5-80%). The average abundances were 6.3 x 10^5 cells/L, with a peak of 1.2 x 10^6 cells/L in March.

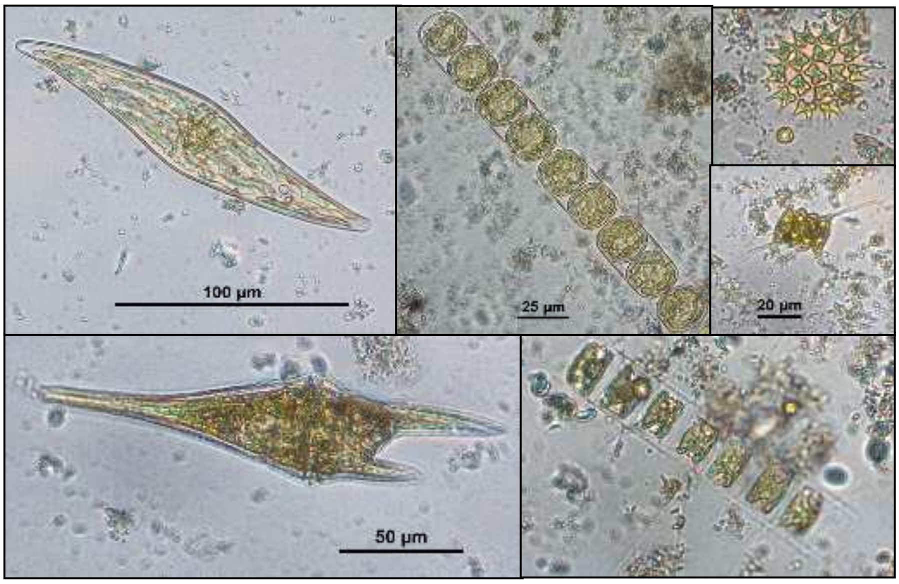


Figure 51. Some microalgae species accounted during the phytoplankton analysis.

Noteworthy, during the microscope analysis, a large amount of microplastic pieces and fabric fibers was noted, mostly in FV station (Figure 52).

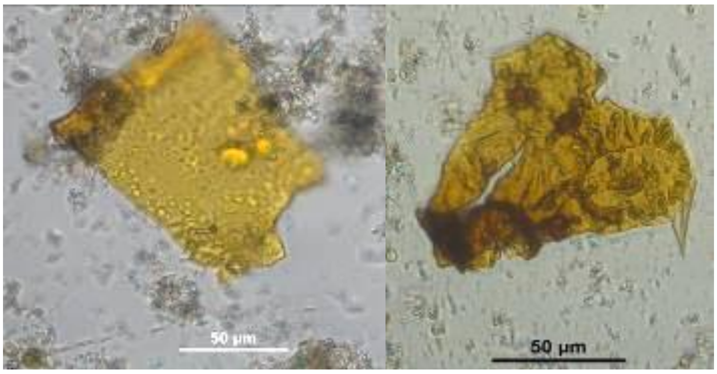


Figure 52. Examples of microplastics observed during microscope analysis.

Lago delle Nazioni is characterized by high chlorophyll-*a* concentration with the dominance of picoplanktonic species that were not quantified during the monitoring; among the micro-phytoplankton, the dominant group in the lake was represented by Dinophyceae (about 60%) followed by cryptophytes and diatoms. Total average abundances were 1.3 x 10^6 cell/L. The presence of algal cells with large dimensions was also recorder, like *Nitzschia* spp., *Dinophysis* sp., *Ceratium fusus* and dinocysts. In addition, it was observed a shift to zooplankton communities with the domination of small species (ciliates and rotifers), which feed on other resources, like bacteria or picoplankton. Furthermore, persistent blooming, mainly due to picocyanobacteria, resulted in typical hypereutrophication effects, such as: increase of turbidity, accumulation of organic matter in the water column and potential anaerobic condition in the bottom layer (Śliwińska-Wilczewska et al., 2018).

4.2.2. Macrobenthic communities in the Sacca di Goro

Macrozoobenthos (henceforth called only macrobenthos) is operatively defined as the invertebrates whose shortest dimension is greater than or equal to 0.5 mm. Macrobenthos includes a vast range of phyla such as Annelida, Mollusca, Crustacea and Cnidaria (Mistri et al., 2001; Munari & Mistri, 2008).

A variety of physico-chemical parameters and biological interactions can affect the macrobenthic communities. Sediment characteristics such as grain size and organic matter content have large influence on macrobenthic assemblages (Gage, 2001; Lalli & Parsons, 1997). In addition to the natural factors, anthropogenic disturbances such as nutrient loading, dredging, dumping of wastewater, land use and aquaculture, etc. can alter or modify the community assemblage and community structure of macrobenthos mostly in a detrimental sense. Many studies have shown the importance of macrobenthic communities in environmental quality assessment and monitoring (Boesch & Rosenberg, 1981.; Clarke & Ainsworth, 1993.; Munari et al., 2009.; Munari & Mistri, 2008., 2010.; Sacchi et al., 2013.).

4.2.2.1. Methods

Macrobenthos and sediment were sampled in four stations: Foce Volano (FV), Centro Sacca (CS), Gorino (GO) and Bocca Mare (BM; Figure 53) in two periods (October 2020 and March 2021). These stations were chosen to represent areas of the same sub-basins with different hydrographic and sedimentological characteristics; three of them are in common with those chosen for the phytoplankton study.

Figure 53. Location of the macrobenthos sampling stations in the Sacca di Goro.

Sampling Points	Latitude (North, WGS84)	Longitude (Est, WGS84)
FV	44°49'11"	12°16'23"
CS	44°49'44"	12°18'03"
GO	44°47'35"	12°21'51"
BM	44°47'47"	12°19'21"

Benthic macrofauna samples were sampled using a Wildo Box-corer (15×15 cm^2), sieved through a 500 µm mesh size, collected into pre-labelled, clean plastic jars and preserved with 90% Ethanol. A sediment sub-sample from the same Wildo Box-corer was simultaneously collected into a small plastic vial and stored immediately under 4°C after carrying to the laboratory for further sediment analysis. The benthos samples were pre-washed with regular tap water to rinse away the remaining mud, and the cleaned benthos specimens were collected into glass beakers added with 70% Ethanol. Then all these specimens were identified to the lowest possible taxonomic level and counted under the stereo microscope. Macrobenthic communities have been analysed by univariate indices (species richness (S), total abundance (N), species diversity as Hill's N1 and evenness as Hill's (N10) and multivariate methods. Hill's diversity index gives the equivalent number of species that would have been found in the sample if all the species have been equally abundant (Hill, 1973). Evenness indicates the distribution of the individuals among species, and ranges in value from 0 to 1 (1 = equally distributed).

Sediment samples were analysed for grain size and organic matter content. The grain size of the sediment was classified according to the Wentworth's Scale for clastic sediments (Wentworth, 1922): >250 µm → coarse sand, 250-63 µm → fine sand, <63 µm → silt and clay. Sediment fractions were separated by wet sieving, then weighed after drying (80°C for 24 h) and expressed as percentage.

Organic matter content, in percentage, was determined by the Loss on Ignition (LOI%) method drying at 80°C for 24 h and then incinerate at 450°C for 8 h (Ponti et al., 2007).

4.2.2.2. Results

A total of 25113 organisms were counted (9755 from October 2020 and 15358 from March 2021), and classified up to the farthest possible taxonomical level. The identified taxa belonged to four main phyla (Figure 55): Annelida (10 taxa), Arthropoda (9 taxa), Mollusca (12 taxa) and Cnidaria (1 taxon). The macrobenthic communities in Goro Lagoon are dominated by the spinoid polychaete *Streblospio shrubsolii* (68-92% of specimens), followed by the nereid polychaete *Hediste diversicolor* (0-14.6%), Oligochaetes (0.1-9.5%), capitellid polychaete *Heteromastus filiformis* (0-8.5%), bivalves: *Ruditapes philippinarum* (0-9%); *Lentidium mediterraneum* (0-4.4%); *Arcuatula senhousia* (0.03-3%), isopod *Cyathura carinata* (0-3.4%), and the amphipods: *Microdeutopus anomalus* (0-2.5%); *Corophium orientale* (0-4.8%), and the gastropod *Hydrobia* sp. (0-2.3%).

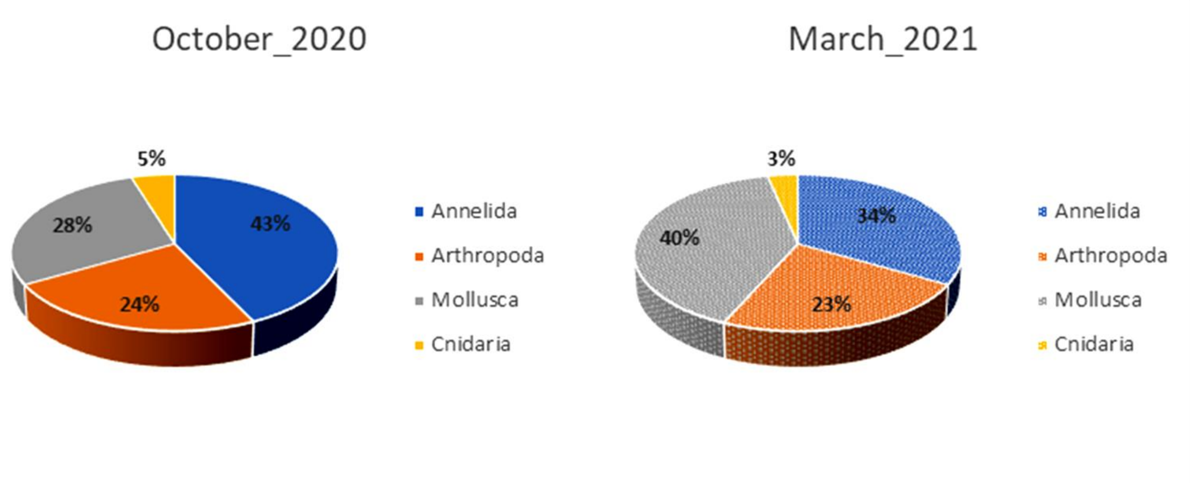


Figure 54. Total abundances as 'percentage per period' of the four main groups in each sampling period.

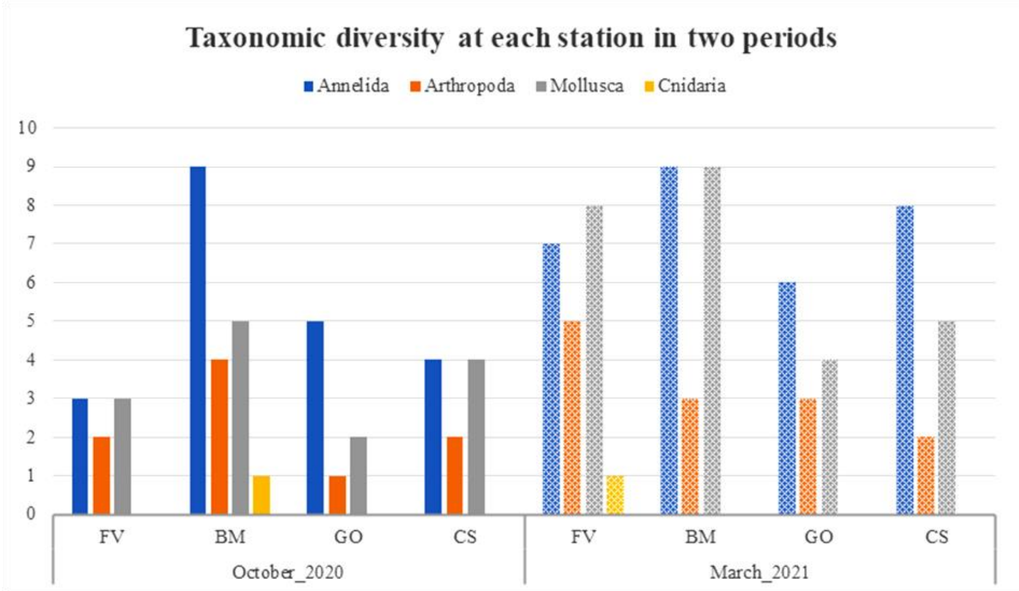


Figure 55. Total number of taxa belonging to the four main microbenthic groups (Annelida, Arthropoda, Mollusca and Cnidaria) present in each location (FV: Foce Volano, BM: Bocca Mare, GO: Gorino, CS: Centro Sacca) in each period (October 2020 and March 2021).

In FV, the number of taxa belonging to all phyla resulted higher in March 2021, compared to those found in October 2020 (Figure 54). In BM, a higher number of taxa in phylum Annelida in comparison with those found in the other stations were found for the two periods, while the number of taxa in phylum Mollusca increases in March 2021. Overall, the taxa distribution into four major phyla at BM in two different periods was different in composition, but more or less similar in the number of taxa. In station GO, phylum Annelida had a higher number of taxa in both sampling periods, followed by phylum Mollusca and a very little taxonomic diversity in phylum Arthropoda. Overall, the number of taxa was higher in March 2021, compared to October 2020. CS had an equally higher number of taxa in phyla Annelida and Arthropoda in October 2020, but phylum Annelida exceeded all the other identified phyla, in taxonomic distribution, in March 2021. Overall, the number of taxa was apparently higher in March 2021 than in October 2020, for station CS.

Species richness (S) ranged between 4±0 at FV in October 2020 to 14.3±1.3 at BM in March 2021. Mean Species richness resulted higher in FV and BM in March 2021; and at BM in October 2020. Total abundance (N) ranged from 20,222±1092 m^{-2} at GO in October 2020 to 91,933±2809 m^{-2} at FV in March 2021. Mean total abundance always resulted higher in March 2021 compared to October 2020. Species diversity given by Hill's N1 had a range from 1.39±0.15 at FV in October 2020 to 3.78±0.65 at BM in March 2021. The values evenness measure (Hill's N10) varied between 0.14±0.01 at BM in October 2020 to 0.37±0.02 at GO in October 2020.

Analysing the sediment grain size, Foce Volano in the westernmost part of the lagoon was characterized by the highest fraction of coarse sand, and relatively lower fractions of fine sand and mud. The Gorino, at the easternmost part of the lagoon was characterized by a higher fraction of silt & clay and lower fractions of sand. In the central part of the lagoon, Centro Sacca had a higher content of silt & clay and lower fractions of coarse sand and fine sand. Finally, Bocca Mare, at the lagoon entrance, was characterized by the highest fraction of fine sand of all stations, higher fraction of coarse sand but lower fractions of mud (silt & clay).

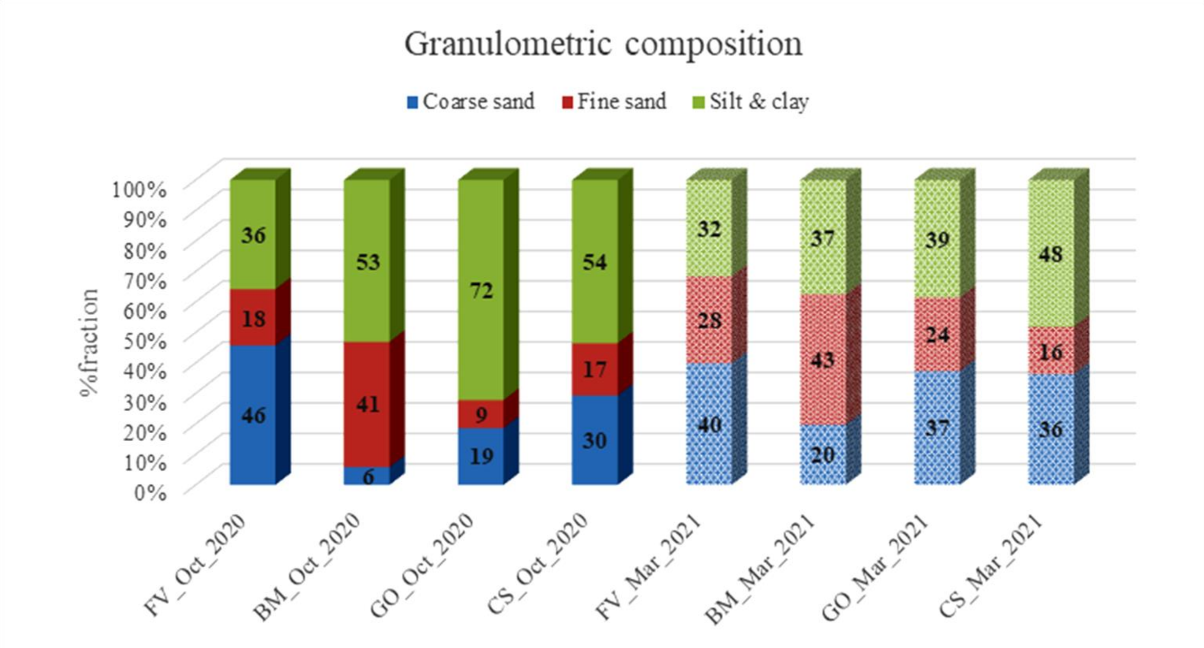


Figure 56. Grain size composition (mean (n=4) %fraction of grain types) at each station in two periods (FV: Foce Volano, BM: Bocca Mare, GO: Gorino, CS: Centro Sacca).

4.2.3. Hypoxic crisis in the Piallassa Lagoon

In the Piallassa Baiona hypoxic events are frequent, particularly during the summer, leading to transitory reductions in the number of species (Ponti & Abbiati, 2004). Hypoxia is functionally defined as [DO] <2 mg DO/L, it is a phenomenon that occurs when [DO] is sufficiently low to have negative (lethal or sub-lethal) effects on all organisms. We quantified in the field the effects of different timing regimes of hypoxia on the structure of benthic communities in the Piallassa Baiona.

4.2.3.1. Methods

We simulated hypoxic events of two, four and eight consecutive days acting on the benthic community and compared them with the responses to normoxic treatments. This allowed threshold responses of the benthic community to the hypoxic events but also on the extent of the normoxic period, ultimately comparing different timing regimes of hypoxia. The study was conducted on a shallow, spatially homogeneous subtidal mudflat in the northern part of the lagoon, which is the furthermost from the urban water discharges (Figure 57).

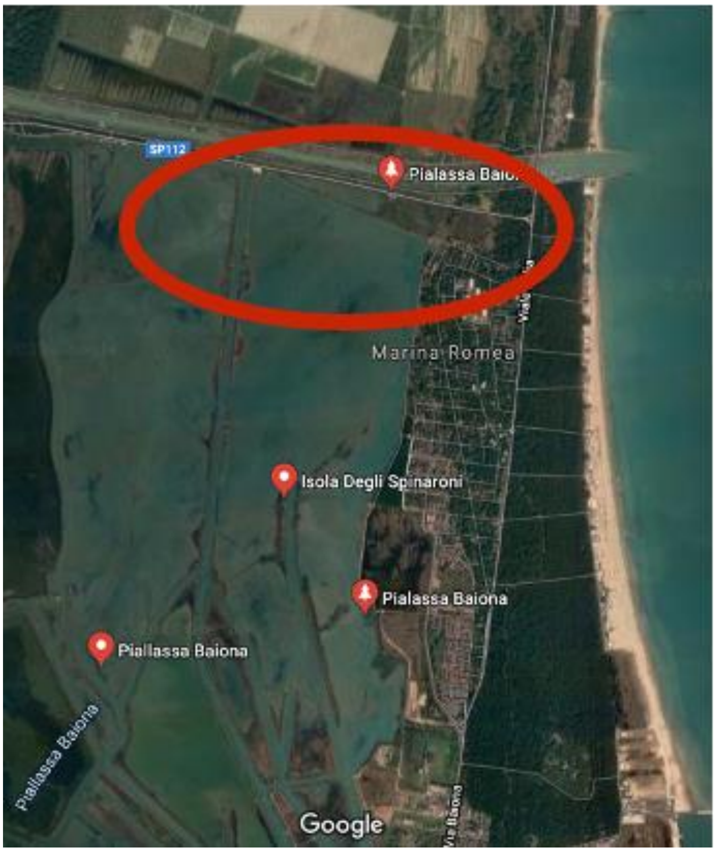


Figure 57. Experimental set up in Piallassa Baiona.

The benthic community was isolated using cylinders 15.5Cm x 20Cm in size (Figure 58). Hypoxic conditions were imposed by covering the treated cylinders with a black plastic bag while control cylinders were left uncovered. We created different timing regimes of hypoxia by manipulating the duration of hypoxia (2, 4, 8 and 20 consecutive days, Figure 59). At the end of each experimental trial, the benthic communities within each pot were retrieved, sieved in the field and subsequently analysed in the laboratory where organisms were identified and counted.

Figure 58. Cylinders used to manipulate oxic conditions in Piallassa Baiona.

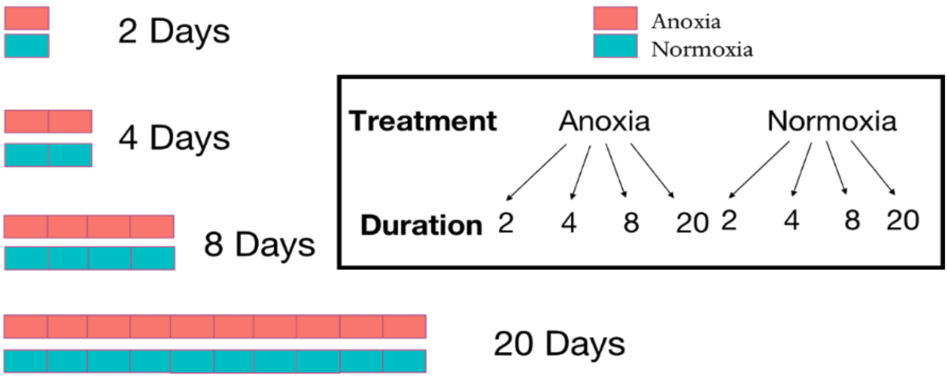


Figure 59. Experimental design to test the effects of duration of anoxia on the benthic community.

4.2.3.2. Results

During the experiment, the seawater temperature ranged between 27.6ºC and 31.1ºC. We found a total of 29355 organisms. When the number of consecutive days of hypoxia increased (2-4-8 or 20 consecutive hypoxic days), the abundance of the benthic fauna decreased (Figure 60). 20 days consecutive days of hypoxia caused almost complete mortality in the benthic community.

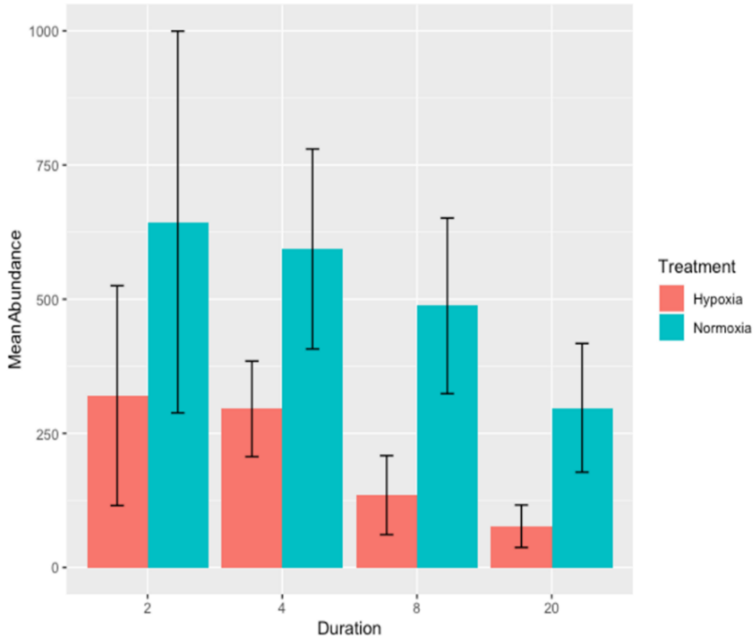


Figure 60. Results of the effects of anoxia on the benthic community.

4.3. P3 - Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT)

4.3.1. Chemical data collected 2000-2018

UniSalento collected obtainable data regarding the chemical parameters of Pilot Area 3, organizing them with metadata. Data were collected from Final Reports and/or dataset of:

- Surface Water body monitoring service of Arpa Puglia (from 2010 to 2018);
- ECODO-NET INTERREG IIIA/ Greece – Italy project (September 2007 – April 2008).

Other data were collected from 2 papers:

- Dell'Anno A., Mei M. L., Ianni C. and Danovaro R. "Impact of bioavailable heavy metals on bacterial activities in coastal marine sediments". *World Journal of Microbiology & Biotechnology.* 2003, 19, p. 93-100;
- Matteucci G., Magagnini M., Armeni M., Giaccaglia L., Fiesoletti F., Ciotti C., Pari P., Riccio S., Rossini P., Ambrosini P., Buongarzone E., Patata L., Trovarelli L. and Tentoni P. "Ecological assessment of a Marine Coastal Area Affected by a Power Plant Water Discharge (Brindisi, Adriatic Sea). *The Open Environmental & Biological Monitoring Journal.* 2011, 4, p. 45-56.

4.3.1.1. Sample collection and preparation of chemical data

Surface water and sediment samples were collected, both in ECODO-NET project and ARPA monitoring service, following the instructions present in: APAT 46/2007, CNR-IRSA and ISPRA-ICRAM guidelines. For ECODO-NET project, sampling points were placed along Canale Reale, inside and outside the MPA.

Dell'Anno et al. collected sediment samples with a multicorer (6 cm inner diameter) Van Veen grab for heavy metal analyses.

Matteucci et al. collected water samples with a "Nyskin type" bottle. Sediment samples were collected with a Van Veen grab.

4.3.1.2. Method description for the determination of chemical data

- ECODO-NET INTERREG IIIA/ Greece – Italy project

In the laboratory, each water sample, after pH measurement, was filtered with a Whatman Schleicher & Schuell 0.45 µm syringe filters. Part of the filtered samples were brought to pH ≤ 2 with concentrated HNO_3. For the simultaneous detection of heavy metals, ASV in pulsed differential mode, as described in the Metrohm 231 method, has been used. For the measurement, 10 ml of sample were added with 1 ml of 0.5 M acetate buffer (pH = 4.6 ± 0.2) with 1.5 M KCl as support electrolyte. The concentrations of the metals were determined by the method of standard additions.

- ARPA monitoring service

Analysis were carried out by ARPA with the protocols shown in the following table:

	TRANSITIONAL and COASTAL MARINE WATERS	COASTAL and TRANSITIONAL SEDIMENTS	BIOTA
Heavy metals	UNI EN ISO 17294-2:2005	EPA 3051-EPA 200,8	UNI EN 13804-13805-15763 (Hg - EPA 7473)
Chlorinated and organophosphate pesticides, IPA and phytosanitary products	RAPPORTI ISTISAN 07/31	EPA 3550/C - EPA 8270	EPA 3545-3640a-8270d
Chlorinated and aromatic solvents	UNI EN ISO 15680:2005	EPA 8260	EPA 5021a+ 8260c
Phenols (pentachlorophenol)	ICRAM 2008 - APAT CNR-IRSA 5090	ICRAM 2008 - APAT CNR-IRSA 5090	EPA 3545-3640a-8270d
Alkylphenols	ICRAM 2008	ICRAM 2008	EPA 3545-3640a-8270d
Carbon tetrachloride	EPA 8260 r.3 2006	EPA 8260	EPA 5021a+ 8260c
PCBs	ICRAM 2008 - EPA 8082/A	EPA 3545/A - EPA 8270	
Phthalates	EPA 3535-EPA 8270c 1996	EPA 3550/C - EPA 8270	

Brominated diphenyl ethers	EPA 1614		EPA 1614
Organotin compounds	Internal method (GC/MS)	Internal method- isotope dilution	Internal method- isotope dilution
Urea herbicides	APAT IRSA-CNR man.29/2003 n.5050		
Dioxins		EPA 1668	

- Dell'Anno et al.

For total heavy metal analysis, sediments were treated with aqua regia in a microwave oven. After centrifugation, the supernatant was analysed by ICP-AES (Cu, Pb and Cr) and ETA-AAS (Cd). Heavy metal speciation was carried out by a selective extraction procedure according to Ph. Quevauviller et al, 1997.

- Matteucci et al.

Water samples for the determinations of inorganic nutrients were filtered immediately after collection through a cellulose acetate filter (0.45 µm) and stored at -20 °C until analyses. Surface sediments (0-2 cm layer) were acid digested and solutions analyzed by ICP-AES for metal determinations.

4.3.1.3. Research results of chemical data

Priority substances

Among the available data those collected by ARPA are the most regular. Data collected in the Surveillance monitorings in 2010-2011 and in 2016 and during the Operational monitorings in 2012-2013, 2013-2014, 2014-2015, and 2017 are available for the river Canale Reale (which output is in MPA Torre Guaceto), the transitional waters Torre Guaceto, Punta della Contessa, Cesine (which is north of the coasts of the municipality of Melendugno), Alimini Grande (which is south of the coasts of the municipality of Melendugno), and for the coastal waters T.Canne-Limite Nord MPA T.Guaceto, MPA Torre Guaceto, Limite Sud MPA T.Guaceto-Brindisi, Brindisi-Cerano, Cerano-Cesine, Cesine-Alimini.

In the following tables there are the chemicals which were not below the maximum permissible concentrations.

	Annual average (SQA-MA)	Maximum permissible concentration (SQA-CMA)
2010-2011		Hg = 0.07 µg/L
2012-2013	Benzo(a)pyrene = 0.6 µg/L	Benzo(a)pyrene = 1.8 µg/L
2013-2014	Hg = 0.10 µg/L	Hg = 1.60 µg/L
2014-2015	Cd = 0.37 µg/L	Cd = 3.3 µg/L; Hg = 0.14 µg/L
2016	Trichloromethane = 3,9 µg/L	

2017	Hg = 0,46 µg/L

Table 6. Canale Reale river, chemicals which were not below the maximum permissible concentrations and relevant concentration (data from ARPA Puglia).

	Water- Annual Average (SQA-MA)					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
Torre Guaceto	PBDE=0.0027 µg/L			Cd= 1.0 µg/L		
Punta della Contessa		As= 19.5 µg/L	As= 5.61 µg/L	As= 9 µg/L	As= 7.60 µg/L	As= 9 µg/L
Cesine			Cd= 0.3 µg/L			
Alimini Grande						

	Water- Maximum permissible concentration (SQA-CMA) (µg/L)					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
Torre Guaceto						
Punta della Contessa						
Cesine						
Alimini Grande						benzo(g,h,i)perylene= 0.001 µg/L

	Sediment - Annual Average (SQA-MA) (µg/kg)					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
Torre Guaceto	Fluorantene= 202 µg/kg p.s.		DDT= 5 µg/kg p.s.; DDE= 5 µg/kg p.s.			
Punta della Contessa			Benzo(a)pyrene = 88 µg/kg p.s.; Benzo(b)fluoranthene= 50 µg /kg p.s.; Benzo(k)fluoranthene= 31 µg/kg p.s.; Anthracene= 724 µg/kg p.s.; Hexachlorobenzene= 7,8 µg/kg p.s.			Pb= 191 mg/kg p.s., As = 15 mg/kg p.s.

Cesine	Pb= 45 mg/kg p.s.
Alimini Grande	

Table 7. Chemical status of transitional waters: chemicals which were not below the maximum permissible concentrations and relevant concentration (data from ARPA Puglia); p.s.= dry weight.

	Water- Annual Average (SQA-MA)					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
MPATorre Guaceto	Hg= 0.02 µg/L					
Lim. sud MPA T. Guaceto-Brindisi	Hg= 0.02 µg/L					
Brindisi-Cerano						Tributyltin = 0.001 µg/L
Cerano-Cesine						
Cesine-Alimini						
	Water- Maximum permissible concentration (SQA-CMA) (µg/L)					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
MPA Torre Guaceto	Hg= 0.08 µg/L					
Lim. sud MPA T.Guaceto-Brindisi	Hg= 0.08 µg/L					
Brindisi-Cerano						Tributyltin = 0.006 µg/L
Cerano-Cesine						
Cesine-Alimini						
	Sediment - Annual Average (SQA-MA) (µg/kg)					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
MPA Torre Guaceto		Cd= 0.4 mg/kg p.s.				As = 17 mg/kg p.s
Lim. sud MPA T.Guaceto-Brindisi						
Brindisi-Cerano		As= 23 mg/kg p.s.; Tributyltin =20 µg/kgp.s	As= 20 mg/kg p.s.;		As= 43 mg/kg p.s.	

Cerano-Cesine	As= 38 mg/kg p.s.; Cd= 0.4 mg/kg p.s.; Tributyltin = 20 µg/kg p.s.	As= 28 mg/kg p.s.;
Cesine-Alimini		

Table 8. Chemical status of coastal waters: chemicals which were not below the maximum permissible concentrations and relevant concentration (data from ARPA Puglia); p.s.= dry weight

Data collected by ARPA showed high levels of Arsenic in Salina Punta della Contessa water samples. This is a long-lasting contaminant as also in 2003 Dell'Anno et al. underlined the presence of Arsenic in Salina Punta della Contessa sediment sample.

ECODO-NET results for inner and outer areas of Torre Guaceto MPA showed for priority substances a value lower than the threshold envisaged by the Italian and European legislation.

Among the other priority substances that were detected at concentration higher than threshold there are Cadmium, Lead and organotin. Deserve attention Canale Reale river whose water also contains mercury above threshold in different surveys. As to the organic priority substances there are not continuous record of exceeding the threshold in water and sediments collected in the reference period in the area in which pilot P3 is found. In particular, Trichloromethane, Benzo(a)pyrene and polybrominateddiphenylethers have been found in water samples whereas DDT, DDE, Benzo(a)pirene, Benzo(b)fluorantene, Benzo(k)fluorantene, Antracene, Esaclorobenzene are the substances exceeding the thresholds in sediments. Details can be found in tables 6, 7 and 8.

For what concern the coastal marine area of Melendugno Municipality, data on the chemical parameters could also be gathered from the offshore survey "ST12904 Trans Adriatic Pipeline – Environmental Survey" carried out by Trans Adriatic Pipeline (TAP) in 2012. This document is available on the Italian Ministry of the Environment's website (https://va.minambiente.it/en-GB/Oggetti/Documentazione/625/841?pagina=3).

In particular, on considering the pilot P3 area, only data relevant to the sampling point within 2 km from the coast and reported in Section 4 of the TAP route were reviewed.

Water samples had total petroleum hydrocarbons (TPH) concentrations lower than the Limit of Detection (<0.1 µg/L). Regarding the concentrations of dissolved metals in water matrix, Cd and Hg values were lower or close to the limit of detection (LOD) (0,1 µg/g) at all sampling points. The concentrations for Ba generally ranged from 8 to 12 µg/g, except for a water sample close to the seabead (ENV_W_37), where a Ba concentration of 43.6 µg/g was recorded. Fe concentrations were generally lower than the LOD (5 µg/g). One point (ENV_W_49) was peculiar as showed a Fe concentration of 98.0 µg/g.

For sediment samples, the total hydrocarbon (TPH) concentrations generally ranged from 10 to 20 µg/g for each sampling points, except for ENV_S_40 station where a concentration of 34,1 µg/g was recorded and for TAP2017 and TAP2019 sampling points where concentration lower than 5 µg/g were recorded. Alkanes concentration values generally ranged from 5 to 8 µg/g for every stations, except for ENV_S_40 where a concentration of 1,397 µg/g was recorded.

For all stations, Polycyclic aromatic hydrocarbons (PAH), PCBs and Organochloride compounds concentrations detected in sediment samples were lower than the LOD. The higher concentrations of Hexachlorobenzene in sediment samples were found in ENV_S_39 and ENV_S_46 sampling stations (0,85 ng/g and 0,86 ng/g respectively), about one hundred-fold the lower concentration recorded in ENV_S_48 station (0,01 ng/g).

Concentrations of metals detected in sediment samples are shown in Table 9.

STATION	Al	As	Ba	Cd	Cr	Cu	Fe	Hg	Ni	Pb	V	Zn
ENV_S_36	53000	3.3	113	<0.1	16	4.3	11321	0.12	8.9	5.1	32	31
ENV_S_37	24000	10.6	292	<0.1	60	15.0	29167	0.21	31.3	17.3	90	88
ENV_S_38	27000	8.3	222	<0.1	35	9.3	20370	0.22	18.5	10.4	57	59
ENV_S_39	35000	8.0	186	<0.1	49	12.6	18571	0.23	24.3	14.0	56	60
ENV_S_40	33000	7.0	212	<0.1	36	9.1	21212	0.21	18.2	10.5	61	62
ENV_S_41	24000	11.3	271	<0.1	42	12.1	16250	0.19	25.0	14.8	46	63
ENV_S_42	14000	18.6	332	<0.1	50	12.9	22500	0.29	29.3	17.5	68	82
ENV_S_43	25000	11.4	220	<0.1	50	13.2	19400	0.18	26.0	14.6	60	64
ENV_S_44	19000	17.4	316	<0.1	42	13.4	18947	0.13	23.9	17.6	53	71
ENV_S_45	56000	6.0	107	<0.1	20	5.6	8929	0.11	10.7	6.2	26	25
ENV_S_46	9200	16.8	402	<0.1	65	13.0	12500	<0.05	48.4	17.9	38	39
ENV_S_47	20000	11.8	475	<0.1	50	10.0	14250	<0.05	25.0	17.5	43	43
ENV_S_48	8500	11.2	1118	<0.1	41	6.5	18824	<0.05	22.9	18.8	55	50
ENV_S_49	16000	9.7	500	<0.1	41	8.8	16250	0.25	22.5	15.6	44	41
ENV_S_49b	17000	2.9	412	<0.1	14	2.6	12059	0.18	7.1	4.7	35	35
TAP2017	24000	15.2	396	<0.1	50	12.9	13958	<0.05	29.2	18.3	44	42
TAP2019	35000	1.7	314	<0.1	8	1.0	2571	0.05	3.0	5.0	9	5

Table 9. Concentrations of dissolved metals in sediment samples of the coastal marine area of Melendugno municipality (data from TAP).

4.3.2. Biological data collected 2000-2018

UniSalento collected obtainable data regarding the biological parameters of Pilot Area 3, organizing them with metadata. Data were collected from Final Reports and/or dataset of:

- Prosecuzione del Monitoraggio Marino-Costiero antistante il Polo Industriale di Brindisi (*Continuation of the Marine-Coastal Monitoring in front of the Industrial Pole of Brindisi*)

- Proposta di classificazione dei Corpi Idrici Superficiali (C.I.S.) nella regione Puglia: analisi integrata a chiusura del primo ciclo triennale di monitoraggio ai sensi del D.M. 260/2010 (*Proposal for the classification of Surface Water Bodies (C.I.S.) in the Apulia region: integrated analysis at the end of the first three-year monitoring cycle pursuant to D.M. 260/2010)*
- "Evaluation of transitional coastal environment health status: Torre Guaceto", Luigi Palmisano Master Thesis

4.3.2.1. Marine area in front of Saline di Punta della Contessa

Marine Plankton

The high biodiversity of zooplankton (2016-2018) and the absence of significant differences between the areas in question allow us to qualify the water column in front of Saline di Punta della Contessa as devoid of obvious environmental problems. Thanks to their capacity for turnover and persistence in the water column, zooplankton communities do not seem to suffer from any anthropogenic impacts (often linked to phenomena of sediment resuspension and silting) which otherwise can affect benthic communities. As regards phytoplankton, the concentrations of chlorophyll a (mg/m^3) in all sampled sites are within the optimal levels, as well as the ISS-Phyto indices (which integrate biomass values, taxonomic composition and size classes of phytoplankton cells) allow to attribute a rank of ecological status varying between "excellent" or good (starting from 2017). In the transect of Brindisi it is worth noting the presence of *Ostreopsis spp.* with an abundance of less than 1%. It is a toxic micro-alga of tropical and subtropical origin that has not been present in the Mediterranean for many years now. The toxin can reach humans (by inhalation of marine aerosols or by contact) which can potentially cause a parainfluenza syndrome (not lethal to humans). The presence of these species is not to be attributed to the anthropic activities in existence in the coastal sector of Brindisi.

Marine Benthos

The analysis of sessile macrobenthic populations has highlighted significant structural and functional differences. These differences, however, do not seem to be attributable to sources of impact: the differences in terms of taxonomic composition and relative abundance of the species are mainly determined by the ecological, edaphic and geomorphological characteristics of the different habitats. Particular attention has been given to particularly important animal taxa as environmental descriptors, such as polychaete annelids, or to taxa of producing organisms such as macroalgae or seagrass *Posidonia oceanica*. The *P. oceanica* meadows in the coastal sector examined revealed a good state of health in the vicinity of the Cerano sector or of weak regression referable to large-scale anthropogenic impacts (e.g. marine garbage, increased sedimentation) which may originate in the central and northern sectors of the Adriatic Sea. An impoverishment of the populations in the control sector manifested itself through the analysis of photographic transects carried out on the coral habitat in 2019. The control sector also appears less diversified here than in the Brindisi and Cerano sectors. Still in the control site, however, the presence of a species of colonial madrepora, the *Cladocora caespitosa*, was reported during 2018 and 2019, which was included by the IUCN (international commission for the conservation of nature) in the red list of endangered species. of extinction, and therefore deserving of protection. This species is usually favored in areas of the Mediterranean with moderately turbid waters but is very sensitive to excessive sedimentation, trophic load and temperature increase. For this reason, monitoring the growth rates and secondary production of this coral reef and the mini-coral reefs it forms will allow us to obtain a sensitive indicator of the health of the marine sector in question.

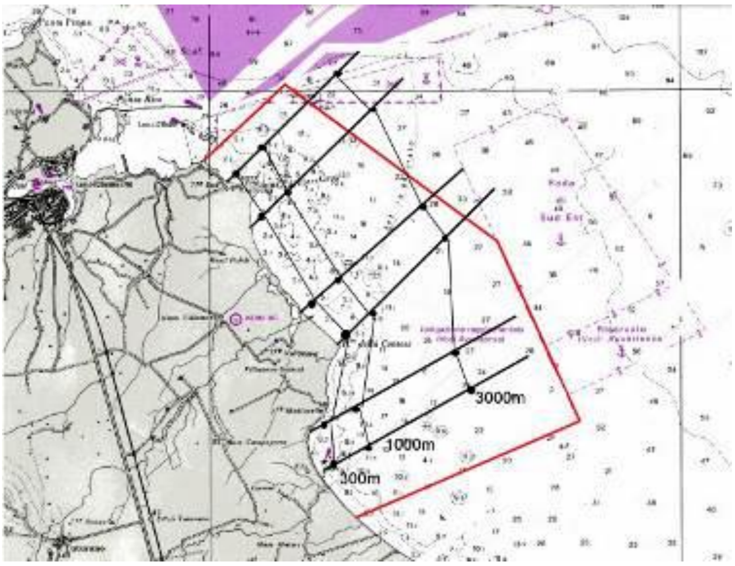


Figure 61. Sampling design used by the project "Prosecuzione del Monitoraggio Marino-Costiero antistante il Polo Industriale di Brindisi" (Continuation of the Marine-Coastal Monitoring in front of the Industrial Pole of Brindisi).

4.3.3. Torre Guaceto

According to different sources, the transitional water area of Torre Guaceto has been classified either with Good (Ponti et al 2008) or Bad (Proposta Classificazione CIS) environmental status, while the marine area has been classified as Sufficient status.

4.3.3.1. Transitional water macrozoobenthos

The analysis has been conducted on the Macrozoobenthos_CadsesProject_2004_2005 dataset, representative of 4 sampling stations for benthos located in the transitional waters located along the coast of the Torre Guaceto Area (Figure 62).

Figure 62. Sampling sites of the CADSES project and Phytoplankton_Torre_Guaceto_2005_2006 dataset within Torre Guaceto.

The transitional water macrozoobentic population of Torre Guaceto is mainly made up of small gastropods (Ecrobia, Bythinia, Theodoxus), insect larvae (mainly *Chironomus plumosus*) and the amphipod *Gammarus insensibilis*. The results obtained by the CADSES project show transitional water macrozoobentic populations characterized by high richness and diversity in species, a trait usually associated with good environmental conditions, with the exception of the site igua114. Species richness tends to be higher in spring than in fall. At the opposite, species diversity tends to be higher in fall than in springtime (Figure 63).

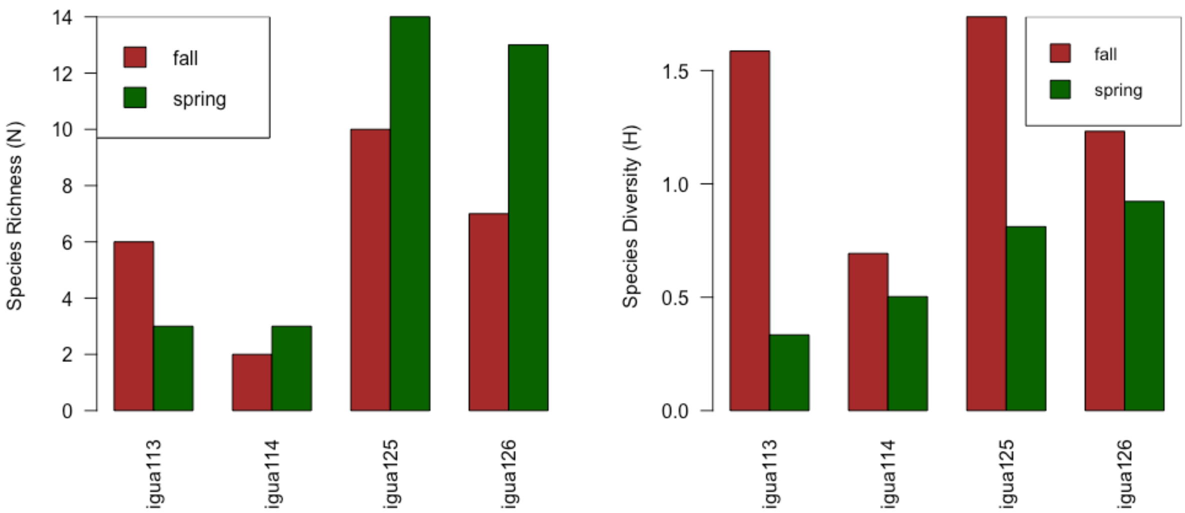


Figure 63. Species Richness and Diversity of Torre Guaceto Transitional Water Macrozoobenthos across different season.

Generally, the abundance of macrozoobenthos is way higher in spring than in fall due to large contribution of mainly small individuals (Ecrobia). The stations igua125 and igua126 are characterized by high individuals' abundance and overall biomass, while only few individuals were sampled in the stations igua 113 and igua 114. Due to the abundance of small individuals, the average individual body mass of the macrozoobenthic community is lower in the igua125 and igua126 (Figure 64).

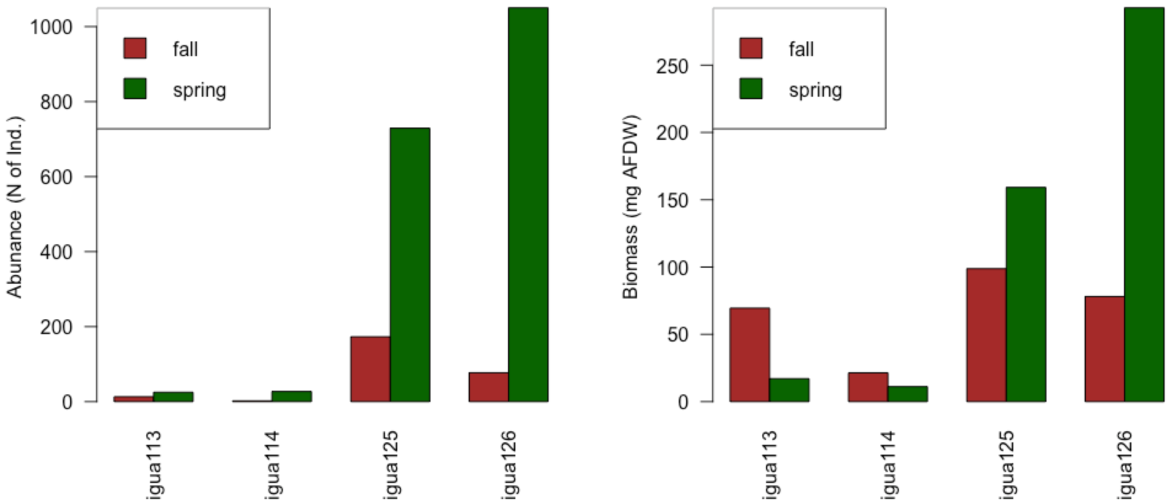


Figure 64. Individual abundances and overall biomass of Torre Guaceto Transitional Water Macrozoobenthos across different season.

4.3.3.2. Marine phytoplankton

The analysis has been conducted on the "Evaluation of transitional coastal environment health status: Torre Guaceto» dataset, representative of 4 sampling stations for benthos located along the Torre Guaceto Area (Figure 65).

The marine water phytoplankton population in the Torre Guaceto Area is mainly made up of Phytoflagellates, Cryptophicea and Chaetoceros. The species Richness and Diversity are higher during the months of September and November and lower in January. The stations m1 and m2 are generally richer in species than the the stations l1 and l2, but not necessarily more diverse (Figure 65) Overall, the diversified phytoplankton community observed in Torre Guaceto is indicative of a good environmental status

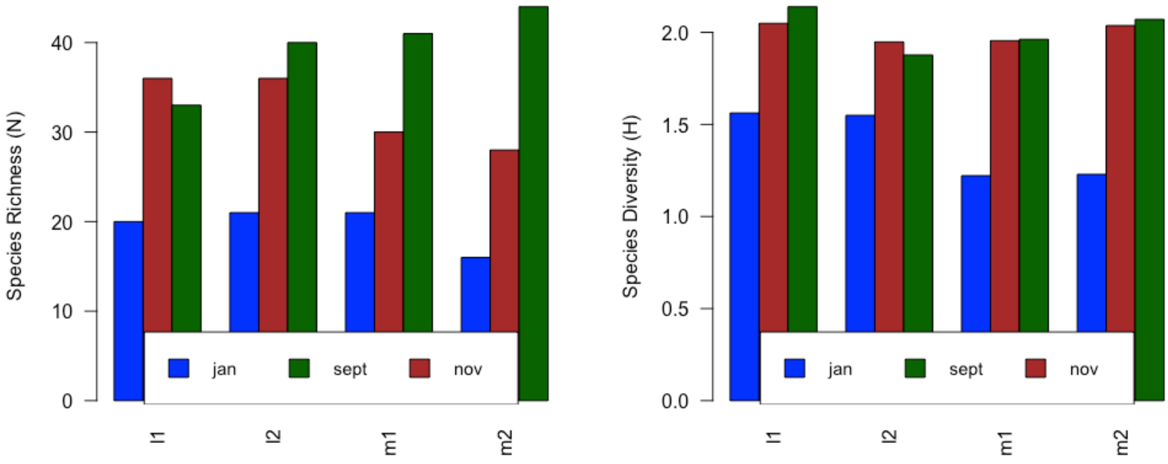


Figure 65. Species Richness and Diversity of Torre Guaceto marine phytoplankton across different months.

The phytoplankton cells' overall biovolume and average individual body mass both peak in September due to the abunances of large individuals belonging to the genus Tripos, Guinardia, Dactyliosolen, Ceratium and Prorocentrum (Figure 66).

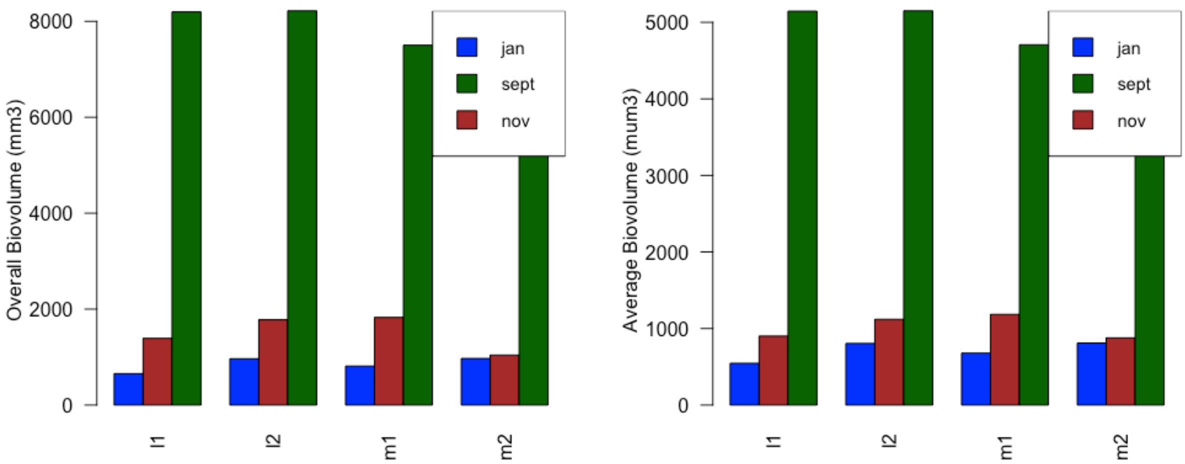


Figure 66. Overall and average biovolume of Torre Guaceto marine phytoplankton across different months.

4.4. P4 - Neretva River

4.4.1. Metals (cadmium, copper, lead, mercury, zinc) in sediment

Hazardous substances can endanger the balance of ecosystems consequently health of human marine organisms in the food chain. Also, river ecosystems are under pressure from several different impacts. Among these, inorganic and organic pollutants are anthropogenic pressures that impacts marine environment and contribute the overall degradation of the ecological status of the aquatic environments.

The main sources of pollution include different industrial activities, untreated effluents from municipal waste waters and intensive agriculture.

In general, contamination with hazardous substances can be:

- risky for the aquatic environment or human,

- adverse effects on the marine environment or the quality of products for human consumption.

In order to assess the ecological status of the Neretva river system, in this report, metals and pesticide in sediments and biota, as well as content of suspended particulate matter (SPM) were studied.

Analyses of trace metal concentrations in digested samples were carried out with an atomic absorption spectrometer (Analyst 800, Perkin-Elmer, Shelton, CT, USA), using graphite furnace (Cd, Cr, Pb and Ni) or flame (Cu, Fe, Mn and Zn) atomic absorption techniques.

The samples are also monitored for organochlorine pesticide residues (Lindan, DDTx, PCBs) using gas chromatography with electron capture detection (GC-ECD).

Furthermore, gravimetric methods are the earliest and most widely used technique for analysis suspended particulate matter. In assessing a water quality dissolved oxygen is measured in sea water samples. Dissolved oxygen refers to the level of free, non-compound oxygen present in water or other liquids. It is an important parameter in assessing water quality because of its influence on the organisms living within a body of water. A dissolved oxygen level that is too high or too low can harm aquatic life and affect water quality.

During the last few decades, rapid urbanization and industrial development have provoked some serious concerns for the aquatic environment. About 80% of the world population is facing an increasing threat regarding water security because sediments in most of the urban rivers have been contaminated by heavy metals. It has been well documented that surface sediment acts as a sink of various contaminants and poses a risk to water quality through biogeochemical exchanges with the overlying water body. Cadmium (Cd), chromium (Cr), copper (Cu) and lead (Pb) are some of the most common heavy metal pollutants in the environment. However, these metals from natural and anthropogenic sources may enter into the aquatic environment and pose serious threats due to their toxicity persistence, and bioaccumulation. Usually, in unaffected environments, the concentration of most of the heavy metals is low and mostly derived from the mineralogy and weathering. Sources such as industrial effluents, agricultural runoffs, transport, burning of fossil fuels, animal and human excretions, geologic weathering, and domestic waste contribute to the accumulation of heavy metals in the water bodies.

4.4.1.1. Sample collection and preparation

Sampling of sediment is carried out once per year. Sediment samples were collected from different locations of the Neretva River. Surface sediment samples (0-2 cm) are collected in plastic sampling bags and stored at -20°C until the further analysis.

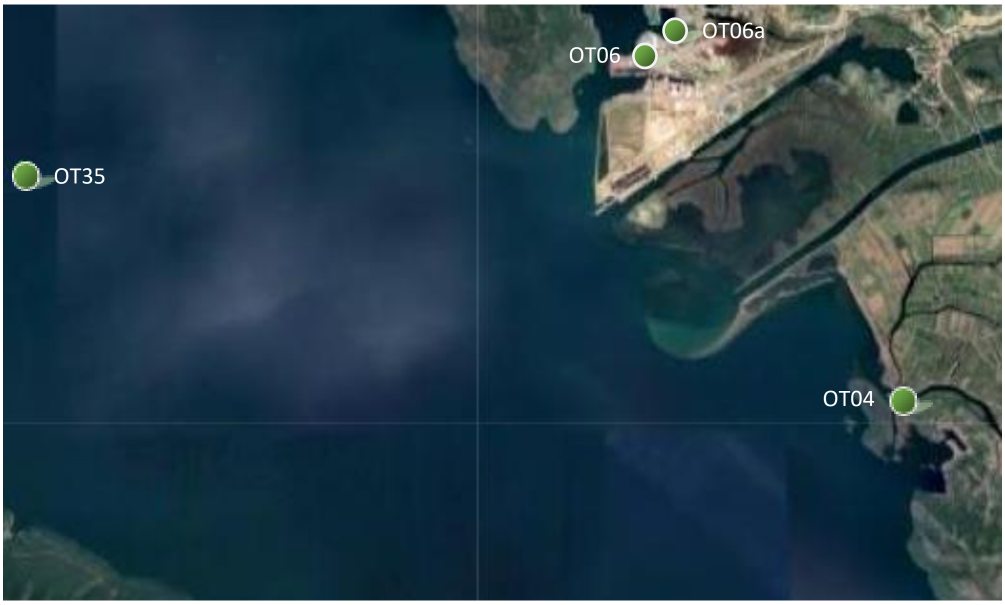


Figure 67. Sediment sampling points.

Sampling points	Bounding Coordinates - west	Bounding Coordinates - east	Bounding Coordinates - north	Bounding Coordinates - south
OT04	17.47027	17.47051	43.00833	43.00841
OT05	17.41333	17.41368	43.02500	43.02515
OT06a	17.43305	17.43309	43.05083	43.0588
OT35	17.328425	17.328431	43.033458	43.033460

Table 10. Longitude and latitude of the sediment sampling points.

4.4.1.2. Method description for determination of metals in sediment samples

Sediment samples are freeze-dried and sieved. Fractions <2 mm are used for further trace metal analysis. Closed vessel microwave digestion with nitric acid and hydrofluoric acid is used for sample wet digestion.

Atomic absorption spectroscopy method (graphite furnace or flame atomization technique) is applied for trace metal analysis in digested samples. Cold vapor atomic absorption technique is used for mercury analysis. Methods are validated by determination of precision (reproducibility, repeatability), accuracy and detection limits. Quality and accuracy of the analytical procedure is controlled by analysis of Certified Reference Materials (CRMs).

4.4.1.3. Research results

Effects Range Low (ERL) is assessment criteria which is used to assess the status of metal concentrations in sediment.

ERLs were developed by the United States Environmental Protection Agency for assessing the ecological significance of sediment concentrations.

ERLs are available for the following metals:

	ERL (mg/kg d.w.)
Cadmium	1.2
Copper	34
Mercury	0.15
Lead	47
Zinc	150

In the period from 1996-2019, the heavy metal contamination of surface sediment samples, collected from 4 stations in the Neretva River, was studied to evaluate their distribution and potential ecological risk. The concentrations of the studied metals decreased in the order Zn>Pb>Cu>Cd>Hg. The Effects Range-Low (ERL) criteria, proposed by the National Oceanic and Atmospheric Administration (NOAA), was used to evaluate the status of metal contamination in sediment. The concentrations of heavy metals in sediment from most of the sampling sites didn't exceeded the proposed ERL values, with the exception of Pb and Zn concentrations in sediment collected at OT05 sampling station. Concentration above ERL may cause adverse effects in marine organisms, therefore, elevated levels of Pb and Zn, in sediment in the OT05 sampling area, may pose a potential risk to marine organisms.

Spatial variations in metal concentrations most likely reflect differences in sources and input rates of heavy metals as well as physicochemical properties of sediment and a variety of environmental factors influencing the metal accumulation in sediments.

The concentration of cadmium in the sediment ranged from 0.187 mg/kg to 0.474 mg/kg, with an average value of 0.315 mg/kg.

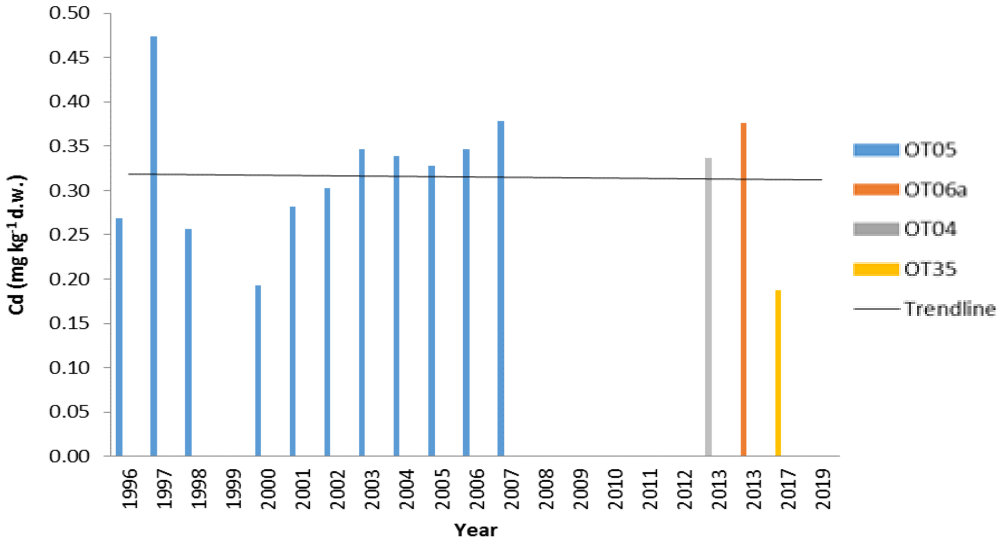


Figure 68. The concentrations of cadmium in sediment samples at OT05, OT06a, OT04 and OT35 stations in period 1996-2019.

The concentrations of lead ranged from 24.37 mg/kg to 65.78 mg/kg, with an average value of 38.02 mg/kg.

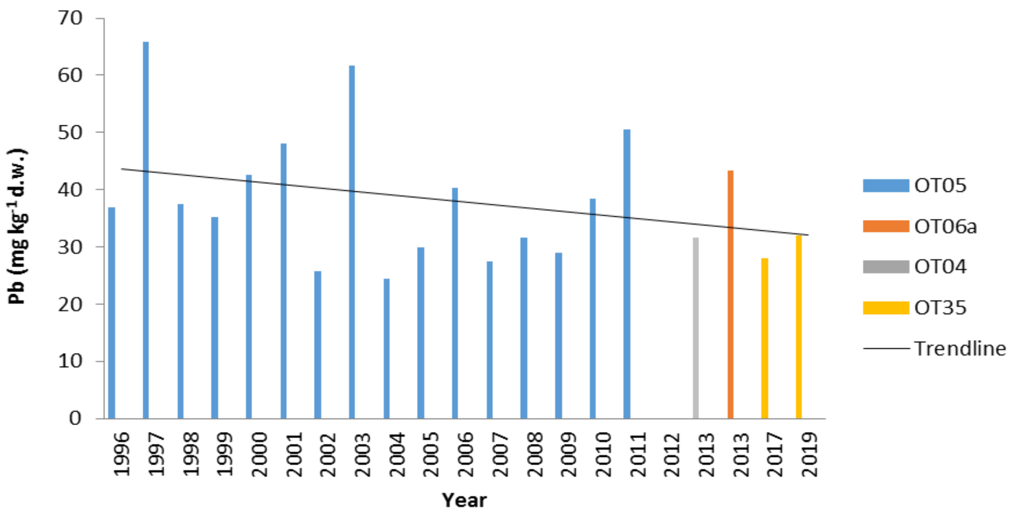


Figure 69. The concentrations of lead in sediment samples at OT05, OT06a, OT04 and OT35 stations in period 1996-2019.

The concentrations of zinc in the sediment ranged from 68.07 mg/kg to 203.14 mg/kg, with an average value of 109.29 mg/kg.

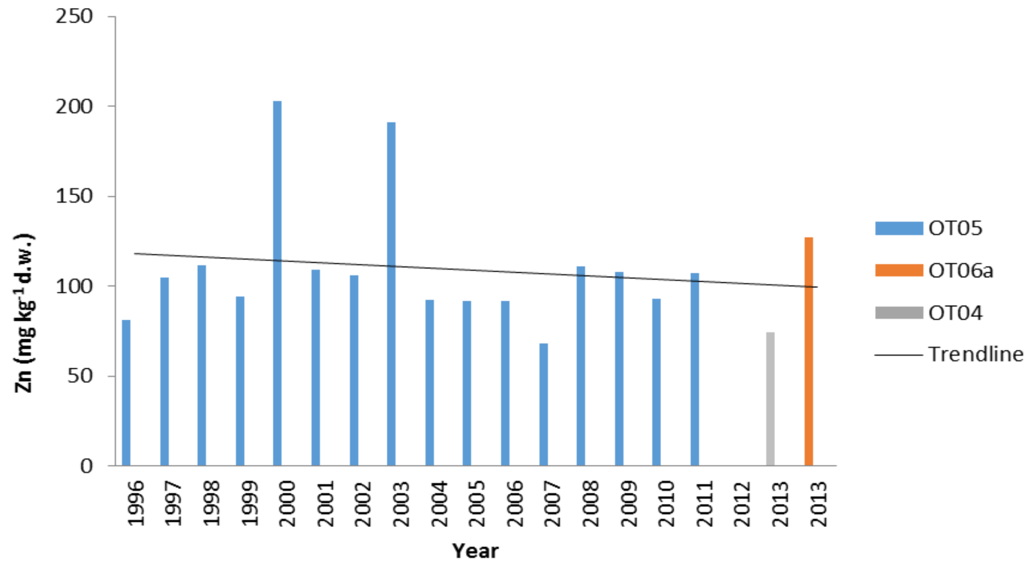


Figure 70. The concentrations of zinc in sediment samples at OT05, OT06a and OT04 stations in period 1996-2013.

The concentrations of copper in the sediment ranged from 19.20 mg/kg to 31.60 mg/kg, with an average value of 25.90 mg/kg.

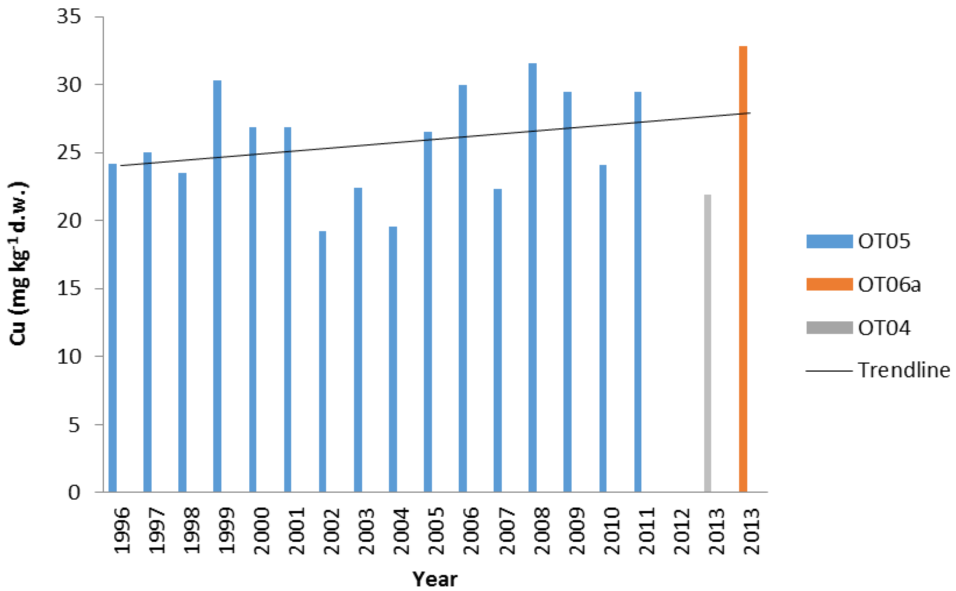


Figure 71. The concentrations of copper in sediment samples at OT05, OT06a and OT04 stations in period 1996-2013.

In 2013. at station 0T04 the value of **total mercury content** was 0.078 µg/kg, while at station OT06a the value of **total mercury content** was 0.167 µg/kg. Furthermore, in 2017. and 2019. concentrations of **total mercury content** were similar (0.06 µg/kg in 2017. and 0.07 µg/kg in 2019.).

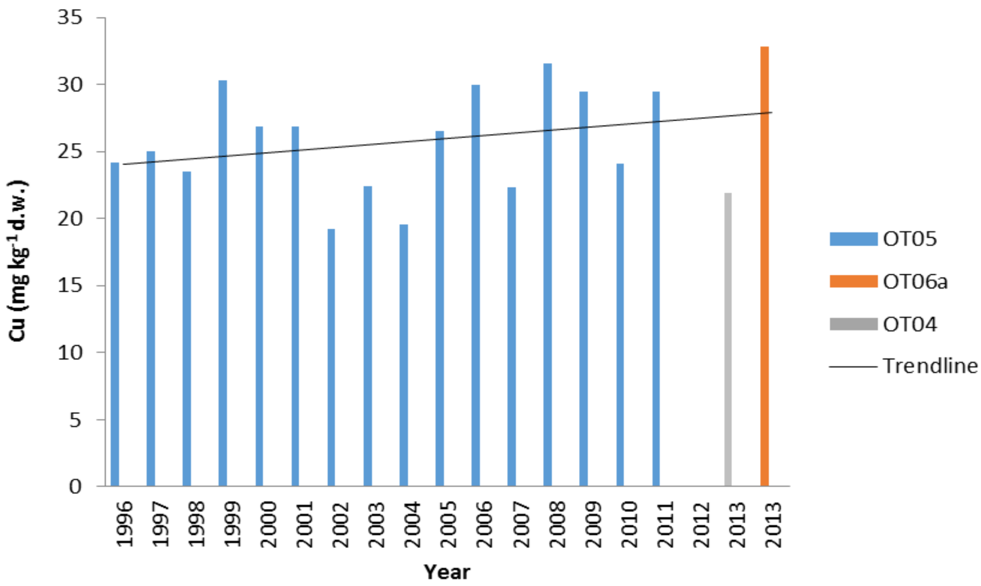


Figure 72. Total mercury contetnt in sediment samples at OT06a, OT04 and OT35 stations for 2013., 2017., 2019..

4.4.2. Metals (cadmium, chromium, copper, lead, mercury, zinc) in shellfish

Shellfish have an important role in the human diet becfause it's rich in proteins, minerals and vitamins, however, an incorrect balance or excess of certain elements in the diet can result in negative health effects. Heavy metals are of particular concern because of their ability to persist and accumulate in the environment. While heavy metals can occur naturally in the environment, human activities and run-off from urban and agricultural land use may increase their concentrations. This is particularly important when considering the growing demands on coastal resources due to increasing populations and the ability of filter feeding bivalve shellfish to bio-accumulate contaminants, whose presence in the food chain can pose a risk to human health. Also, contaminant in shellfish is reflect of ecological status of marine inhabited areas.

Due to its environmental toxicity and threat to human health, cadmium and mercury are classified as a Priority Hazardous Substance under the Environmental Quality Standards Directive (2008/105/EC), (2013/39/EU) which requires that all discharges, emissions and losses cease over time.

4.4.2.1. Sample collection and preparation

Sampling of biota is carried out once per year. Shellfish samples were collected from commercial aquaculture areas every year between 2000 and 2013 from Neretva River, as part of an ongoing monitoring program. Samples were representative of the main species harvested in that area. This was predominantly mussels (*M. galloprovincialis*). Shellfish samples are collected in plastic sampling bags and stored at -20°C until the further analysis.

Figure 73. Shellfish sampling points.

Sampling points	Bounding Coordinates - west	Bounding Coordinates - east	Bounding Coordinates - north	Bounding Coordinates - south
OT04	17.47027	17.47051	43.00833	43.00841
OT06	17.42843	17.42849	43.04867	43.04860

Table 11. Longitude and latitude of the shellfish sampling points.

4.4.2.2. Method description for determination of metals in shellfish samples

Whole body tissue samples are freeze-dried. Closed vessel microwave digestion with nitric acid is used for sample digestion.

Atomic absorption spectroscopy method (see chapter 4.4.1.2.).

4.4.2.3. Research results

Datasets were statistically analyzed. All data were presented as mg/kg wet weight. The moisture content of the shellfish was calculated by recording the difference between fresh and dry weights. The average moisture content in the soft tissue of bivalve molluscs is **80%**.

Recorded ***copper concentrations*** ranged from 0.87 – 12.06 mg/kg wet weight, with an average value of 2.49 mg/kg, while ***lead concentrations*** ranged from 0.07 – 0.52 mg/kg wet weight. Average value of lead was 0.24 mg/kg. The maximum reported concentration for Pb (0.52 mg/kg) and Cd (0.39 mg/kg) were considerably below the EU Regulative 1881/2006.

According to EU Regulative 1881/2006, maximum permitted levels for Cd is 1.0 mg/kg and for Pb is 1.5 mg/kg wet weight.

Concentrations of Cd and Pb were less than these permitted values in all analyzed shellfish, so, high concentrations i.e. concentrations that are not suitable for human consumption, were not found in any station. The average concentrations of heavy metals in shellfish were in the following decreasing order: Zn > Cu > Cr > Pb > Cd > Hg. Elevated values of the investigated metals were found at stations OT06 (Ploče port), in areas of larger ports, marinas and near industrial plants, where the anthropogenic impact is more pronounced, while the lowest concentrations were measured in samples from the mouth of the Mala Neretva (OT04).

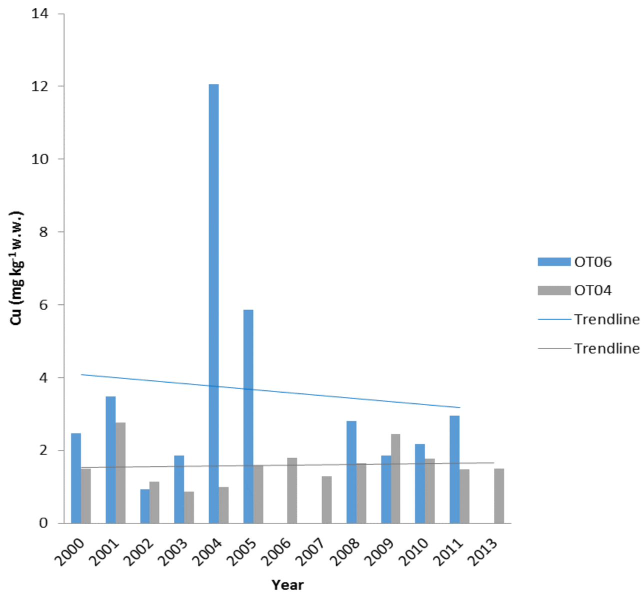


Figure 74. The concentrations of copper in shellfish samples at OT06 and OT04 stations in period 2000-2013.

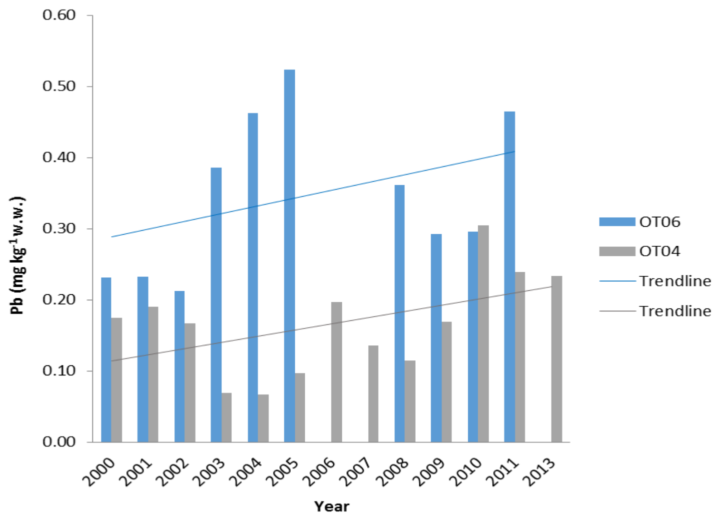


Figure 75. The concentrations of lead in shellfish samples at OT06 and OT04 stations in period 2000-2013.

The measured values of ***zinc content*** ranged from 11 – 41 mg/kg wet weight, with an average value of 21 mg/kg.

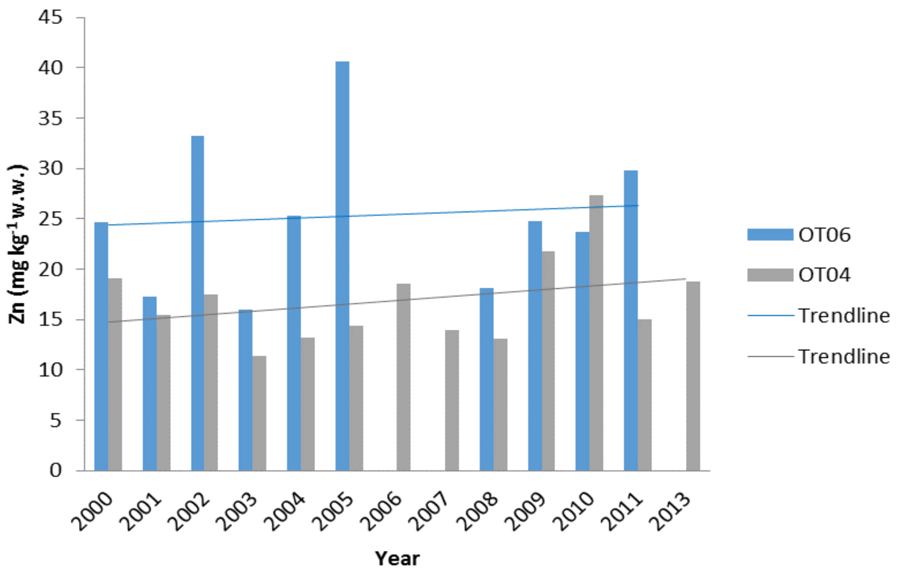


Figure 76. The concentrations of zinc in shellfish samples at OT06 and OT04 stations in period 2000-2013.

Concentrations of mercury in the soft tissue of shellfish ranged from 0.01 – 0.06 mg/kg wet weight. Average value of mercury was 0.02 mg/kg.

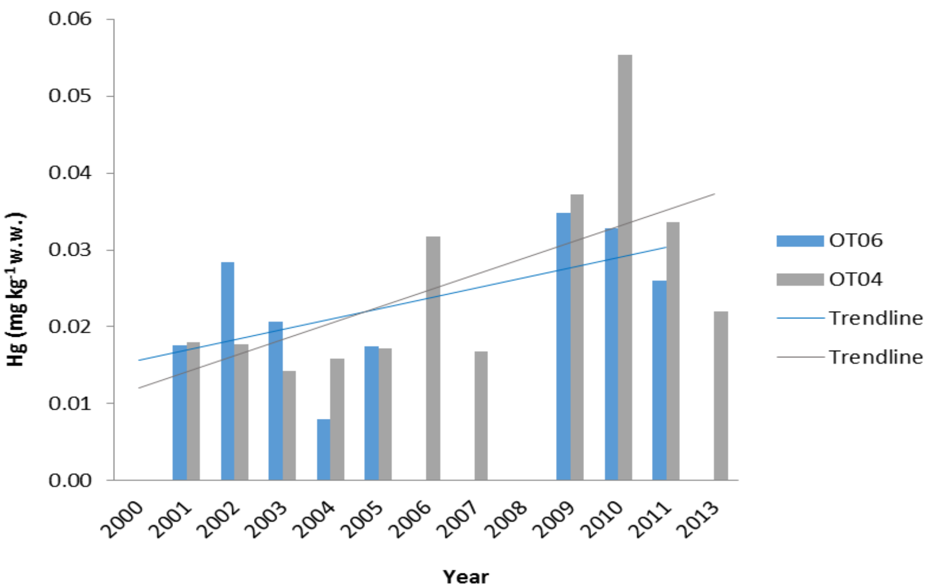


Figure 77. The concentrations of mercury in shellfish samples at OT06 and OT04 stations in period 2000-2013.

Measured ***cadmium concentrations*** ranged from 0.04 to 0.39 mg/kg wet weight, with an average value of 0.16 mg/kg.

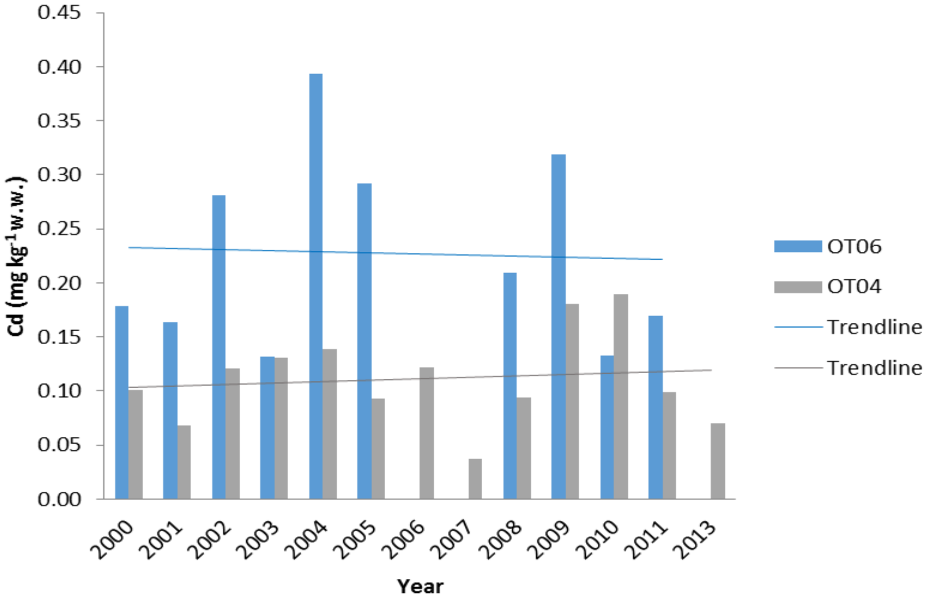


Figure 78. The concentrations of cadmium in shellfish samples at OT06 and OT04 stations in period 2000-2013.

Chromium concentrations ranged from 0.10 – 0.94 mg/kg wet weight. Average value of chromium was 0.38 mg/kg.

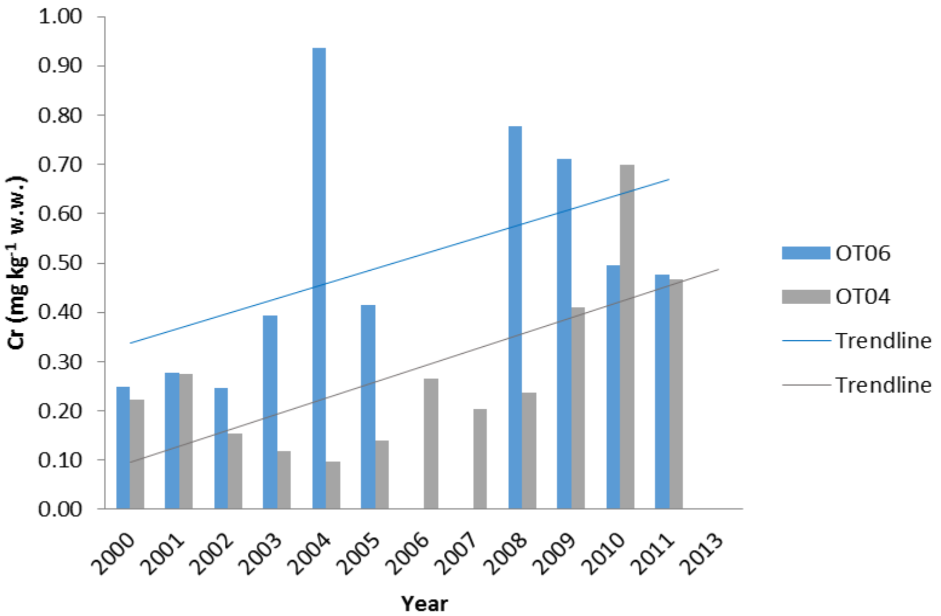


Figure 79. The concentrations of chromium in shellfish samples at OT06 and OT04 stations in period 2000-2013.

4.4.3. Pesticide in shellfish samples

LINDANE, PCB and DDT are synthetic substances that are not found naturally in the environment. The most commonly used pesticides are the organochlorine pesticides and they are considered to be responsible for the various environmental consequences. In addition to changes in the marine environment as habitat disturbance, human health effects of pesticides can caused by inhalation and ingestion through skin contact, handling of pesticide products, breathing of dust or spray and pesticides consumed on/in food, water and aquatic organisms.

4.4.3.1. Sample collection and preparation

Sampling of biota is carried out once per year. Shellfish samples were collected from commercial aquaculture areas every year between 2002 and 2013 from Neretva River, as part of an ongoing monitoring program. Samples were representative of the main species harvested in that area. This was predominantly mussels (*M. galloprovincialis*). Shellfish samples are collected in plastic sampling bags and stored at -20°C until the further analysis. Longitude and latitude and map of the sampling sites is shown in chapter 4.4.4.1.

4.4.3.2. Method description for determination of Lindan, DDTs and PCBs in shellfish samples

After sampling, the mussels were cleaned, washed in seawater, and transferred to a laboratory where soft tissue was dissected. The weight and length of the shell and the mass of the total soft tissue were determined for each individual. After dissection, the soft tissue composite samples were lyophilized and homogenized. Lyophilized tissue samples (~5g) were extracted by the Soxhlet method and purified with sulfuric acid. The concentration of lindane, DDT compounds (p, p'-DDE + p, p'-DDD + p, p'-DDT) and PCB compounds (arochlor 1254 + arochlor 1260) were determined by gas chromatography with electron capture detector (GC-ECD) on the capillary column. The internal standard and reference material IAEA-406 were used to verify the analytical procedure. The concentration of chlorinated hydrocarbons in the shellfish samples were expressed in µg/kg in relation to the dry weight (d.w.) of the sample. The limit of detection (LOD) of the applied method for lindane is 0.05 µg/kg d.w., DDT compounds 0.09 g/kg d.w. and PCB compounds 0.11 µg/kg d.w.

<u>Gas chromatography with an electron capture detector (GC-ECD) on the capillary column</u>

One microliter each of processed sample for GC analysis was injected in turns into the GC-ECD system. The carrier gas was 99.999% nitrogen gas. The extraction efficiencies at the different temperature were determined by comparison of the peak areas sample extract with those of the pesticide standard mixture. The response factors were determined according to standard method.

All compounds were well resolved and eluted within a reasonable time of less than 30 minutes under the optimized gas chromatograph-electron capture detector (GC-ECD) conditions. The identities of the OCPs in sample extracts were confirmed comparing their retention times with those of standards and concentrations were determined by computer calculation making use of both the response factors of the OCPs and the internal standard.

4.4.3.3. Research results

PCBs

Polychlorinated biphenyls (PCBs) are a group of theoretically 209 different compounds (congeners) of which 150-160 are found in the environment.

All PCBs are man-made, but are now found all over world due to their persistence and relative volatility. PCBs have been previously widely used in electrical equipment, and also as a plasticizer and paint additive. Aquatic organisms can accumulate hydrophobic compounds like PCBs and reach concentrations considerably higher than those of the surrounding waters. Therefore mussels and fish are suitable and commonly used for monitoring of PCBs. PCBs have extreme mobility and the ability to bioaccumulate and magnify in marine food webs, where long-lived animals at high trophic levels appear to be most at risk from PCBs.

The station-by-station overview of 2002-2013 concentrations of PCB for mussels (*M. galloprovincialis*) indicated that concentrations were generally low. At this station anthropogenic impact is less pronounced.

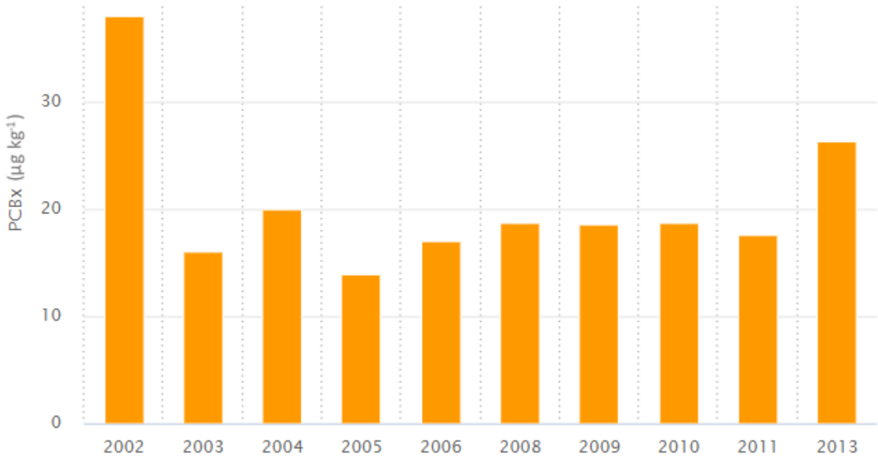


Figure 80. PCBx in shellfish samples at OT04 station in period 2002-2013.

Lindane

Lindane or HCH (1,2,3,4,5,6-hexachlorocyclohexane), also known as benzenehexachloride (BHC), is a pesticide that is still used in some parts of the world. Non-agricultural use of lindane includes use for wood preservation, as an insecticide, as rodenticide and for medicinal purposes (scab and louse ointments). Lindane is an irritant in humans and may affect mucus membranes, immune and nervous systems following exposure.

The station-by-station overview of 2002-2013 concentrations of lindane for mussels (*M. galloprovincialis*) indicated that concentrations were very low at all stations in Neretva River.

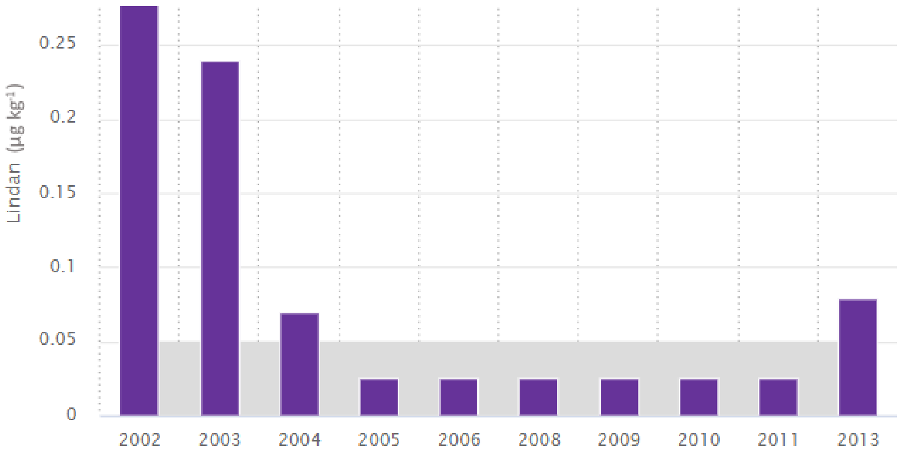


Figure 81. Lindan in shellfish samples at OT04 station in period 2002-2013 (concentrations in bivalve tissue expressed as a dry weight of 0.025 indicate that the amount is below the detection threshold (<0.05)).

<u>DDTx</u>

The presence of DDT and some of its degradation residues in the matrix can be attributed to their wide usage before their banning.

The concentrations of contaminants in shellfish samples were generally low.

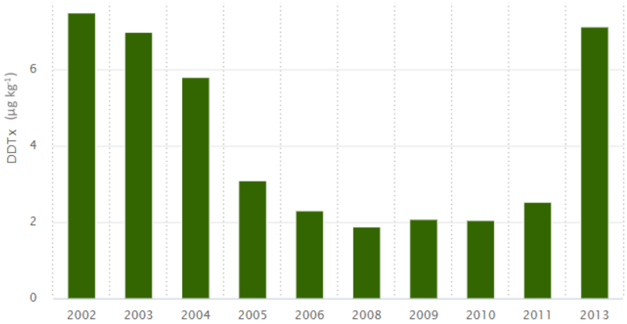


Figure 82. DDTx in shellfish samples at OT04 station in period 2002-2013.

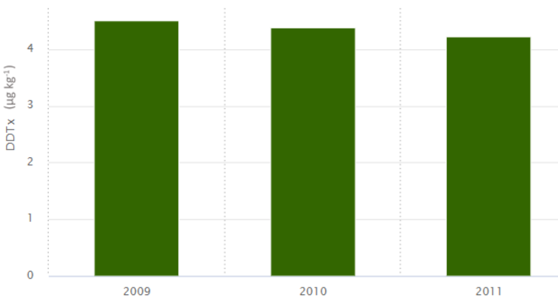


Figure 83. DDTx in shellfish samples at OT06 station in period 2009-2011.

4.4.4. Pesticide in sediment samples

The distribution of various contaminants in sediments depends mainly on the physico-chemical properties of the ecosystem, the partition coefficients of individual contaminants, organic contents, and microflora activities. In fact, river sediments are substrates that can be used to infer the agricultural history of a region, since the wastes that are added to the ecosystem are accumulated in sediments, according to the agricultural practices of an area and the properties of agrochemicals used. Usually, the surface layers of sediment represent the entry of anthropogenic substances of the system.

4.4.4.1. Sample collection and preparation

Sampling of sediment is carried out once per year. Sediment samples were collected from two locations of the Neretva River (OT04 and OT06 stations). Surface sediment samples (0-2 cm) are collected in plastic sampling bags and stored at -20 °C until the further analysis. Map of the sampling sites is shown in chapter 4.4.4.1.

4.4.4.2. Method description for determination of Lindan, DDTs and PCBs in sediment samples

Sediment samples were collected from the 0–3 cm depth from the same site as water samples, wrapped up in aluminum foil and then put in a polyethylene bag. Samples were kept cool during transportation to the laboratory. At the laboratory, they were frozen at -20°C until analysis, to avoid degradation. Samples were lyophilized and processed through <250 µm stainless steel sieve.

Samples of sieved sediment (10 g) were extracted by the Soxhlet method, with removal of sulfur, and separation of components into fractions using a column with activated florisil. The concentration of lindane, DDT compounds (p, p`-DDE + p, p`-DDD + p, p`-DDT) and PCB compounds (arochlor 1254 + arochlor 1260) were determined by gas chromatography with electron capture detector (GC-ECD) on the capillary column. The internal standard and reference material IAEA-408 were used to verify the analytical procedure. The concentration of hazardous substances are expressed in µg/kg in relation to the dry weight (d.w.).

<u>Gas chromatography with an electron capture detector (GC-ECD) on the capillary column</u>

Description of GC-ECD system is shown in the chapter 4.4.5.2.

4.4.4.3. Research results

<u>PCBs</u>

Marine sediments, in particular those with a high organic carbon content, may accumulate hydrophobic compounds like PCBs to considerably higher levels than those of the surrounding waters. The sampling strategy depends on the purpose of the monitoring programme and the natural conditions of the region to be monitored.

The station-by-station overview of 2006-2013 concentrations of PCB in sediment samples indicated that concentrations were generally low. At these stations anthropogenic impact is less pronounced.

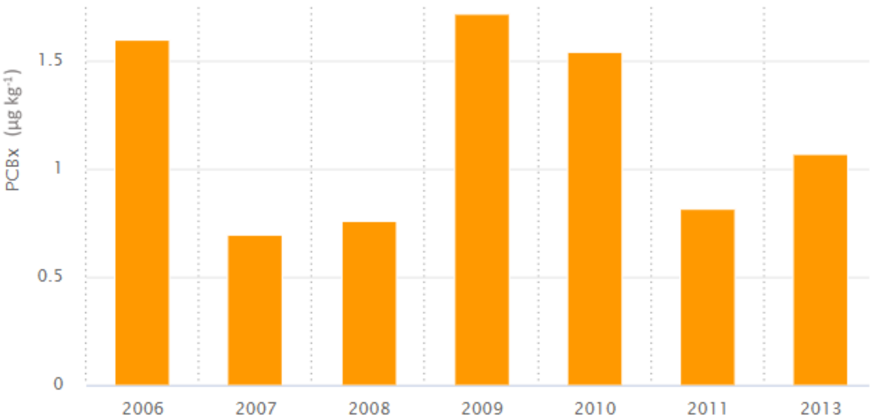


Figure 84. PCBx in sediment samples at OT04 station in period 2006-2013.

Lindane

Lindane is an organochlorine pesticide, which is under a great deal of regulatory pressure around the world. The usage of lindane has been banned in many countries of the world.

Lindane is highly persistent in most soils, with a field half-life of approximately 15 months. When sprayed on the surface, the half-life was typically much shorter than when incorporated into the soil. It shows a low affinity for soil binding, and may be mobile in soils with especially low organic matter content or subject to high rainfall.

All sample results were reported below the limit of detection (<0.05) at OT04 station in Neretva River.

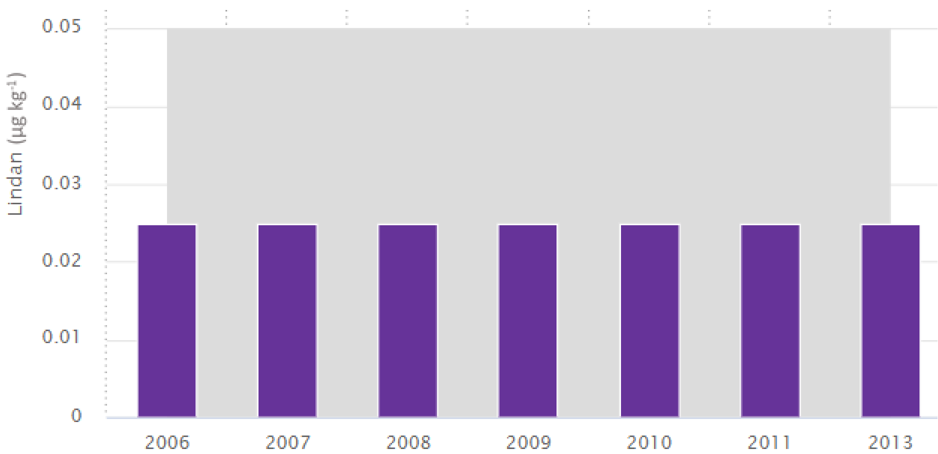


Figure 85. Lindane in sediment samples at OT04 station from 2006-2013 (concentrations in sediment expressed as a dry weight of 0.025 indicate that the amount is below the detection threshold (<0.05)).

DDTx

Since DDTx are persistent enough and degrade slowly and easily accumulate in the soil, the transportation of these pesticides both sorbed onto solids and dissolved by the surface water down to the water sources is expected.

Based on the data gathered during continuous monitoring in the period from 2006 to 2013 for the station OT04 indicates that the concentrations of contaminants (lindane) in shellfish samples (*M. galloprovincialis*) are mostly below the detection limit (<0.09), which indicates the absence of lindane in the environment.

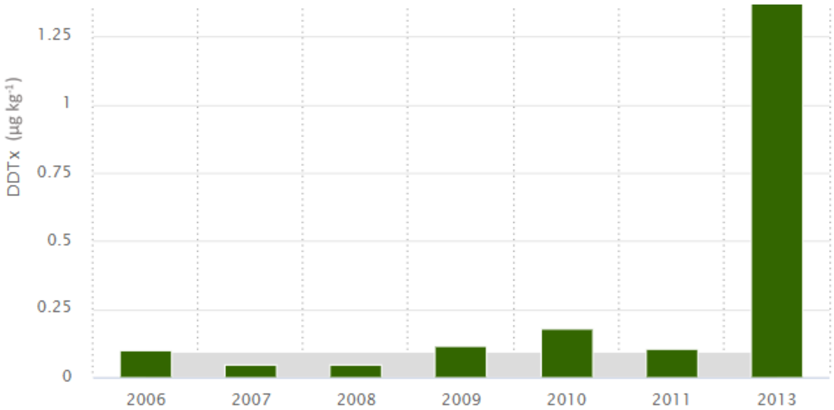


Figure 86. DDTx in sediment samples at OT04 station in period 2006-2013 (concentrations in sediment expressed as a dry weight of 0.045 indicate that the amount is below the detection threshold (<0.09)).

4.4.5. Suspended particulate matter

In the present report, suspended particulate matter (SPM) of the Neretva River were studied in order to assess the pollution status of this river system.

4.4.5.1. Sample collection

During oceanographic cruises, samples are taken to determine the concentration of suspended solids at selected stations, at a depth of 10 m, and in the bottom layer. Two dm^3 of sea water are sampled at stations where the transparency exceeds 10 m and 1 dm^3 of sea water if the transparency at the station is less than 10 m. Samples was collected from one location of the Neretva River (OC03 station).

Measurements are performed 3 times a year (February, April and July).

Figure 87. Sea water sampling point.

Sampling points	Bounding Coordinates - west	Bounding Coordinates - east	Bounding Coordinates - north	Bounding Coordinates - south
OC03	17.41333	17.41368	43.02500	43.02515

Table 12. Longitude and latitude of the sea water sampling point.

4.4.5.2. Method description

In period before 2014. samples are filtered through (pre-weighed) glass fiber filters (Whatman GF/F) with 0.45 µm pore size. After 2014. total suspended matter for the Central Adriatic station - OC03 was made according to the standard for the determination of suspended solids "Water quality - Determination of suspended solids - Method by filtration through a glass fiber filter (EN 872: 2005)" using a glass filter of pore size (0.6 - 0.8) micrometers (Whatman GF/F filter).

The filters are washed after filtration with 200 ml of distilled water.

Gravimetric methods are quantitative methods that are based upon determining the mass of a pure compound to which the analyte is related.

4.4.5.3. Research results

The sampling method changed in the period from 2005 to 2019, due to which it is not possible to create a homogeneous time series of data. Therefore, it is not possible to analyze the trend for a longer period of time. The trend can be analyzed for the period in which the sampling method did not change, i.e. after 2014.

In period from 2005 until 2011 at the coastal station (OCO3), the average suspended matter was 4 mg/dm^3 and was in the expected interval.

At the same station no measurements of suspended solids were performed for the years 2012 and 2013, while the concentrations measured in 2015 and 2016 were within the expected and in accordance with previous values.

The highest concentration (6.68 mg/dm^3) of suspended solids was measured at the station OC03 during 2016.

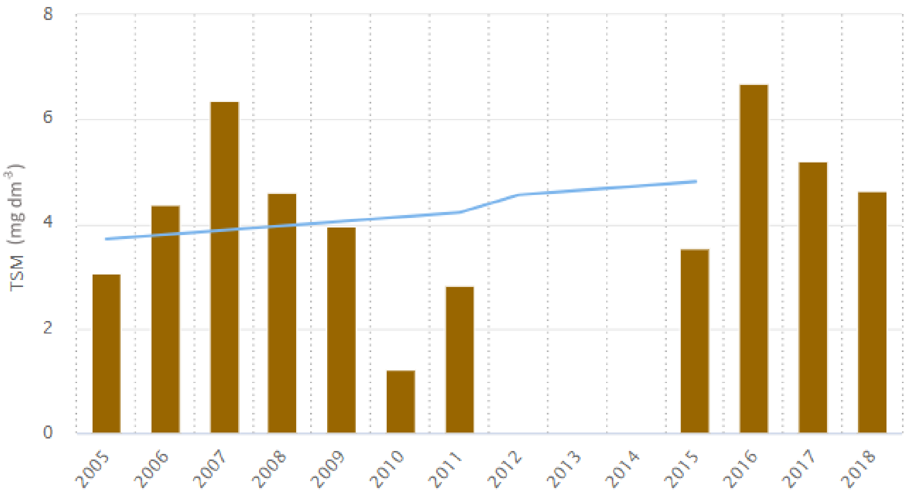


Figure 88. Total suspended matter at OC03 station in period 2005-2018.

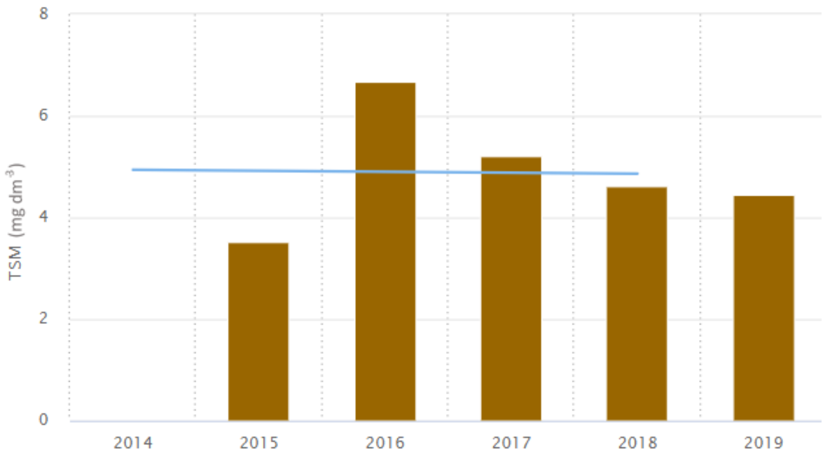


Figure 89. Total suspended matter at OC03 station from 2014-2019.

The share of organic and inorganic matter is within the limits. Also, the share of inorganic matter at station OC03 has a positive trend because the share of inorganic phase increased for approximately 50% in the period from 2005 to 2019. At the same station the share of organic matter has a negative trend because the share of organic phase decreased for approximately 50% in the same period.

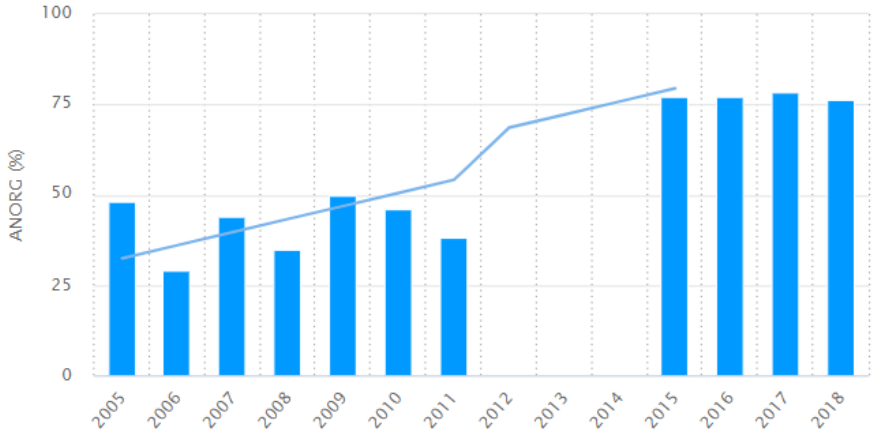


Figure 90. Part of inorganic matter in total suspended matter at OC03 station from 2005-2018.

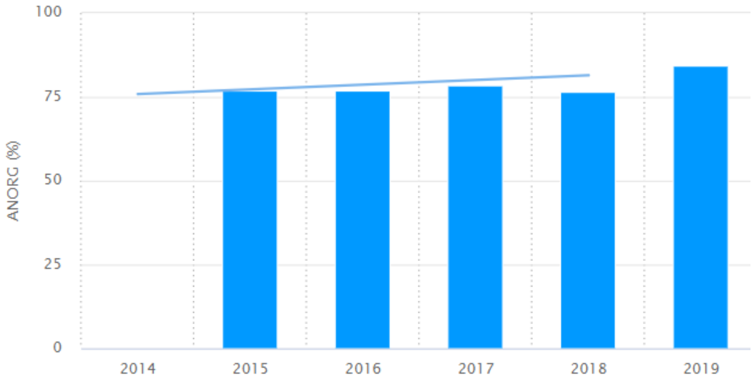


Figure 91. Part of inorganic matter in total suspended matter at OC03 station from 2014-2019.

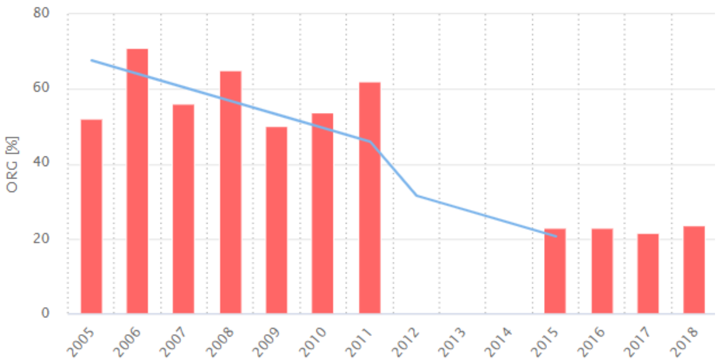


Figure 92. Part of organic matter in total suspended matter at OC03 station from 2005-2018.

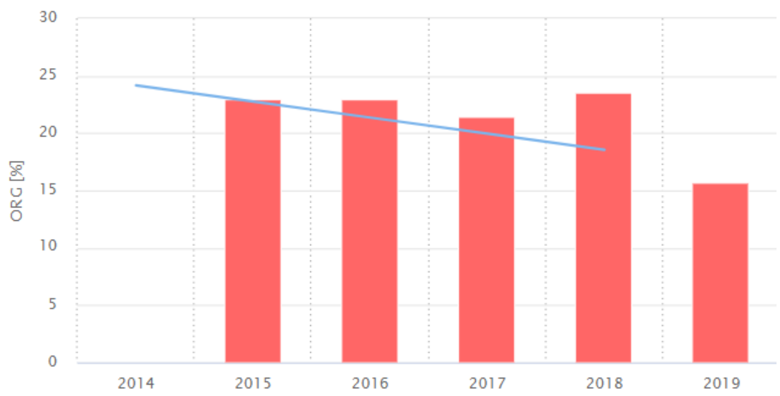


Figure 93. Part of organic matter in total suspended matter at OC03 station in period 2014-2019.

4.4.6. Low oxygen concentrations (oxygen saturation) in bottom layer

Around the world, marine hypoxia — a shortage of dissolved oxygen (below 2 mg/L in the bottom layer) — is a growing problem that can have dramatic impacts on marine life conditions and affected the ecosystems, including direct loss of habitat or habitat compression, altered trophic (food web) relationships, changes in migration patterns, and changes in biodiversity.

A decline in oxygen saturation in seawater is now recognized as one of the likely consequences of global warming, because oxygen saturation in warmer water are decreased. This condition may be naturally occurring or be exacerbated and can be directly caused by human influences. In either case, the processes involved and the resulting environmental stresses are essentially similar.

Surface waters are always rich in dissolved oxygen. Hypoxia occurs in deeper waters when oxygen is removed from the water much faster than it is replenished from the atmosphere or by photosynthesis. Microbial respiration due to the decay of organic material in deep water and in sediments can also contribute to hypoxia. This condition may be transient, seasonal or permanent depending on a variety of factors including local oceanographic conditions.

The impact of hypoxia on living organisms depends on the concentration of dissolved oxygen as well as temperature, salinity and the tolerance of each particular species to low oxygen.

There is an upper limit to the amount of oxygen that can be dissolved in seawater, which depends on water temperature, salinity and atmospheric pressure. Oxygen saturation is a measure of how much oxygen is dissolved, as a percentage of the maximum oxygen concentration in seawater.

4.4.6.1. Sample collection

Water samples are collected from OCO3 station at three relevant depths (usually 0, 5 and 10 m) once a month. Longitude and latitude and map of the sampling sites is shown in chapter 4.4.8.1.

4.4.6.2. Method description

The dissolved oxygen content in seawater samples was determined titrimetrically with thiosulfate.

Titrimetric analysis is a method of analysis in which a solution of the substance being determined is treated with a solution of a suitable reagent of exactly known concentration. The reagent is called the titrant. The reagent is added to the substance until the amount added is equivalent to the amount of substance to be determined.

4.4.6.3. Research results

In this report the values of oxygen concentrations are given according to analytical laboratory methods, while the degree of saturation is calculated from the gathered analytical data, and is expressed as the ratio of the established and theoretical oxygen content at a given temperature and salinity.

In the period from 1999 to 2019, oxygen saturation in the bottom layer at the station OCO3 ranged from 0.8407 to 1.0576, while oxygen concentrations in the bottom layer at the same station ranged from 6.4241 to 7.5559 mg/L. Ecologically critical oxygen concentration (2-3 mg/L) was not found at station OC03 in the period from 1999 to 2019, which could negatively affect the life of benthic organisms.

Also, no significant trends in concentrations were observed at OC03 station during the period 1999-2019.

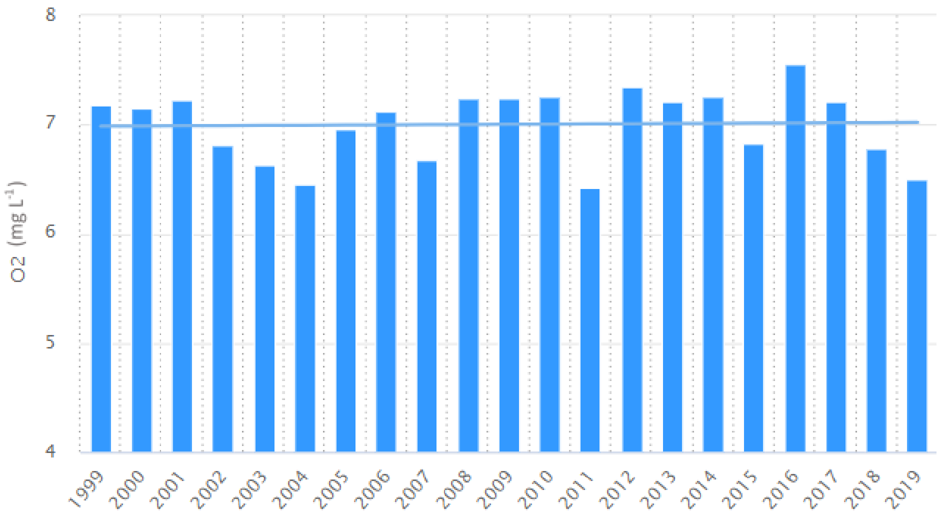


Figure 94. Dissolved oxygen at OC03 station in period from 1999 to 2019.

4.5. P5 - Teqnue coralligenous

4.5.1. Hydrological parameters and water quality

The first data on the chemical-physical environment of two rocky outcrops off the coast of Chioggia were collected, through SCUBA dives, between August 2002 and June 2003 (Casellato et al., 2005). The two stations investigated were named station 1, located at about 8 NM from the coast (45°13,16'N; 012°29,67'E), and station 2, located closer to the coast, at 4.2 NM (45°11,70'N; 012°25,20'E). Water depth at the stations ranged between 22 and 24 m. The bottom water temperature showed similar values at both stations (Table 13), and the oxygenation of the water reaches high supersaturation values in winter (Table 13) (Casellato et al., 2005). These two stations presented different conditions of turbidity of the waters, more turbid near the coast; even in the most distant seabed, although not deep, they are reached by a residual amount of light (Casellato et al., 2005). In both stations, the highest transparency values were recorded during the summer period. Generally speaking, the PAR penetration at the bottom for the Tegnùe located in the Northern Adriatic is strongly affected by fluvial runoff, current transport of sediments and phytoplankton blooms. Measures undertaken at two stations located at 25 m depth (Ponti et al., 2011), indicated an irradiance of about 30–150 MJ m^{-2} $year^{-1}$, corresponding to a light attenuation at 1–2% of the mean surface irradiance, estimated in 5,800 MJ m^{-2} $year^{-1}$.

	Summer		Winter		Spring	
	Station 1	Station 2	Station 1	Station 2	Station 1	Station 2
T [°C]	22	22	8	7	11	14,5
Dissolved Oxygen Concentration [mg/L]	4,49	2,90	13	14,80	7,42	6,41
Dissolved Oxygen [% saturation]	65,79	42,49	141,70	159	86,78	80,73

Table 13. Water bottom temperature and oxygenation (data from Casellato et al., 2005).

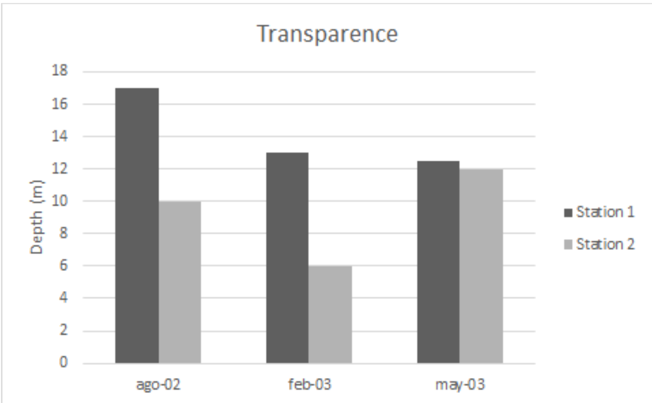


Figure 95. Light penetration (Secchi disc) along the water column (modified from Casellato et al. 2005).

In 2009 ARPAV made new surveys, by subdividing the tegnùe's areas in the Veneto Region, between the rivers Brenta and Tagliamento, into inshore (< 4 km from the cost) and offshore ones (> 4 km from the cost). The arbitrary limit of 4 km was chosen based on abiotic data available from the Veneto region (Secchi disc, total P and total N concentration) (Figure 96) (Curiel et al. 2017).

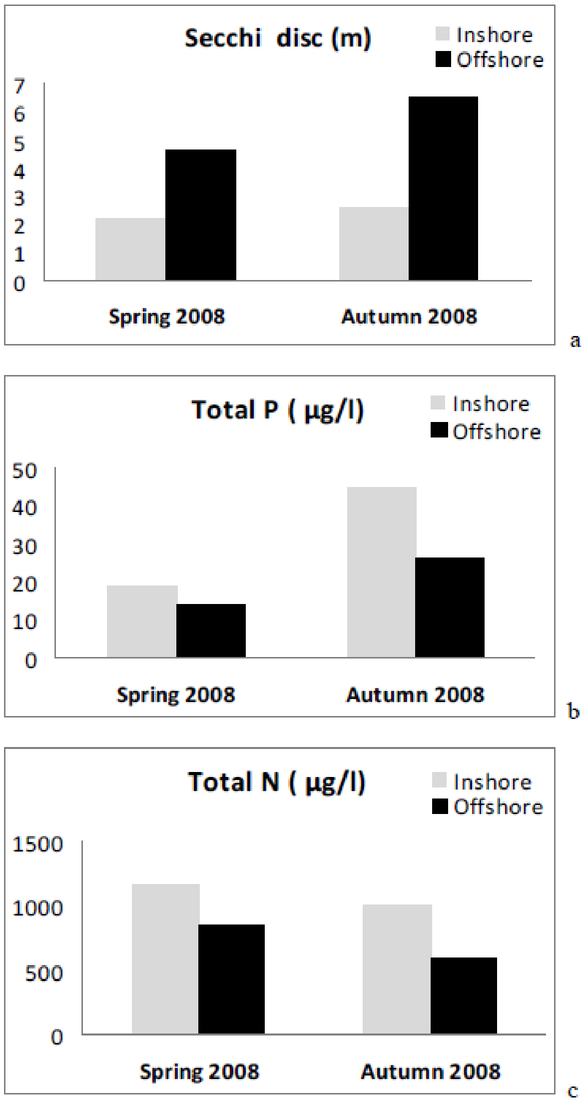


Figure 96. Value of transparency (Secchi disc) (a), concentration of total P (b) and total N in spring and autumn 2008 (Curiel et al., 2017).

As far as water currents is concerned, as part of the VI Three-year Fisheries and Aquaculture Plan, an ICRAM multiparametric probe has been active at Tegnùe di Chioggia site since 2004, which collecting data on direction and module of the current. As shown in Figure 97, currents in the area present average daily values oscillating between 5 and 7 cm/sec, with average daily values achieving 25-30 cm/sec during localized storm events.

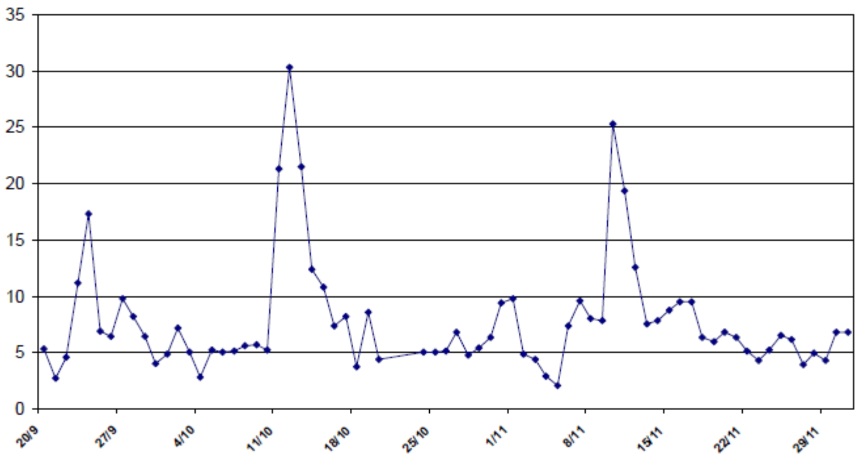


Figure 97. Current speed [cm/sec] measured by probe; daily average of autumn period (20/09 - 30/11) of 2004 (Franceschini and Giovanardi, 2005).

Tegnùe di Chioggia fall within the area of pertinence for monitoring coastal marine waters, carried out by the Veneto region, according to the Water Framework Directive 2000/60 / EC, Legislative Decree 152/2006, Ministerial Decree 131/08, DM 56/2009 and Ministerial Decree 260/2010, which allows to define the ecological state (understood as the quality of the structure and functioning of the marine ecosystem) and the chemical state of the marine area overlooking the Venetian coast (ARPAV, 2015). The rocky outcrops fall within the water bodies called "ME2_1" and "ME2_2" (Table 14), of which the first classification is shown in Table 13, relating to the three-year operational monitoring 2010-2012, integrated with the data for the year 2013.

		Station	
		ME2_1	ME2_2
Ecological status	Phytoplankton and phytoplankton biomass (Chlorophyll a)	High	High
	Benthic macroinvertebrate fauna	High	High
	Supporting physico-chemical quality elements (TRIX trophic index)	Good	Sufficient
	Supporting chemical elements: substances not belonging to the priority list (water matrix)	Good	Good

	Supporting chemical elements: substances not belonging to the priority list (sediment matrix)	Sufficient	Sufficient
Chemical status	Water matrix (Environmental Quality Standard (EQS) for priority and priority hazardous substances)	Good	Good
	Water and sediment matrix (Environmental Quality Standard (EQS) for priority and priority hazardous substances)	Not Good	Not Good
	Water sediment and biota matrix (Environmental Quality Standard (EQS) for priority and priority hazardous substances)	Not Good	Not Good
Ecological status classification	Water (Figure 98 left)	**Good**	**Sufficient**
	Water and sediment (Figure 98 right)	**Sufficient**	**Sufficient**

Table 14. Ecological and chemical state class, and overall classification of the ecological state.

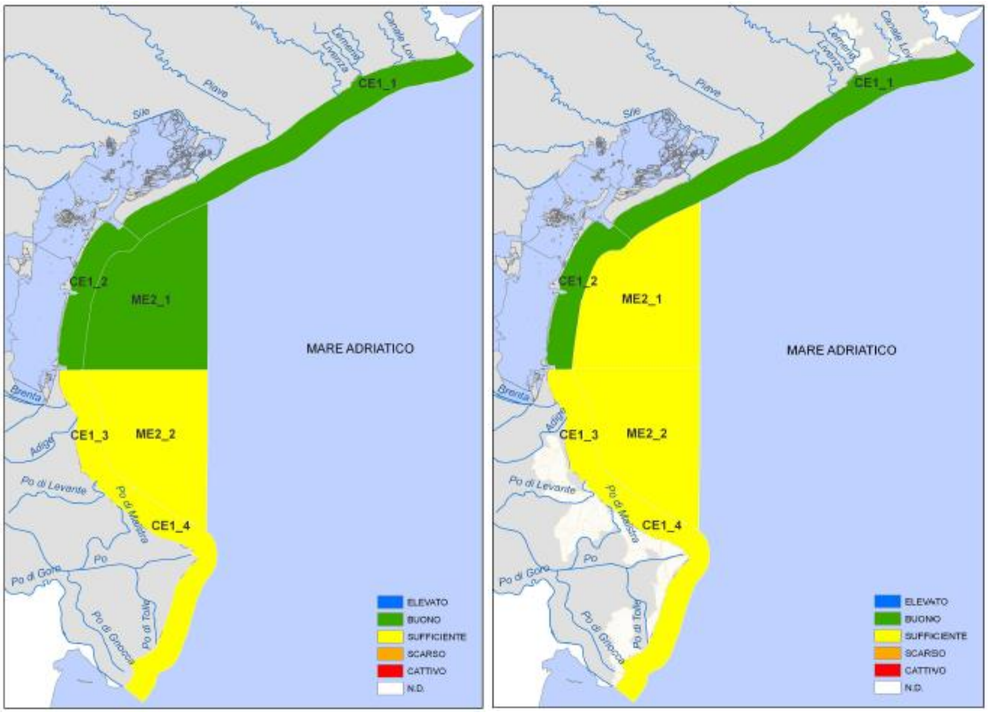


Figure 98. Ecological status of coastal marine water bodies in Veneto (years 2010-2013, water matrix on the left; water and sediment matrices on the right). ARPAV, 2015.

4.5.2. Ecosystem characterization

Several reef formations are found sparse in the area within few miles seaward from the Italian coast of the Northern Adriatic Sea. Each reef differs in extension and benthic assemblages, depending from a number of key factors: depth, abiotic features (e.g. mineralogical characteristics of the substrata (ARPAV 2011.), distance from coast, geographic position and other outcrops proximity (Ingrosso et al. 2018.).

The calcareous bioconstructions of the Northern Adriatic, including the Tegnùe di Chioggia, form the habitat that supports the life of several flora and fauna species. Among the species identified, several are prioritized for conservation.

The specific habitat found on top of the Northern Adriatic rocky outcrops presents many similarities with typical coralligenous habitat. However, they differ in composition and overall structure from most coralligenous outcrops found in the Mediterranean basin (Curiel et al. 2012.; Falace et al. 2015.), and based on EUNIS classification, as reported in Annex I of the Habitat Directive, they are classified as Reef habitat (habitat code 1170).

Concerning the focus of this report, the reef formation included in the SCI of Tegnùe di Chioggia, is the biogenic reef with the largest extension of the entire Northern Adriatic marine area (Casellato and Stefanon 2008.).

4.5.2.1. Marine Biocenosis

Calcareous bioconstructions of the Tegnùe di Chioggia site are composed mainly by calcareous algae, together with molluscs, Bryozoas, polychaetes and madrepores. These sessile organisms form an unique coralligenous-like habitat which provides trophic resources, reproduction niches and shelter for several benthic, demersal and pelagic invertebrate and vertebrate species through their different life stages. The great availability of nutrients in the water column typical of the northern Adriatic Sea, represents a main trophic source for several filter and suspension feeders' organisms living on the reef (Ingrosso et al. 2018.).

Within the homogeneous sea bottom of the Northern Adriatic Sea, the Tegnùe di Chioggia ecosystem function as collectors of organic matter, which is then made available through the trophic chain to macrophage organisms that feed directly on the bioconstructor organisms of the reef as well as detrivorous species (Casellato et al. 2005.).

4.5.2.2. Floral species

<u>Benthic</u>

190 different macroalgae taxa have been identified in the Northern Adriatic rocky outcrops, including the SCI of Tegnùe di Chioggia. Red algae, are the most represented taxa, both in terms of species number and abundance). However, algal assemblages in the area variate consistently in terms of species composition and structure of the communities (Curiel et al. 2012). The main factor affecting such variability is light availability, which in turn depends from water turbidity and depth,

and the sedimentation rate (Ponti et al. 2006.). Morphology of the outcrops, distance from the coast and geographical location are also influencing the composition and structure of macroalgae assemblages. Compared to other coralligenous assemblages of the Mediterranean the biodiversity found is high while the mean total algal coverage is relatively low, probably as a consequence of the unique condition of these outcrops, within the Mediterranean context (Falace et al. 2015.).

Concerning the SCI of Tegnùe di Chioggia the main benthic macroalgae found are from the genus of the Corallinaceae family, typical of areas with limited light intensity, namely: *Lithophyllum* and *Pseudolithophyllum* (Casellato et al. 2005) as well as *Peyssonnelia* genus (ISPRA 2010). With minor frequency, Rodophyceae of the Ceramiaceae family were also found (Casellato et al. 2005.)

Planktonic

Phytoplankton growth and concentration in the Northern Adriatic is strongly influenced by seasonal variations in nutrients availability, temperature and solar radiation. Peaks in chlorophyll-a, which reflects phytoplankton biomass variations are observed at the end of the winter, in spring and autumn when river inputs are higher. During the rest of the year, phytoplankton concentration varies mainly depending from nutrients availability and the grazing pressure of zooplankton (Pugnetti et al. 2007.). In summer great input of nutrients from rivers enhance bloom of phytoplanktonic organisms, which tend to develop close to the surface. Blooms found in the areas more influenced by river inputs are composed mainly by diatoms species, such as *Skeletonema costatum, Chaetoceros spp*. e *Pseudonitzschia delicatssima* among the others. In coastal waters influenced by Po River nutrients input, species of the dinoflagellate group, such as the toxic *Dinophysis spp*., tend to spread in winter and peak during summer, causing the so called "red tides" (AA.VV. 2005.). The spread of different types of mucilage in the summer season, a complex phenomenon still partially unexplained, is also typical of this area (Pugnetti et al. 2007.).

4.5.2.3. Faunal species

Plankton

The Northern Adriatic represents a hotspot in terms of zooplankton biomass, in particular due to the influence of the Po River and its large input of nutrients. On the other hand, biodiversity of zooplanktonic organisms in the area has been reported to be relatively low compared to the Southern Adriatic Sea. The zooplanktonic community in the area of the Tegnùe di Chioggia is composed mainly by Copepods and Cladocera during spring and summer and larvae of benthic invertebrates and Appendicularia in winter. Main copepods species found in the area include: the epipelagic genus *Oithona, Clausocalanus, Ctenocalanus, Calanus, Oncaea*; neritic species *Acartia clausi*, *Paracalanus parvus*, *Calanus helgolandicus*, *Ctenocalanus vanus*, *Temora longicornis* and *Pseudocalanus elongatus*; other species presented in lower abundance are *Euterpina acutifrons* and those of the genus *Centropages spp.* and *Corycaeidae* (Busatto, 2007.).

Local zooplanktonic communities are also partially formed by larvae of invertebrate species. The thesis work by Bertasi (2007.) made a taxonomic analysis of the zooplanktonic invertebrate species found above the Tegnùe di Chioggia site. This study identified several species mainly from the Mollusca and Anellida phyla and the Crustacea subphyla, namely: from the Bivalvia class *Chlamys spp., Pectinidae sp., Lima spp.,* Anomiidae sp*., Barbatia spp., Glycymeris sp., Arcidae sp., Ensis spp., Solenidae sp., Cardiidae sp., Lucinidae sp., Spisula spp., Mactridae sp., Tellinidae sp., Veneridae sp., Hiatella arctica, Hiatellidae sp., Xylophaga sp., Teredinidae sp., Mytilidae sp., Ostreidae sp.*; from the gasteropoda class, common in the area, no taxonomic identification of their planktonic phase has been reported; from the Annelida phylum, Polychaeta class were found including Pomatoceros triqueter, Nereididae sp., Phyllodocidae sp., Syllidae sp., Paraonidae sp., Orbiniidae sp., Pilargiidae sp., Spionidae sp.; From the Arthropoda philum, Malacostraca class were identified *Gammaridi sp., Coriphiidae sp., Gnathiidae sp., Isopoda sp., Diastylis sp.*; from the Ostracoda class *Ostracodi sp*.; from the Cirripedia class nauplii di cirripedi; from the Copepoda class nauplii di copepoda, *Acartia spp., Paracalanus parvus, Temora spp., Oithona spp., Oncaea spp., Corycaeus spp., Caligoidae, Ameira sp., Canuella perplexa, Euterpina acutifrons, Harpacticus sp., Microsetella norvegica, Porcellidium sp., Tegastidae sp., Thalestris sp., Tisbe spp., Harpactoide sp.; from the Branchiopoda class Penilia avirostris, Podon sp., Evadne spp.*; from the Malacostraca class *Palaemon spp., Anomura zoea, Brachiura zoea*, unidentified Decapoda, *Chetognata sp*.; from the Echinodermata phylum, class Ophiuroidea, were found ofioplutei, echinoplutei; In the Chordata phylum from class Larvacea were found *Larvacei sp*., from the class Ascidiacea, *Ascidiacei sp*.; eggs of *Engraulidae sp*. (Actinopterygii) were also found.

<u>Nekton</u>

The Tegnùe di Chioggia are particularly rich in fish species. The complex habitat built by sessile organisms represents an ideal shelter and it is a main trophic source. A study from Cenci and Mazzoldi (2005) identified in the area 32 fish species from 14 different families (Blennidae, Gobiidae, Scorpaenidae, Congridae, Scophthalamidae, Serranidae, Sciaenidae, Labridae, Gadidae, Pomacentridae, Sparidae, Centrachantidae, Mullidae, Carangidae), including nektobenthic, demersal, small and large pelagic species. Several fish species found in the area are of commercial interest, namely: *Diplodus annularis (*the most abundant), *Chelidonichthys lucernus, Merlangius merlangus, Squilla mantis, Spicara smaris, Trisopterus minutus, Pagellus erythrinus, Diplodus annularis* (ISPRA 2010.)*.*

The study by ISPRA (2010) assessed the species of commercial interest through a two years sampling campaign within and around the SCI both above the outcrops and the surrounding soft bottom. In total were found 50 species of bony fishes from 22 different families, 5 species of cartilaginous fishes, 2 species of crustacea and 2 of cephalopods. Of these most of the species found (33) are demersal, 10 benthopelagic (all from the Sparidae family), 9 pelagic, 3 benthonic, 2 bathydemersal, 1 benthodemersal and 1 reef associated.

Demersal and benthopelagic species represented together around the 80% of the abundance and 88% of the total biomass. Some species were found to be distributed almost exclusively above the coralligenous outcrops, nemely *P. erythrinus* and *D. annularis.*

The species commonly found in the area, which are targeted for conservation under the Habitat Directive include two large migratory species *Tursiops truncatus* and *Caretta caretta*, and two sessile bivalves *Lithophaga lithophaga and Pinna nobilis* (which local population severely decreased due to the recent spread of a parasitic disease). Further, the occasional presence of *Chelonia mydas* has been reported, although with non-significant populations.

Other species relevant for conservation found in the area include: *Carcharodon carcharias, Cetorhinus maximus, Hippocampus hippocampus, Hippocampus ramulosus, Isurus oxyrinchus, Lamna nasus, Mobula mobular, Prionace glauca, Raja alba, Sciaena umbra, Squatina squatina, Umbrina cirrosa, Aplysina aerophoba, Astroides calycularis, Axinella cannabina, Axinella polypoides, Geodia cydonium, Hippospongia communis, Homarus gammarus, Maja squinado, Pholas dactylus*, *Spongia agaricina, Spongia officinalis, Tethya aurantium, Tethya citrina (*Natura 2000 SDF, 2021*).*

Benthic species on hard substrata

A study conducted between 2002 and 2003 on two sampling stations within the SCI, by Casellato and Masiero recognized a total of 318 taxa of benthic organisms from 10 different phyla, namely: Anellida, Bryozoa, Crustacea, Chaetognata, Cnidaria, Echinodermata, Mollusca, Porifera, Sipunculida and Tunicata. Among these phyla, Molluska, Crustacea and Polychaeta were found with homogeneous distribution, while Tunicate and Cnidaria were found to have a much more heterogeneous distribution. Concerning the trophic categories, filter feeders and suspensivorous are the more represented while herbivorous and detrivorous are present in much smaller abundance.

Based on ISPRA study (2010) Porifera present several encrusting forms (e.g. *Dictyonella incisa* e *Antho (Antho) inconstans*), erected forms (e.g. *Geodia cydonium, Ircinia variabilis, Dysidea avara, Chondrosia reniformis, Tedania anhelans, Ulosa stuposa, Axinella damicornis, A. polipoides, Aplysina aerophoba*) and perforating type (*Cliona spp.*). Cnidaria species are represented mainly by *Cereus pedunculatus, Cerianthus membranaceus, Cornularia cornucopiae, Epizoanthus arenaceus, Parazoanthus axinellae*. Isolated colonies of *Maasella edwardsi* were also found. Among Ascidiae species the most abundant are *Polycitor adriaticus, Aplidium conicum* e *Aplidium tabarquensis*. Endobionts such as porifera and burrowing bivalves can also settle within the rocks and calcareous shells. Many mobile species can also find refuge in the cavities and interstices present, including crustaceans and echinoderms, among which *Ophiothrix fragilis* prevails; other taxa found within the hard substrata include nudibranchs, flatworms, sipunculids, nemertines and echiurids.

Some patterns have been identified in the distribution of benthic species: The abundance of some organisms have been found to be correlated with the distance from coast, among this sponge species *Dictyonella incisa* (negatively correlated) (Ponti et al. 2006.) and Ascidiacea *Polycitor adriaticus* (positively correlated) (Ponti et al., 2006.). Concerning, the Ascidiacea population in the area, the most abundant are *Polycitor adriaticus*, *Aplidium conicum*, *Aplidium tabarquensis*, *Aplidium* cfr. *densum* and *Cystodytes dellechiajei*, while *Phallusia mammillata*, *Phallusia fumigata*, Pyuridae sp. and *Clavelina sabbadini* were also found in smaller amount (Ponti and Mastrotaro 2006.).

<u>Benthic species on soft substrata</u>

A study from Boscolo et al. (2005.) identified 140 different taxa on the soft substrata surrounding the rocky outcrops of Tegnùe di Chioggia and found that abundance and biodiversity on soft substrata was higher in area close to the rocky outcrops than the more distant areas. The most represented phylum has been found to be Annelida, followed in decreasing order by Mollusca, Echinodermata, Arthropods and Sipunculids. Another study from ISPRA (2010.) found that the 95% in species and number of individuals is represented mainly by Mollusca species, followed in decreasing order by Annelida, Echinodermata and Arthropods.

It is worth noting that some molluscs species of economic importance are found in the soft substrata surrounding the rocky outcrops, namely *Pecten jacobaeus*, *Chlamys varia*, *Chamelea gallina* and *Paphia aurea*.

4.5.2.4. Socio economic characterization: main human uses and activities

The area surrounding the SIC of Tegnùe di Chioggia has a great socio-economic value. Many uses and activities interact and compete for space in this marine area, generating pressures and direct and indirect impacts on the SIC ecosystem. An overlay map of the main uses present in the area is shown in Figure 99, and the following sub-sections will briefly describe the most important uses present in this area.

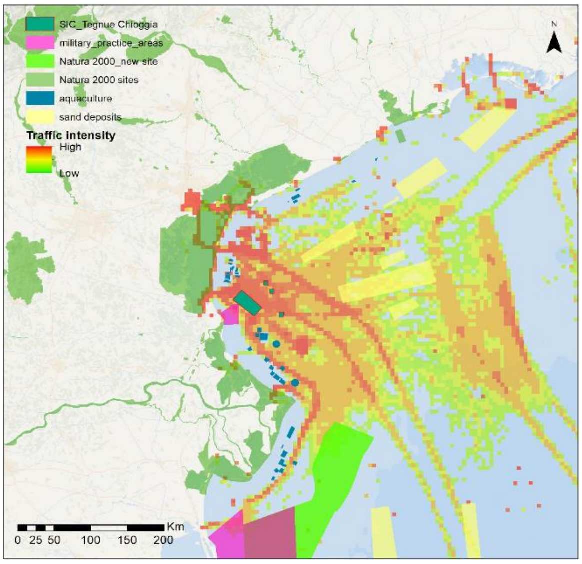


Figure 99. Overview of main human uses and activities in the coastal and marine area surrounding the Tegnue di Chioggia SIC.

Marine traffic

Marine traffic is intense in the area (Figure 100) due to the closeness to the Venice and Chioggia Port, two hotspots in terms of commercial shipping, nautical tourism, including cruise traffic among the others, and fisheries.

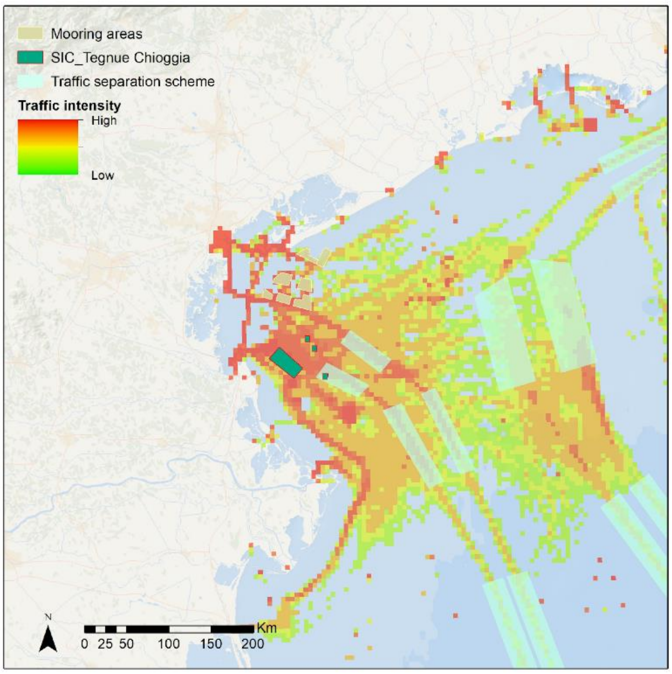


Figure 100. Spatial distribution of marine traffic related uses and activities.

Notably one of the denser areas of marine traffic partially overlap with the SIC of Tegnùe di Chioggia. Traffic lanes have been defined by IMO regulation (1972/COLREGs) to canalize the traffic. The port of Venice, develops an industrial traffic linked to different supply chains (agri-food, steel, chemical, energy), in addition to the commercial one linked to the logistics of different sectors (break bulk, container reefer / perishable products, containers, dry bulk, etc.) and to tourism (both cruise passengers, passengers on Ro-pax ships and both river boats and yachts). In terms of the socio-economic contribution to the reference area, consider that the companies active in the port system of the Northern Adriatic are 1,260 in Venice and 322 in Chioggia, for a total of over 21,000 employees (Assoporti, 2021.). The total shipping tonnage for the port of Venice amounted to 26.500.228 in 2018, while for the Port of Chioggia it was equivalent to 1.021.968 on the same year (Port of Venice, 2018.). The port of Chioggia, which also handles dry bulk and various goods and offers services to tourism by sea, integrates this wide system offer with the fishery industry.

Fisheries and aquaculture

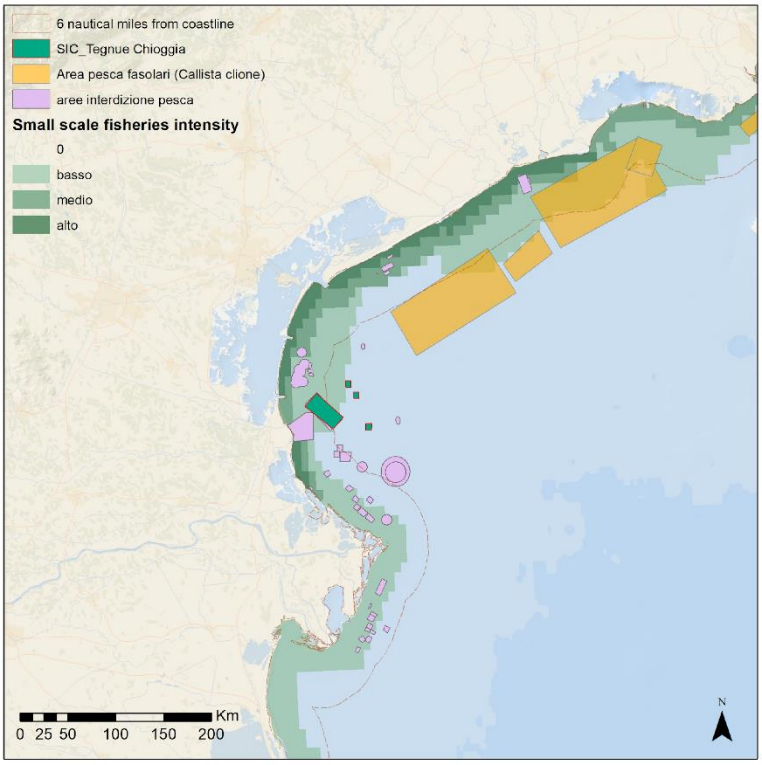


Figure 101. Spatial distribution of fishing activities.

The total fish production of Veneto in 2018, consisting of both sea fishing catches and products deriving from fish farms, is estimated at approximately 61,223 tons. In 2018, the value of Veneto fishing production was approximately 185 million euros. Fish companies for Veneto in the last year equal to 3,787 units, which correspond to 15.4% of companies in the national fishing sector. The regional fishery and aquaculture sector alone represent 2.9% of the primary Veneto production and 3.1% of the total added value. In 2018 the number of boats in the Veneto fleet stood at 658 units. Divided between the following fishing techniques: Hydraulic dredger (164), Fixed longlines (69), Bottom trawl (195), Purse seine (16), Gill net (214). These boats are located in 4 main harbours: Caorle (159), Chioggia (222), Polesine (170), Venice (107). It is worth remarking that many of the registered boats are not currently active.

In Veneto, professional or industrial fishing is flanked by a thriving small-scale fishing activity, carried out both at sea and in lagoon with specific gears. The tools that are used in this type of fishing are the pots, the bertovelli, the cogolli and the seragie, with which cuttlefish, shrimp, lobster, mullet and octopus have been fished for centuries.

Small-scale coastal fishing is defined, by convention, "that exercised by boats of less than 12 meters overall length (LFT), with tackle gear, ferret, longline, lines and harpoons, operating within 12 miles from the coast, as well as with other systems that are used locally in the coastal strip... ".

Of the 658 fishing boats detected in Veneto in 2018, as many as 327 (almost 50%) have an "overall" length of less than 12 meters. In addition, they have an average tonnage of 2.3 GT, an engine power of 35 kW and an average age of over 38 years. These 327 boats, appear to have: 21 licenses for hydraulic dredges, 68 longlines, 16 purse seines (system not present in reality), 199 gillnets and other 23 trawlers. Among the main species that together make up the total fish production of Veneto we find Mediterranean mussels with 15,623 tons, followed by fish landed in fish markets with 15,456 tons (25% st) and Manila clams which add up to 17% (10,274 tons) of the total. Still, below 10,000 tons we find fish farming (15%), lagoon mussels (7%), and clams *Chamelea gallina* (6%). Concerning the smooth clam, *Callista chione*, the quantity of annual catch amounted to 728 tons (Osservatorio Socio Economico della Pesca e dell'Acquacoltura, 2019.).

Tourism

In the Veneto Region coastal counties undergo the greatest tourism pressure (Figure 102). High peaks of tourist incidence on the population have been reported: there are on average 366 tourists per 1,000 inhabitants every day of the year, but the ratio doubles (826) in the months from May to September and becomes three times higher, or even more, in August (1,296 tourists out of 1,000 residents), to the point that the number of tourists every day exceeds that of inhabitants by as much as 30% (Regione del Veneto, 2018.). The total amount of tourism presence on coastal counties for the year 2016 has been estimated to be 34.608.353 (Tools4MSP, 2018.).

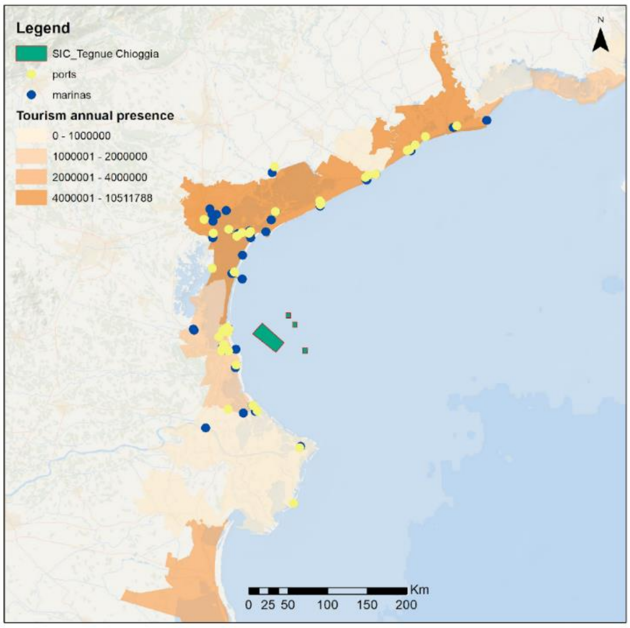


Figure 102. Spatial distribution of tourism presence and key tourism areas.

Aquaculture

The aquaculture sector of Veneto, one of the largest nationally, features clam farming (farming of Manila clam in lagoon waters), mussel farming, and extensive fish farming in enclosures (Valli da pesca). The areas most suited for the cultivation of Manila clam (*Tapes philippinarum*) are located in the Venice lagoon and in the Po Delta. In 2018, the total Venetian production of Manila clam was 10,274 tons, with a decrease in production of -30.5% compared to 2017, a decrease that rises to -45.4% comparing the latest data with that recorded in 2009 (18,827 t). The production of Veneto mussels in 2018, is estimated at 19,786 tons, of which 15,623 produced in offshore plants in the open sea (Figure 103) and the remaining 4,164 in lagoon waters. Aquaculture of marine finfish species, mainly seabass, seabream and mullets, produced 550 t in 2018 and provides for about 1% of the fishing products of the Region (Osservatorio Socio Economico della Pesca e dell'Acquacoltura, 2019).

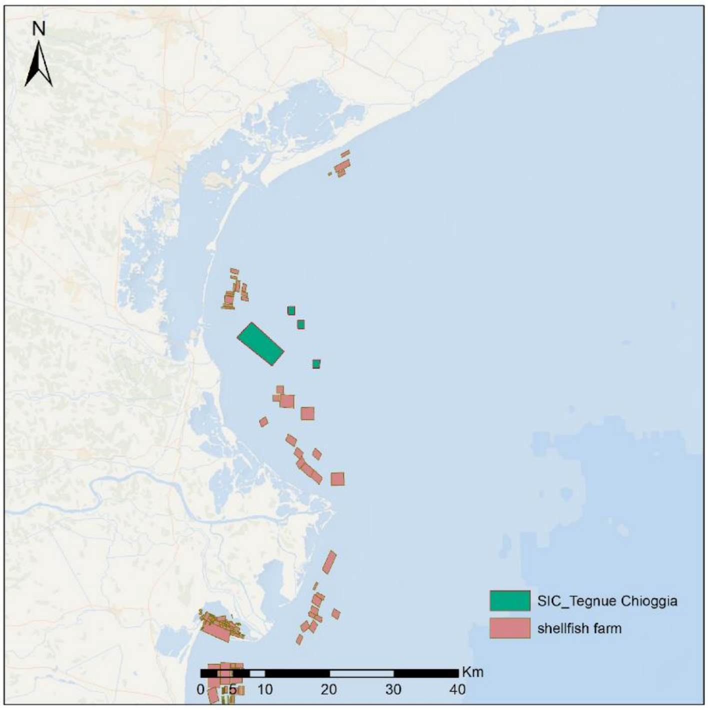


Figure 103. Zones dedicated to shellfish farming.

Extraction of non-living resources

Sand extraction

The amount of sand dredged each year varies depending from the needs and the projects developed. Sand extraction is used mainly for coastal nourishment to mitigate the effect of coastal erosion. This activity is regulated and limited to specific areas (Figure 104). However, it is worth noting that dredging activities take place in these zones with seasonal variations and only within limited spots of the entire areas. Dredging is also undertaken for maintaining the navigability of inland waterways and access to fluvial ports of Venice and Chioggia.

Hydrocarbons extraction

Hydrocarbons are also extracted in the area (Figure 104). Veneto marine waters are included in the so-called marine Zone A define by Italian low (Legislative Decree 21 luglio 1967, n. 613) specifically for hydrocarbons extraction. Zone A covers approximately 13,300 km^2 and constitutes approximately 2% of the Italian continental shelf. The legal jurisdiction over this area is under the UNMIG Bologna.

Over the years, for the purposes of coastal protection and environmental protection, some limitations have been introduced to the areas where new mining activities can be carried out.

In particular, the Legislative Decree of 3 April 2006 no. 152 establishes a ban in sea areas located within twelve miles of the coast lines along the entire national coastal perimeter and from the external perimeter of protected marine and coastal areas.

Other limitations are set by Article 4 of the Law of 9 January 1991, n. 9 (prohibition in the waters of the Gulf of Venice, in the stretch of sea between the parallel passing through the mouth of the Tagliamento river and the parallel passing through the mouth of the Goro branch of the river Po).

These regulatory changes have in fact reduced the area in which it is possible to present new applications for the conferment of new extraction licenses even if Zone A remains the one defined by Law 613/67 as all the limitations subsequently imposed have only applied to extraction licenses conferred after their enactment (DM 9 Agosto 2013).

In year 2020 natural gas production amounted to 1,358,606,581 scm while gasoline production amounted to 181.369 kg (Ministero dello sviluppo economico, 2020.).

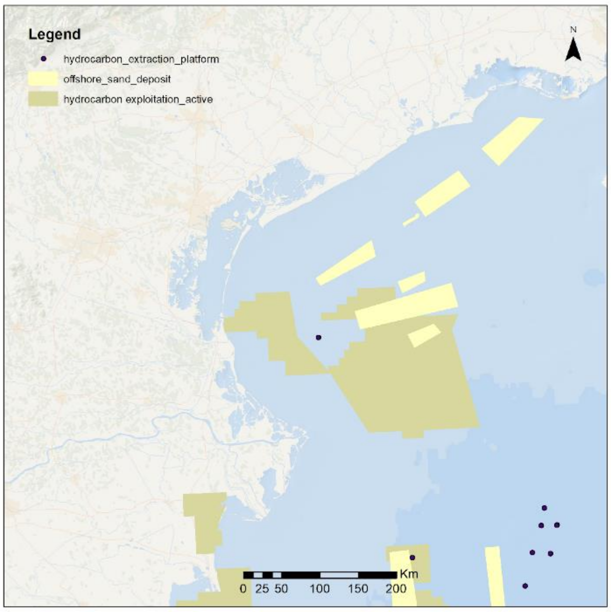


Figure 104. Areas dedicated to extraction of non-living resources.

Nature conservation

Several coastal areas in the region, including the Po Delta, the Venice Lagoon, the Marano and Grado Lagoon, the Caorle Lagoon and Tagliamento river mouth are included in the Natura 2000 Network. In the lagoon of Venice the Ramsar site of Valle Averto is also present. The Tegnùe di Caorle, together with the Tegnùe di Chioggia, are the two Natura 2000 areas located at sea. A third area was established at the boundaries between Veneto and Emilia Romagna Region mainly for the protection of large migratory pelagic species such as the sea turtle *Caretta caretta* and the common bottlenose dolphin *Tursiops truncates* (marked as "Natura 2000 new site" in Figure 105).

Figure 105. Areas dedicated to nature conservation.

Defense

In Veneto area, close to the Tegnùe di Chioggia site, a military area called "Zona Echo 357" for Land-sea shooting exercises have been defined (Figure 106). In this zone navigation, fishing and any activity connected to the public use of the sea are prohibited during shooting exercises.

Another larger area (Zona Echo Echo 346) is defined southern on the waters in front of Emilia Romagna coast (Figure 106). This area includes both subareas where mooring and fishing are permanently prohibited for the presence of explosive weapons on the sea bottom and others where navigation, fishing and any activity connected to the public use of the sea are prohibited during shooting exercises.

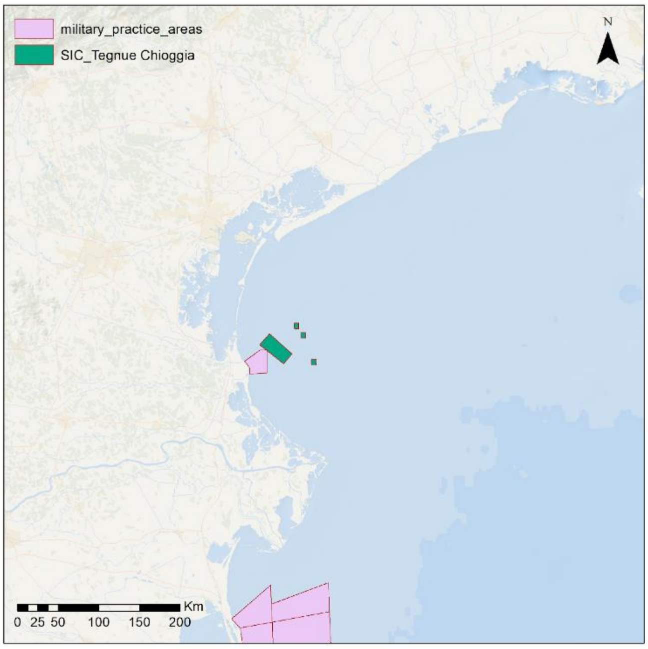


Figure 106. Marine areas dedicated to defense related activities.

4.6. P6 – Miljašić jaruga

4.6.1. Specific results of in situ monitoring of the molluscs at the mouth of the river Miljašić

Monitoring of the area around the river mouth of the Miljašić channel began in 1996, which, among many others, proved to be one of the most interesting malacological sites in the Adriatic Sea. In the described area, 369 species of Mollusca (without class of Cephalopoda where 4 species are present) has been recorded. Of those 369 species of Mollusca, 272 belong to Gastropoda, 90 to Bivalvia, 5 species to Polyplacophara and 2 species are Scaphopoda. Of the recorded approx. 1200 species in the Adriatic Sea, almost a quarter Mollusca species live in this micro-location, which is an

exceptional case. The most valuable part are the rocks on the right side of the dock and the area next to it where 2/3 of the recorded species of this micro-location were founded.
This area has been monitored sporadically since 1996, once per year since 2002, and more detailed since 2016. Sediment was collected on the beach itself and also after strong storms and winds accompanied by high tide, some individuals were collected by diving, but also by brushing the rocks and shaking the algae. In the period up to 2015, the results did not change significantly. The number of species and abundance were constant with the usual fluctuations of 10-20%. In malacology, this can be considered constant because not every year the same conditions are favour to different species. Since 2015, there has been a significant reduction of certain individuals. In the last 5 years, the number of individuals has decreased by about 50%, which we attribute to global climate change. The sea temperature has changed significantly, so in summer there is extreme warming of the seawater, which reaches up to 28°C at that micro-location. For spawning of certain species, this is very devastating and they are not able to survive such newly created conditions. Spawning takes place for most molluscs during the winter and spring periods when the sea temperature is lowest. Regarding the reduction in the number of species, minor variations were found, however some of the species were absent in the results of the last three years of the study. One of these species is the pelagic snail *Akera bullata*. During previous samplings it was regularly present in the study area during March or April due to reproduction. On the underwater wall it should be mentioned the shellfish habitat *Gregariella petagnae* which is not common in the Mediterranean or in the Adriatic, but is present at this location, which classifies this micro-location as a rare habitat of the Mediterranean.
Of all the gastropods found, the first finding for the Adriatic Sea should be mentioned *Doto koenneckeri,* then a potentially new species not found anywhere else in the Adriatic or the Mediterranean, and that is Eatonina sp., and species that were found in only three locations in the Zadar area, such as *Alvani*a cfr*. rudis*, Turbonilla sp. i Ondina sp. Additionaly, findings of some rare species are also valuable *Ondina modiola, Rissoella diaphana, Auristomia nofronii, Odostomia lukisii* and *Ebala nitidissima.*
Regarding the ichthyofauna community on the research site, we found total of 14 species of fish belonging to the 7 different families using the UVC method. The most represented is the Sparidae family with total of 7 species. Since the monitoring was performed in the coldest part of the year (February 2022.), we expect that the number of species in other periods with higher sea temperature will increase, as well as the abundance of noticed species. Therefore, we suggest continuation of *in situ* monitoring by using the BRUV method, which is far more successful in detecting fish species distribution and abundance.
From all the above, it is clear that this is a very specific micro-location that is an endemic habitat of potentially new and rare mollusc species, a breeding ground for some species, and this location should be under permanent monitoring in the future and/or scientific research.

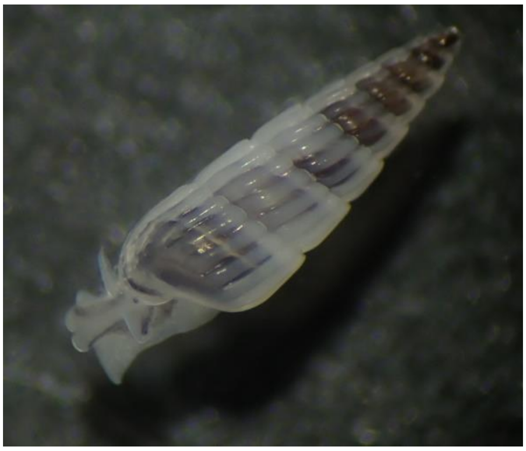


Figure 107. A rare gastropod Turbonilla sp. found at the research site

Figure 108. A rare gastropod Ondina modiola found at the research site

Figure 109. A rare gastropod Alvania rudis found at the research site

Classis **BIVALVIA** Linné, 1758
Subclassis PROTOBRANCHIA Pelseneer, 1889
Ordo SOLEMYOIDA Dall, 1889
Familia SOLEMYIDAE Gray, 1840
Solemya togata (Poli, 1791)
Ordo NUCULOIDA Dall, 1889
Familia NUCULIDAE Gray, 1824
Nucula nucleus (Linné, 1758)
Ordo NUCULANOIDA Carter, Campbell & Campbell, 2000
Familia NUCULANIDAE Adams H. & A., 1858
Nuculana pella (Linné, 1767)
Subclassis PTEROMORPHIA Beurlen, 1944
Ordo ARCOIDA Stolizka, 1871
Familia ARCIDAE Lamarck, 1809
Arca noae Linné, 1758
Barbatia barbata (Linné, 1758)
Familia NOETIIDAE Stewart, 1930
Striarca lactea (Linné, 1758)
Familia GLYCYMERIDIDAE Newton, 1916
Glycymeris nummaria (Linné, 1758)
Ordo MYTILOIDA de Fèrussac, 1822
Familia MYTILIDAE Rafinesque, 1815
Mytilus galloprovincialis Lamarck, 1819
Mytilaster lineatus (Gmelin, 1791)
Mytilaster minimus (Poli, 1795)
Gregariella petagnae (Scacchi, 1832)
Modiolarca subpicta (Cantraine, 1835)
Lithophaga lithophaga (Linné, 1758)
Modiolus barbatus (Linné, 1758)
Gibbomodiola adriatica (Lamarck, 1819)
Ordo PTERIOIDA Newell, 1965
Familia PINNIDAE Leach, 1819
Pinna nobilis Linné, 1758
Ordo PECTINOIDA Gray, 1854
Superfamilia PECTINOIDEA Rafinesque, 1815
Familia PECTINIDAE Rafinesque, 1815
Subfamilia PECTININAE Rafinesque, 1815
Pecten jacobeus (Linné, 1758)
Flexopecten flexuosus (Poli, 1795)
Flexopecten glaber (Linné, 1758)
Flexopecten hyalinus (Poli, 1795)
Subfamilia CHLAMYDINAE Teppner, 1922
Talochlamys multistriata (Poli, 1795)
Subfamilia PEDINAE Bronn, 1862
Aequipecten opercularis (Linné, 1758)
Mimachlamys varia (Linné, 1758)

Superfamilia ANOMIOIDEA Rafinesque, 1815
Familia ANOMIIDAE Rafinesque, 1815
Anomia ephippium Linné, 1758
Heteranomia squamula (Linné, 1758)
Order LIMOIDA Moore, 1952
Familia LIMIDAE Rafinesque, 1815
Lima lima (Linné, 1758)
Limaria hians (Gmelin, 1791)
Limaria tuberculata (Olivi, 1792)
Ordo OSTREOIDA de Fèrussac, 1822
Familia OSTREIDAE Rafinesque, 1815
Ostrea edulis Linné, 1758
Subclassis HETERODONTA Neumayr, 1884
Ordo CARDITOIDA Dall, 1889
Familia CARDITIDAE Fleming, 1828
Cardita calyculata (Linné, 1758)
Glans trapezia (Linné, 1767)
Goodallia triangularis (Montagu, 1803)
Ordo LUCINOIDA Gray, 1854
Familia LUCINIDAE Fleming, 1828
Ctena decussata (Costa O.G., 1829)
Lucinella divaricata (Linné, 1758)
Ordo VENEROIDA Gray, 1854
Superfamilia CHAMOIDEA Lamarck, 1809
Familia CHAMIDAE Lamarck, 1809
Chama gryphoides Linné, 1758
Pseudochama gryphina (Lamarck, 1819)
Superfamilia GALEOMMATOIDEA Gray, 1840
Familia KELLIIDAE Forbes & Hanley, 1849
Bornia sebetia (Costa O.G., 1829)
Familia LASAEIDAE Gray, 1847
Lasaea adansoni (Gmelin, 1791)
Superfamily CARDIOIDEA Lamarck, 1809
Familia CARDIIDAE Lamarck, 1809
Acanthocardia aculeata (Linné, 1758)
Acanthocardia paucicostata (Sowerby G.B. II, 1834)
Acanthocardia spinosa (Lightfoot, 1786)
Acanthocardia tuberculata (Linné, 1758)
Parvicardium exiguum (Gmelin, 1791)
Parvicardium scriptum (B., D. & D., 1892)
Laevicardium crassum (Gmelin, 1791)
Laevicardium oblongum (Gmelin, 1791)
Cerastoderma glaucum (Bruguiére, 1789)
Superfamilia MACTROIDEA Lamarck, 1809
Familia MACTRIDAE Lamarck, 1809
Mactra stultorum (Linné, 1758)

Spisula subtruncata (Da Costa, 1778)
Familia MESODESMATIDAE Gray, 1840
Donacilla cornea (Poli, 1795)
Superfamilia TELLINOIDEA de Blainville, 1814
Familia TELLINIDAE de Blainville, 1814
Tellina albicans Gmelin, 1791 ***= Tellina nitida*** Poli, 1791
Tellina donacina Linné, 1758
Tellina fabula Gmelin, 1791
Tellina incarnata Linné, 1758
Tellina planata Linné, 1758
Tellina tenuis da Costa, 1778
Arcopagia balaustina (Linné, 1758)
Gastrana fragilis (Linné, 1758)
Familia DONACIDAE Fleming, 1828
Donax trunculus Linné, 1758
Familia PSAMMOBIIDAE Fleming, 1828
Gari depressa (Pennant, 1777)
Gari fervensis (Gmelin, 1791)
Familia SEMELIDAE Stoliczka, 1870
Abra alba (Wood W., 1802)
Abra nitida (Müller O.F., 1776)
Scrobicularia plana (Da Costa, 1778)
Familia SOLECURTIDAE d'Orbigny, 1846
Solecurtus strigilatus (Linné, 1758)
Azorinus chamasolen (Da Costa, 1778)
Familia VENERIDAE Rafinesque, 1815
Venus verrucosa Linné, 1758
Chamelea gallina (Linné, 1758)
Clausinella brongniartii (Payraudeau, 1826)
Gouldia minima (Montagu, 1803)
Dosinia exoleta (Linné, 1758)
Dosinia lupinus (Linné, 1758)
Pitar rudis (Poli, 1795)
Callista chione (Linné, 1758)
Irus irus (Linné, 1758)
Polititapes aureus (Gmelin, 1791)
Polititapes rhomboides (Pennant, 1777)
Venerupis corrugata (Gmelin, 1791)
Ruditapes decussatus (Linné, 1758)
Ordo MYOIDA Stoliczka, 1870
Superfamilia MYIOIDEA Lamarck, 1809
Familia CORBULIDAE Lamarck, 1818
Corbula gibba (Olivi, 1792)
Superfamilia PHOLADOIDEA Lamarck, 1809
Familia PHOLADIDAE Lamarck, 1809

Pholas dactylus Linné, 1758
Barnea candida (Linné, 1758)
Familia SOLENIDAE Lamarck, 1809
Solen marginatus Pulteney, 1799
Familia PHARIDAE Adams H. & A., 1858
Ensis ensis (Linné, 1758)
Ensis minor (Chenu, 1843)
Familia HIATELLIDAE Gray, 1824
Hiatella arctica (Linné, 1767)
Hiatella rugosa (Linné, 1767)
Ordo ANOMALODESMATA Dall, 1889
Superfamilia PHOLADOMYIOIDEA Gray, 1847
Superfamilia THRACIOIDEA Stoliczka, 1870
Familia THRACIIDAE Stoliczka, 1870
Thracia corbuloidea de Blainville, 1827
Thracia pubescens (Pulteney, 1799)
Superfamilia PANDOROIDEA Rafinesque, 1815
Familia PANDORIDAE Rafinesque, 1815
Pandora pinna (Montagu, 1803)

Table 15. List of bivalves found at the site

Subclassis PATELLOGASTROPODA Lindberg, 1986
Superfamilia PATELLOIDEA Rafinesque, 1815
Familia PATELLIDAE Rafinesque, 1815
Patella caerulea Linné, 1758
Subclassis VETIGASTROPODA Salvini-Plawen, 1980
Superfamilia FISSURELLOIDEA Fleming, 1822
Familia FISSURELLIDAE Fleming, 1822
Diodora gibberula (Lamarck, 1822)
Diodora graeca (Linné, 1758)
Diodora italica (Defrance, 1820)
Emarginula huzardii Payraudeau, 1826
Emarginula octaviana Coen, 1939
Superfamilia SCISSURELLOIDEA Gray, 1847
Familia SCISSURELLIDAE Gray, 1847
Scissurella costata D'Orbigny, 1824
Sinezona cingulata (Costa O.G., 1861)
Superfamilia HALIOTOIDEA Rafinesque, 1815
Familia HALIOTIDAE Rafinesque, 1815
Haliotis tuberculata tuberculata Linné, 1758
Superfamilia TROCHOIDEA Rafinesque, 1815
Familia TROCHIDAE Rafinesque, 1815
Subfamilia TROCHINAE Rafinesque, 1815
Clanculus cruciatus (Linné, 1758)
Clanculus jussieui (Payraudeau, 1826)
Subfamilia CANTHARIDINAE Gray, 1857
Jujubinus exasperatus (Pennant, 1777)

Jujubinus striatus (Linné, 1758)
Gibbula adansonii (Payraudeau, 1826)
Gibbula adriatica (Philippi, 1844)
Gibbula albida (Gmelin, 1791)
Gibbula fanulum (Gmelin, 1791)
Gibbula rarilineata (Michaud, 1829)
Gibbula turbinoides (Deshayes, 1835)
Gibbula nebulosa (Philippi, 1848)
Gibbula varia (Linné, 1758)
Phorcus mutabilis (Philippi, 1846)
Phorcus turbinatus (Von Born, 1778)
Familia CALLIOSTOMATIDAE Thiele, 1924
Calliostoma laugieri (Payraudeau, 1826)
Calliostoma* cfr. *virescens
Familia TURBINIDAE Rafinesque, 1815
Bolma rugosa (Linné, 1767)
Superfamilia PHASIANELLOIDEA Swainson, 1840
Familia PHASIANELLIDAE Swainson, 1840
Subfamilia TRICOLIINAE Woodring, 1928
Tricolia tenuis (Michaud, 1829)
Superfamilia NERITOIDEA Rafinesque, 1815
Familia NERITIDAE Rafinesque, 1815
Smaragdia viridis (Linné, 1758)
Subclassis CAENOGASTROPODA Cox, 1959
Superfamilia CERITHIOIDEA Fleming, 1822
Familia CERITHIIDAE Fleming, 1822
Cerithium lividulum Risso, 1826
Cerithium protractum Bivona Ant. in Bivona And., 1838
Cerithium vulgatum Bruguière, 1792
Bittium latreillii (Payraudeau, 1826)
Bittium reticulatum (Da Costa, 1778)
Bittium jadertinum (Brusina,1865)
Bittium scabrum (Olivi, 1792)
Familia PLANAXIDAE Gray, 1850
Subfamilia FOSSARINAE Adams A., 1860
Fossarus ambiguus (Linné, 1758)
Familia TURRITELLIDAE Lovèn, 1847
Turritella communis Risso, 1826
Superfamilia TRIPHOROIDEA Gray, 1847
Familia TRIPHORIDAE Gray, 1847
Cheirodonta pallescens (Jeffreys, 1867)
Marshallora adversa (Montagu, 1803)
Monophorus erythrosoma (Bouchet & Guillemot, 1978)
Monophorus perversus (Linné, 1758)
Similiphora similior (Bouchet & Guillemot, 1978)
Metaxia metaxae (Delle Chiaje, 1828)

Familia CERITHIOPSIDAE Adams H. & A., 1853
Cerithiopsis minima (Brusina, 1865)
Cerithiopsis oculisfictis Prkić & Mariottini, 2010
Cerithiopsis scalaris Locard, 1892 (***group scalaris***)
Cerithiopsis tubercularis *sensu* auct., non (Montagu, 1803) = ***C. buzzurroi*** (Cecalupo & Robba, 2010); ***C. nana*** Jeffreys, 1867
Superfamilia EPITONIOIDEA Berry S.S., 1910
Familia EPITONIIDAE Berry S.S., 1910
Epitonium clathrus (Linné, 1758)
Epitonium turtonis (Turton, 1819)
Superfamilia EULIMOIDEA Philippi, 1853
Familia EULIMIDAE Philippi, 1853
Eulima glabra (Da Costa, 1778)
Melanella boscii (Payraudeau, 1827)
Parvioris ibizenca (Nordsieck, 1968)
Vitreolina curva (Monterosato, 1874)
Vitreolina incurva (Bucquoy, Dautzenberg & Dollfus, 1883) = ***V. philippi*** (De Rayneval & Ponzi, 1854) *sensu auct.* >> ***V. philippi*** (De Rayneval & Ponzi, 1854) = *nomen dubium*
Superfamilia LITTORINOIDEA Children, 1834
Familia LITTORINIDAE Children, 1834
Subfamilia LITTORININAE Children, 1834
Melaraphe neritoides (Linné, 1758)
Superfamilia CINGULOPSOIDEA Fretter & Patil, 1958
Familia CINGULOPSIDAE Fretter & Patil, 1958
Eatonina cossurae (Calcara, 1841)
***Eatonina* sp.**
Superfamilia RISSOOIDEA Gray, 1847
Familia RISSOIDAE Gray, 1847
Rissoa decorata Philippi, 1846
Rissoa frauenfeldiana Brusina, 1868
Rissoa labiosa (Montagu, 1803)
Rissoa lia (Monterosato, 1884)
Rissoa rodhensis Verduin, 1985
Rissoa scurra (Monterosato, 1917)
Rissoa similis Scacchi, 1836
Rissoa splendida Eichwald, 1830
Rissoa variabilis (Megerle von Müehlfeldt, 1824)
Rissoa ventricosa Desmarest, 1814
Rissoa violacea Desmarest, 1814
Pusillina ehrenbergi (Philippi, 1844)
Pusillina inconspicua (Alder, 1844)
Pusillina marginata (Michaud, 1830)
Pusillina philippi (Aradas & Maggiore, 1844)
Pusillina radiata (Philippi, 1836)
Pusillina gemmula (Fischer P. in De Folin, 1871)

Setia fusca (Philippi, 1844)
Setia turriculata Monterosato, 1884
Setia* cfr. *turriculata
Alvania aspera (Philippi, 1844)
Alvania cancellata (Da Costa, 1778)
Alvania carinata (Da Costa, 1778)
Alvania cimex (Linné, 1758)
Alvania discors (Allan, 1818)
Alvania lactea (Michaud, 1830)
Alvania lanciae (Calcara, 1845)
Alvania lineata Risso, 1826
Alvania mamillata Risso, 1826
Alvania cfr. rudis
Crisilla semistriata (Montagu, 1808)
Manzonia crassa (Kanmacher, 1798)
Rissoina bruguieri (Payraudeau, 1826)
Familia CAECIDAE Gray M.E., 1850
Caecum auriculatum De Folin, 1868
Caecum subannulatum De Folin, 1870
Caecum trachea (Montagu, 1803)
Familia ASSIMINEIDAE Adams H. & A., 1856
Paludinella globularis (Hanley in Thorpe, 1844)
Paludinella sicana (Brugnone, 1876)
Familia HYDROBIIDAE Troschel, 1857
Hydrobia acuta acuta (Draparnaud, 1805) << Vio & De Min (1999)
Ecrobia ventrosa (Montagu, 1803)
Hydrobiidae sp. 1
Hydrobiidae sp. 2
Hydrobiidae sp. 3
Familia TORNIDAE Sacco, 1896
Tornus subcarinatus (Montagu, 1803)
Circulus striatus (Philippi, 1836)
Circulus tricarinatus (Wood S., 1848) form of C. striatus ?
Familia TRUNCATELLIDAE Gray, 1840
Truncatella subcylindrica (Linné, 1767)
Superfamilia STROMBOIDEA Rafinesque, 1815
Familia APORRHAIDAE Gray, 1850
Aporrhais pespelecani (Linné, 1758)
Superfamilia CALYPTRAEOIDEA Lamarck, 1809
Familia CALYPTRAEIDAE Lamarck, 1809
Calyptraea chinensis (Linné, 1758)
Superfamilia CAPULOIDEA Fleming, 1822
Familia CAPULIDAE Fleming, 1822
Capulus ungaricus (Linné, 1758)
Superfamilia VELUTINOIDEA Gray, 1840
Familia TRIVIIDAE Troschel, 1863

Subfamilia TRIVIINAE Troschel, 1863
Trivia mediterranea (Risso, 1826)
Superfamilia NATICOIDEA Guilding, 1834
Familia NATICIDAE Guilding, 1834
Natica hebraea (Martyn, 1786)
Natica stercusmuscarum (Gmelin, 1791)
Euspira guilleminii (Payraudeau, 1826)
Euspira nitida (Donovan, 1804)
Superfamilia MURICOIDEA Rafinesque, 1815
Familia MURICIDAE Rafinesque, 1815
Subfamilia MURICINAE Rafinesque, 1815
Bolinus brandaris (Linné, 1758)
Hexaplex trunculus (Linné, 1758)
Subfamilia OCENEBRINAE Cossman, 1903
Ocenebra erinaceus (Linné, 1758)
Ocinebrina aciculata (Lamarck, 1822)
Subfamilia MURICOPSINAE Radwin & d'Attilio, 1971
Muricopsis cristata (Brocchi, 1814)
Subfamilia TYPHINAE Cossman, 1903
Typhinellus labiatus (De Cristofori & Jan, 1832)
Familia MARGINELLIDAE Fleming, 1828
Subfamilia GRANULININAE Coovert G.A. & H.K., 1995
Granulina marginata (Bivona Ant., 1832)
Familia CYSTISCIDAE Stimpson, 1865
Gibberula miliaria (Linné, 1758)
Familia MITRIDAE Swainson, 1831
Mitra cornicula (Linné, 1758)
Familia COSTELLARIIDAE Mac Donald, 1860
Vexillum ebenus (Lamarck, 1811)
Vexillum savignyi (Payraudeau, 1826)
Vexillum tricolor (Gmelin, 1791)
Superfamilia BUCCINOIDEA Rafinesque, 1815
Familia BUCCINIDAE Rafinesque, 1815
Euthria cornea (Linné, 1758)
Engina leucozona (Philippi, 1844)
Pisania striata (Gmelin, 1791)
Pollia dorbignyi (Payraudeau, 1826)
Familia NASSARIIDAE Iredale, 1916
Nassarius corniculum (Olivi, 1792)
Nassarius cuvierii (Payraudeau, 1826)
Nassarius incrassatus (Ström, 1768)
Nassarius mutabilis (Linné, 1758)
Nassarius nitidus (Jeffreys, 1867)
Nassarius pygmaeus (Lamarck, 1822)
Cyclope neritea (Linné, 1758)
Familia COLUMBELLIDAE Swainson, 1840

Columbella rustica (Linné, 1758)
Mitrella scripta (Linné, 1758)
Familia FASCIOLARIIDAE Gray, 1853
Fusinus syracusanus (Linné, 1758)
Superfamilia CONOIDEA Fleming, 1822
Familia CONIDAE Fleming, 1822
Conus mediterraneus Hwass in Bruguière, 1792
Family MANGELIIDAE P. Fischer, 1883
Mangelia attenuata (Montagu, 1803)
Mangelia brusinae van Aartsen & Fehr de Wal, 1978
Mangelia costulata Risso, 1826
Mangelia paciniana (Calcara, 1839)
Mangelia scabrida Monterosato, 1890 << Vio & De Min (1999)
Mangelia taeniata (Deshayes, 1833)
Mangelia unifasciata (Deshayes, 1835)
Mangelia vauquelini (Payraudeau, 1826)
Bela zonata (Locard, 1892)
Family RAPHITOMIDAE Bellardi, 1875
Raphitoma andrehoaraui Pelorce & Horst, 2020
Raphitoma concinna (Scacchi, 1836)
Raphitoma densa (Monterosato, 1884)
Raphitoma horrida (Monterosato, 1884)
Raphitoma laviae (Philippi, 1844)
Raphitoma leufroyi (Michaud, 1828)
Raphitoma linearis (Montagu, 1803)
Raphitoma petanii Prkić,Gianuzzi-Savelli & Pusateri, 2020
Subclassis HETEROBRANCHIA Gray, 1840
HETEROBRANCHIA unassigned to Order
Superfamilia RISSOELLOIDEA Gray M.E., 1850
Familia RISSOELLIDAE Gray M.E., 1850
Rissoella diaphana (Alder, 1848)
Rissoella opalina (Jeffreys, 1848)
Superfamila OMALOGYROIDEA Sars G.O., 1878
Familia OMALOGYRIDAE Sars G.O., 1878
Omalogyra simplex (Costa O.G., 1861)
Ammonicera fischeriana (Monterosato, 1869)
Superfamilia VALVATOIDEA Gray, 1840
Familia HYALOGYRINIDAE Warén & Bouchet, 1992
Xenoskenea pellucida (Monterosato, 1874)
Superfamilia PYRAMIDELLOIDEA Gray, 1840
Familia PYRAMIDELLIDAE Gray, 1840
Parthenina emaciata (Brusina, 1866)
Parthenina excavata (Philippi, 1836)
Parthenina incerta (Milaschewitsch, 1916)
Parthenina intermixta (Monterosato, 1884)
Parthenina interstincta (Adams J., 1797)

Parthenina indistincta (Montagu, 1808)
Parthenina juliae (De Folin, 1872)
Odostomella doliolum (Philippi, 1844)
Eulimella acicula (Philippi, 1836)
Eulimella ventricosa (Forbes, 1844)
Odostomia erjaveciana Brusina, 1869
Odostomia eulimoides Hanley, 1844
Odostomia lukisii Jeffreys, 1859
Odostomia nofronii Buzzurro, 2002
Odostomia plicata (Montagu, 1803)
Odostomia turrita Hanley, 1844
Odostomia unidentata (Montagu, 1803)
Megastomia conoidea (Brocchi, 1814)
Ondina diaphana (Jeffreys, 1848)
Ondina modiola (Monterosato, 1884)
Ondina vitrea (Brusina, 1866)
Ondina warreni (Thompson W., 1845)
***Ondina* sp.**
Turbonilla jeffreysi (Forbes & Hanley, 1850)
Turbonilla pusilla (Philippi, 1844)
Turbonilla rufa (Philippi, 1836)
Turbonilla sinuosa (Jeffreys, 1884)
***Turbonilla* sp.**
Superfamilia MURCHISONELLOIDEA Casey, 1905
Familia EBALIDAE Warén, 1994
Ebala nitidissima (Montagu, 1803)
Ebala pointeli (de Folin, 1867)
HETEROBRANCHIA unassigned to Superfamily
Familia CIMIDAE Warén, 1993
Graphis albida (Kanmacher, 1798)
Superfamilia ACTEONOIDEA d'Orbigny, 1842
Familia ACTEONIDAE d'Orbigny, 1842
Acteon tornatilis (Linné, 1758)
Infraclassis OPISTHOBRANCHIA Milne-Edwards, 1848
Ordo CEPHALASPIDEA Fisher P., 1883
Superfamily BULLOIDEA Gray, 1827
Familia BULLIDAE Gray, 1827
Bulla striata Bruguière, 1792
Superfamily HAMINOEOIDEA Pilsbry, 1895
Familia HAMINOEIDAE Pilsbry, 1895
Haminoea navicula (Da Costa, 1778)
***Haminoea* orteai** Talavera**,** Murillo & Templado, 1987
***Haminoea* sp. 1** (book: Opisthobranchia of the Adriatic sea, Prkić, Petani, Iglić i& Lanča)
Weinkauffia turgidula (Forbes, 1844)
Superfamily PHILINOIDEA Gray, 1850
Familia PHILINIDAE Gray, 1850

Philine catena (Montagu,1803)
Philine intricata Monterosato, 1884
Familia AGLAJIDAE Pilsbry, 1895
Chelidonura africana Pruvot-Fol, 1953
Melanochlamys seurati (Vayssiere, 1926)
***Melanochlamys* sp. 2** = new species
Family SCAPHANDRIDAE G.O. Sars, 1878
Scaphander lignarius (Linné, 1758)
Familia RETUSIDAE Thiele, 1925
Retusa crebrisculpta (Monterosato, 1884)
Retusa crossei (Bucquoy, Dautzenberg & Dollfus, 1886)
Retusa laevisculpta (Granata-Grillo, 1877)
Retusa leptoeneilema (Brusina, 1866)
Retusa mammillata (Philippi, 1836)
Retusa truncatula (Bruguière, 1792)
Retusa umbilicata (Montagu, 1803)
Familia RHIZORIDAE Dell, 1952
Volvulella acuminata (Bruguière, 1792)
Ordo RUNCINACEA Burn, 1963
Superfamily RUNCINOIDEA H. & A. Adams, 1854
Familia RUNCINIDAE H. & A. Adams, 1854
Runcina adriatica Thompson T., 1980
Runcina brenkoae Thompson T., 1980
Runcina ferruginea Kress, 1977
Runcina zavodniki Thompson T., 1980
Ordo THECOSOMATA de Blainville, 1824
Superfamilia CAVOLINIOIDEA Gray, 1850
Familia CLIIDAE Jeffreys, 1869
Clio cuspidata (Bosc, 1802)
Clio pyramidata Linné, 1767
Superfamilia LIMACINOIDEA Gray, 1840
Familia CRESEIDAE Rampal, 1973
Creseis acicula Rang, 1828
Ordo SACOGLOSSA Von Ihering, 1876
Superfamilia OXYNOOIDEA Stoliczka, 1868
Familia VOLVATELLIDAE Pilsbry, 1895
Ascobulla fragilis (Jeffreys, 1856)
Superfamilia PLAKOBRANCHOIDEA Gray, 1840
Familia PLAKOBRANCHIDAE Gray, 1840
Elysia timida (Risso, 1818)
Elysia viridis (Montagu, 1804)
Thuridilla hopei (Vérany, 1853)
Familia BOSELLIIDAE Marcus ev., 1982
Bosellia mimetica Trinchese, 1891
Superfamilia LIMAPONTIOIDEA Gray, 1847
Familia LIMAPONTIIDAE Gray, 1847

Ercolania coerulea Trinchese, 1892
Ercolania viridis (Costa A., 1866)
Limapontia capitata (O. F. Müller, 1774)
Ordo ANASPIDEA Fisher P., 1883
Superfamilia AKEROIDEA Mazzarelli, 1891
Familia AKERIDAE Mazzarelli, 1891
Akera bullata Müller O.F., 1776
Superfamilia APLYSIOIDEA Lamarck, 1809
Familia APLYSIIDAE Lamarck, 1809
Aplysia fasciata Pioret, 1789
Aplysia punctata (Cuvier, 1803)
Ordo PLEUROBRANCHOMORPHA Deshayes, 1832
Superfamilia PLEUROBRANCHOIDEA Gray, 1827
Familia PLEUROBRANCHIDAE Gray, 1827
Berthella stellata (Risso, 1826)
Berthellina edwardsi (Vayssière, 1896)
Ordo NUDIBRANCHIA de Blainville, 1814
Subordo DORIDINA Odhner, 1934
Superfamilia DORIDOIDEA Rafinesque, 1815
Familia DORIDIDAE Rafinesque, 1815
Doris bertheloti (d'Orbigny, 1839)
Doris ocelligera (Bergh, 1881)
Familia DISCODORIDIDAE Bergh, 1891
Discodoris stellifera (Ihering in Vayssière, 1904)
Paradoris indecora (Bergh, 1881)
Jorunna tomentosa (Cuvier, 1804)
Platydoris argo (Linné, 1767)
Baptodoris cinnabarina Bergh,1884
Taringa armata Swennen, 1961
Rostanga rubra (Risso, 1818)
Familia CHROMODORIDIDAE Bergh, 1891
Felimare picta (Schultz in Philippi, 1836)
Felimare villafranca (Risso, 1818)
Felimida krohni (Vérany, 1846)
Felimida purpurea (Risso in Guérin, 1831)
Superfamilia PHYLLIDIOIDEA Rafinesque, 1814
Familia DENDRODORIDIDAE O'Donoghue, 1924
Dendrodoris grandiflora (Rapp, 1827)
Dendrodoris limbata (Cuvier, 1804)
Superfamilia ONCHIDORIDOIDEA Gray, 1827
Familia GONIODORIDIDAE Adams H. & A., 1854
Goniodoris castanea Alder & Hancock, 1845
Trapania maculata Haefelfinger, 1960
Familia AEGIRIDAE Fischer P.., 1883
Aegires leuckartii Verany, 1853
Superfamilia POLYCEROIDEA Alder & Hancock, 1845

Familia POLYCERIDAE Alder & Hancock, 1845
Subfamilia POLYCERINAE Alder & Hancock, 1845
Palio nothus (Johnston, 1838)
Subfamilia TRIOPHINAE Odhner, 1941
Limacia clavigera (Müller O.F., 1776)
Subordo DENDRONOTINA Odhner, 1934
Superfamilia TRITONIOIDEA Lamarck, 1809
Familia TRITONIIDAE Lamarck, 1809
Tritonia manicata Deshayes, 1853
Marionia blainvillea (Risso, 1818)
Familia TETHYDIDAE Rafinesque, 1815
Tethys fimbria Linné, 1767
Melibe viridis (Kelaart, 1858)
Familia DOTIDAE Gray, 1853
Doto coronata (Gmelin, 1791)
Doto koenneckeri Lemche, 1976
Familia PROCTONOTIDAE Gray, 1853
Janolus cristatus (delle Chiaje, 1841)
Subordo AEOLIDIINA Odhner, 1934
Superfamilia AEOLIDIOIDEA Gray, 1827
Familia AEOLIDIIDAE Gray, 1827
Aeolidiella alderi (Cocks, 1852)
Berghia coerulescens (Laurillard, 1830)
Spurilla neapolitana (delle Chiaje, 1841)
Familia FACELINIDAE Bergh in Carus, 1889
Facelina annulicornis (Chamisso & Eisenhart, 1821)
Facelina fusca Schmekel, 1966
Facelina rubrovittata (Costa A., 1866)
Caloria elegans (Alder & Hancock, 1845)
Cratena peregrina (Gmelin, 1791)
Favorinus branchialis (Rathke, 1806)
Superfamilia FLABELLINOIDEA Bergh, 1889
Familia FLABELLINIDAE Bergh, 1889
Flabellina affinis (Gmelin, 1791)
Flabellina pedata (Montagu, 1815)
Calmella cavolini (Vérany, 1846)
Superfamilia FIONOIDEA Gray, 1857
Familia TERGIPEDIDAE Bergh, 1889
Trinchesia caerulea (Montagu, 1804)
Trinchesia foliata (Forbes & Goodsir, 1839)
Infraclassis PULMONATA Cuvier, 1817
Superfamilia ELLOBIOIDEA Pfeiffer, 1854
Familia ELLOBIIDAE Pfeiffer, 1854
Myosotella myosotis (Draparnaud, 1801)
Ovatella firminii (Payraudeau, 1826)

Table 16. List of gastropods found at the site

Classis **POLYPLACOPHORA** Gray, 1821
Subclassis NEOLORICATA Bergenhayn, 1955
Ordo LEPIDOPLEURIDA Thiele, 1909
Familia LEPTOCHITONIDAE Dall, 1889
Lepidopleurus cajetanus (Poli, 1791)
Ordo CHITONIDA Thiele, 1910
Superfamilia CHITONOIDEA Rafinesque, 1815
Familia CHITONIDAE Rafinesque, 1815
Chiton olivaceus Spengler, 1797
Family ISCHNOCHITONIDAE Dall, 1889
Ischnochiton rissoi (Payraudeau, 1826)
Familia LEPIDOCHITONIDAE Iredale, 1914
Lepidochitona cinerea (Linnaeus, 1767)
Superfamilia CRYPTOPLACOIDEA H. & A. Adams, 1858
Familia ACANTHOCHITONIDAE Pilsbry, 1893
Acanthochitona fascicularis (Linnaeus, 1767)
Classis **SCAPHOPODA** Bronn, 1862
Ordo DENTALIIDA Da Costa, 1776
Familia DENTALIIDAE Gray, 1847
Antalis inaequicostata (Dautzenberg, 1891)
Antalis vulgaris (da Costa, 1778)

Table 17. List of species from Polyplacophora & Scaphopoda found on the site

Classis **ACTINOPERI**
Subclassis TELEOSTEI
Ordo GOBIFORMES
Familia GOBIIDAE
Gobius geniporus Valenciennes, 1837
Gobius cruentatus Gmelin, 1789
Ordo PERCIFORMES
Familia SERRANIDAE
***Serranus scriba* (Linnaeus, 1758)**
Familia TRACHIIDAE
***Trachinus draco* Linnaeus, 1758**
Ordo MUGILIFORMES
Familia MUGILIDAE
***Mugil cephalus* Linnaeus, 1758**
Ordo EUPERCARIA
Familia SPARIDAE
***Sparus aurata* Linnaeus, 1758**
***Sarpa salpa* (Linnaeus, 1758)**
Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)
Lithognathus mormyrus (Linnaeus, 1758)
Spicara maena (Linnaeus, 1758)
Diplodus annularis (Linnaeus, 1758)
Familia MORONIDAE

***Dicentrarchus labrax* (Linnaeus, 1758)**
Familia LABRIDAE
***Symphodus tinca* (Linnaeus, 1758)**
Symphodus ocellatus (Linnaeus, 1758)

Table 18. List of fish species noticed on the site

4.6.2. Assessment of the impact of the reconstruction of the Miljašić Jaruga on the coastal bay ecosystem and the river ecosystem

Most of the planned rough work will take place close to the current coastline and the existing canal. The impact during construction will be manifested in the partial destruction of the infralittoral algae community during backfilling and excavation for deepening purposes. Given that the project area within is mainly composed of green algae of the genus *Ulva* spp., this impact is not of significant importance for biodiversity in this area. Sandy bottoms permanently covered by the sea will be less directly affected by works during construction in the form of raised sediment. It is to be expected that a small part of the area in the area where excavation or backfilling is planned will be completely destroyed by deepening the canal. No protected or endangered species have been observed in this part, but a muddy bottom with more or less gravel and occasional stones. The impact on the seabed in this habitat is possible to a lesser extent in the enhanced sedimentation settlement on seagrass *Cymodocea nodosa* in the places where the excavation will be carried out. This phenomenon should not have a major impact on this species as it adapts well to muddy bottom conditions and increased sedimentation that regularly occurs in this area due to north and west winds (Cabaco & Rui, 2014.; Mokos, 2017.). There should be no direct physical destruction of this species with regard to the planned intervention, given that it was observed on both sides of the existing canal exit to the sea and outside the planned intervention.

Of the protected species, the only species of mussel *Pinna nobilis* is present, but all individuals observed during the diving inspection were already dead, probably due to mass mortality in the Mediterranean and Adriatic in the period from 2016 to 2020 (Cabanellas-Reboredo et al. 2019). Given the fouling on the shells, it is possible to assume that the death occurred during 2020.

The construction of facilities in the sea should be done in such a way that the impact is limited in space and time to the shortest possible period or as small an area outside the construction area. As much as possible, the intensity of works should be adjusted to avoid excessive turbidity of seawater and sedimentation of marine flora and fauna. In this regard, it is very useful that the project area is characterized by increased flow and the impact during construction will not be long-lasting. After the deepening and excavation of the sediment, it is to be expected that the sediment will completely settle in a few months period. Therefore, no further impact of the planned intervention on the surrounding area and the present biocenoses is expected.

Special attention should be placed on the protection of benthic fauna on the right side of the channel within the large rocks, where rare and possibly endemic gastropods species have been noticed in the past monitoring.

4.7. P7 - Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC)

4.7.1. Coastal dune and halopsamophile vegetation

Being the P7 an LTER site, it hosts the experimentation of different monitoring approaches implemented for several aspects of ecosystem structure and function. Here, we focused on vascular plants and vegetation as bioindicators, briefly describing the methodological approach consisting on re-visitation of random stratified paired plots distributed across the coastal dune sea-inland gradient. In this example, we investigated the diversity patterns of some species guilds (e.g. focal, ruderals and aliens' species) and structural features (life growth forms) in relation to plant invasion on coastal dune Mediterranean maquis. Specifically, we focused on two woody habitat types of European conservation concern (EU 2250*: Coastal dunes with Juniperus spp and EU 2260 Cisto-Lavanduletalia dune sclerophyllous scrubs), which are particularly affected by *A. saligna* invasion (Del Vecchio et al., 2013.; Marzialetti et al., 2019.).

4.7.2. Plant species. Bioindicators of ecosystem functioning and health

Several threats as the spread of alien plants, trampling and beach litter accumulation, impinge coastal ecosystem diversity and functioning, each one exerting specific impact on certain native plant groups, while not on others. In this context, the EC Directive 92/43/EEC (Habitats Directive) (EEC 1992), one of the major steps towards a European strategy for nature conservation, lists a series of "diagnostic species" for the habitats of conservation interest. Diagnostic species are those taxa that play a major role in determining the structure and functioning of these ecosystems by directly or indirectly controlling the availability of resources for other species. In our re-visitation study, we consider as focal species the diagnostic native plant species mentioned in the Interpretation Manual of habitats (Biondi et al., 2009.; Angelini et al., 2016.).

We considered focal species because being strictly adapted to coastal environments (Santoro et al., 2012.) are particularly sensitive to disturbance (Del Vecchio et al. 2019). On the other hand, we considered opportunistic taxa as ruderals because favored by human pressure. We also considered life growths cover because, depicting vegetation structure (Pignatti 2005) they are a good proxy of ecosystem response to human pressure.

4.7.2.1. Sample collection and preparation

Each vegetation plot is georeferenced in a GIS environment. Plots are 16 m^2 wide (4m x 4m) and are carried out during the vegetative period (April – June). Since this example describes the effects of *A. saligna* invasion on woody habitat types 2250* and 2260, we considered two sets of vegetation plots of comparable size: the first collected on well preserved woody habitats types, and the second occurring in invaded areas (e.g. with *A. saligna* cover > 70%). In each plot, the complete list of vascular plants is reported, along with their cover percentage, using the Braun-Blanquet scale of abundance/dominance (Braun-Blanquet 1964). Vascular plants taxonomy conforms Conti et al. (2005) for natives and Celesti-Grapow et al. (2009) for exotic species.

To explore the effects of *A. saligna* invasion on vegetation composition, structure and ecological characteristics, we classified plant species according to plant functional groups (del Vecchio et al. 2019) and growth forms (Pignatti et al. 2005). In particular, we classified species in focal, ruderal and aliens as such guilds showed to react in a different way to alien's invasions (Santoro et al. 2011). Focal species play a major role in determining the structure and functioning of EU habitats and could be considered as reliable indicators of conservation status (Chytrý and Tichý, 2003.). Ruderals are native species not strictly considered as "dune flora" as they are opportunistic and well adapted to disturbed habitats. Consequently, they could be favoured in invaded and disturbed habitats (Malavasi et al., 2016.). Aliens are those species growing outside their natural range and dispersal potential (Richardson et al., 2000.), causing severe environmental changes and ecosystem functioning.

We defined focal species as the characteristic taxa according to the Italian Interpretation Manual of the 92/43/EEC Directive habitats (Biondi et al., 2009.). Alien species were identified following the inventory of the non-native flora of Italy (Celesti-Grapow et al., 2009.), while ruderal species were identified based on previous phytosociological studies in the same area (Del Vecchio et al., 2019.). We also identified the following growth forms: arboreal phanerophytes (PhS), bushy phanerophytes (PhC), lianose phanerophytes (PhL), nano-phanerophytes (NP), geophytes (Geo), caespitose hemicryptophytes (HC), scapose hemicryptophytes (HS), biennial hemicryptophytes (HB), suffruticosa chamaephytes (ChS) and therophytes (Th). Pignatti et al. (2005.).

4.7.2.2. Data analysis

We compare species composition among natural and invaded plots by means of an analysis of similarities (ANOSIM) through a one-way ANOSIM test (9999 permutations). We compared invaded and not invaded habitats in terms of species richness and cover of the considered plant functional groups (Wilcoxon test). The similarity percentage procedure (SIMPER—Clarke1993) was performed to determine which species contribute most consistently to differences between the invaded and not invaded plots (R Core Team 2017.; vegan package Oksanen et al., 2018.).

4.7.2.3. Research results

The analysis of plant functional groups richness and cover evidenced significant differences between natural and invaded plots, reporting higher values of focal species and lower values of ruderals in natural plots. In *A. saligna* invaded plots, we observed a significant ingression of ruderal-nitrophilous plants, as along with other IAPs, besides *A. saligna*. These species are perennial and annual grasses and forbs, which benefit of soil nutrients enrichment in invaded plots and tolerate dry substrates, as in the case of *Oloptum miliaceum, Reseda alba, Erigeron canadensis* and *Xanthium orientale subsp. italicum*.

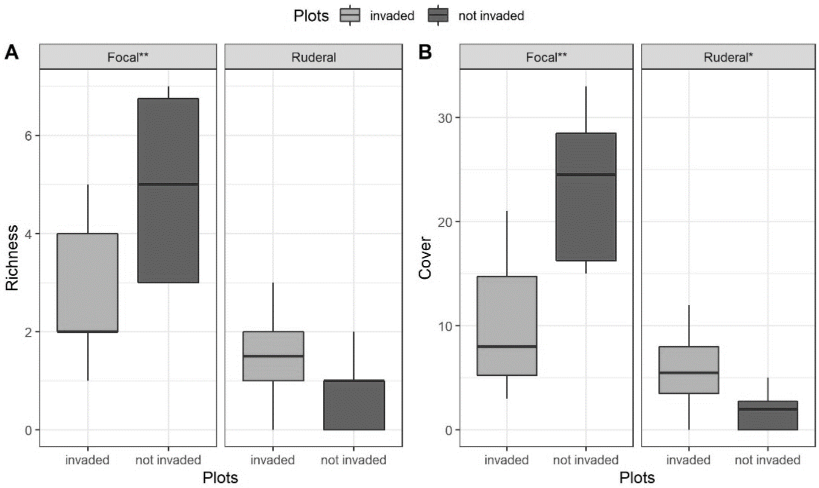


Figure 110. Boxplots comparing species richness (A) and cover (B) of focal and ruderal species for the EU woody habitats. Asterisks indicate significant differences according to the Wilcoxon test for paired samples (p<0.05, ** p<0.01).*

Growth form cover is significantly different among invaded and not invaded plots (Figure 111). The species that mainly contribute to differences in vegetation composition between invaded and not invaded plots were *Phillyrea angustifolia, Pistacia lentiscus, Cistus creticus, Rhamnus alaternus, Rosmarinus officinalis uniperus macrocarpa, Pinus halepensis, Smilax aspera, Phillyrea angustifolia* and *Cistus creticus*, which were more abundant in not invaded plots. Regarding structural changes occurring in invaded plots of EU habitats 2250* and 2260, we observed that *A. saligna* forms a high and dense monospecific stand with a new dominant tree layer which shades the wood floor and causes a significant reduction of bushy phanerophytes and nanophanerophytes cover (i.e. the focal sclerophylls' scrubs). The herbaceous layer, very limited in not invaded stands, becomes more abundant in invaded plots with the ingression of ruderal caespitose hemicryptophytes.

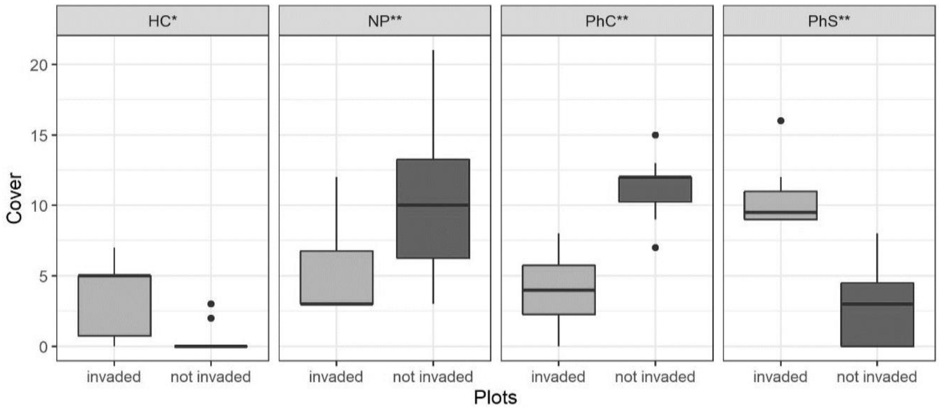


Figure 111. Boxplots comparing the growth forms cover on invaded and not invaded plots for the woody EU habitats. Asterisks indicate significant differences according to the Wilcoxon test for paired samples (p<0.05, ** p<0.01).*

4.8. P8 – The northern eastern Adriatic coastal area (HR)

4.8.1. Nutrients

Station RV001 is 1 nautical mile west off Rovinj, and represents well most of the west Istrian coast. The annual nutrient cycle is characterized by higher values during late autumn and winter, and lower values during the late spring and summer, and is associated to dominant processes in the sea. Thus, lower values are usual during the stratification period and predominant assimilation processes when nutrients are mainly used. Consequently, higher values of oxygen saturation, originated in these processes, are observed. Also, a maximum concentration of chlorophyll *a,* as a measure of increasing phytoplankton biomass, appears.

During late autumn, while the water column is still stratified, nutrient concentrations increase mainly due to intensive decomposition processes in the bottom layer, oxygen consumption, and a yearly minimum of oxygen saturation appears in this layer (Djakovac et al., 2006.). With the establishment of a mixed water column in November, the values are uniformly distributed, and up to spring gradually decrease due to the intensification of assimilation processes. The nitrogen and phosphorus ratio (N/P; Figure 112) is maximal during winter. These high values (>100) suggest an unfavourable relationship of these two nutrient for phytoplankton growth, and that phosphorus is the limiting factor in this ecosystem (*e.a.* Degobbis et al., 2000).

In some years we observed values for all chemical parameters (Figure 112) not consistent with the previously described annual cycle. Specifically, during the period from October - April orthophosphate values were systematically lower and total inorganic nitrogen more than the average. It could be related to a longer period (since 2003) of very low orthophosphate values related to significantly lower annual phosphorus inflow in a wider area, and to the accumulation of total inorganic nitrogen in the ecosystem (Precali and Djakovac, 2009). That indicates too, a very unfavourable N/P relationship (>100, Figure 112). The May-October period was characterized by a different distribution of values of nutrient concentrations and their relations. Thus, concentration values of orthophosphate, total inorganic nitrogen and orthosilicate were significantly below normal ones. Oxygen saturation values were systemically higher than the average (especially in the deeper layers), as well as chlorophyll *a* concentration. Also, the N/P ratio was lower than usual (Degobbis et al., 2000.). These facts suggest that there was a shift in the functioning of the ecosystem in relation to the previous period as well as in relation to its average behaviour. Probably a rather higher inflow of freshwater in a wider area, after a longer period, was favourable for such processes. The Po River, after 6 years, had in 2008 for the first time a significant flow in spring (>6000 m^3/s). Changes in the northern Adriatic open waters reflected along the western Istrian coast, which could be observed from the appearance of a maximum of chlorophyll *a* concentration in the bottom layer from July onwards with a significant decrease in nutrient concentrations.

A significant variability of the ecosystem along the western Istrian coast, as well as the occasional important impact of open northern Adriatic waters, makes it an especially interesting ecosystem. Monitoring and modelling of such an ecosystem, especially due to occasionally more significant external than local influences, is particularly demanding. In such areas the ecosystem approach becomes significant, and we suggest to extend the research and monitoring area also to the open waters in the next phase of the project.

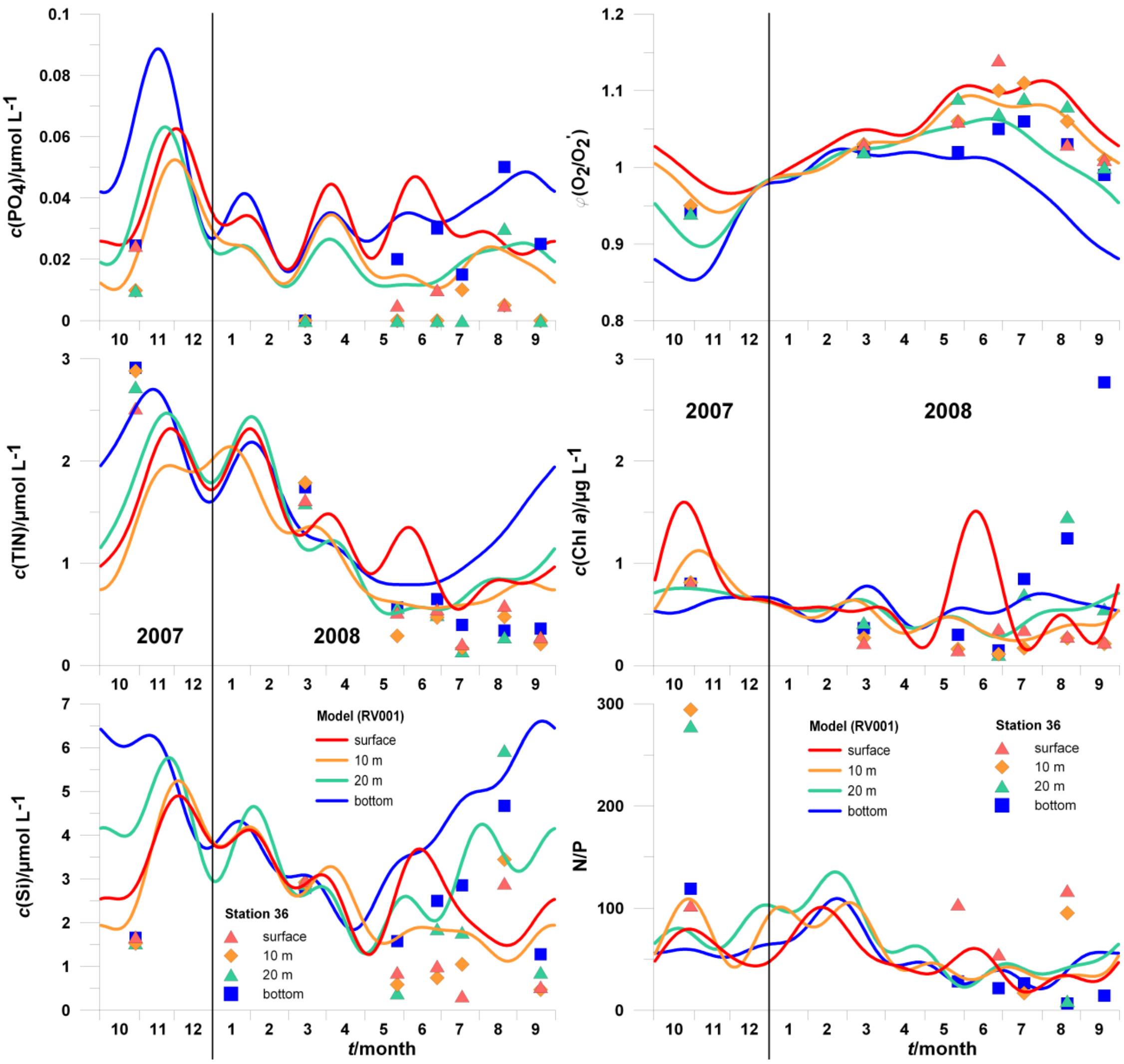


Figure 112. Values measured at station 36 or RV001 and average seasonal model of concentration (c) of orthophosphate (PO_4), total inorganic nitrogen (TIN), orthosilicate (Si) and chlorophyll a (Chl a), oxygen saturation ratio ($\varphi(O_2/O_2')$), and nitrogen and phosphorus ratio (N/P) for distinct layers in the period 1972-2006. A function with three harmonics was fit to the data.

4.8.2. Phytoplankton

Phytoplankton plays a central role in the health and productivity of marine ecosystems, as well as being considered a sensitive indicator of speed and severity of global climate change (Widdicombe et al., 2010.). Cloern and Jassby (2010.) proposed the hypothesis that year to year variability of phytoplankton is a response to anthropogenic activities or shifts in the climate system.

The abundances, biomass, productivity and seasonality of phytoplankton in the northern Adriatic (NA) were extensively studied earlier (Revelante et al., 1985; Mozetič et al., 1998.; Bernardi Aubry et al., 2004.; Cabrini et al., 2004.; Bernardi Aubry et al., 2006., Viličić et al., 2009.), however the majority of those studies mostly refer to the western part of the basin, the Gulf of Trieste and the Venice coastal area, and to lesser extend to the eastern part which is characterized by different hydrological regime (Revelante and Gilmartin, 1980; Revelante, 1984). Phytoplankton abundances and species composition in the NA are related to the annual regime of the Po River outflow and to atmospheric forcing (Viličić et al., 2009.). However, the circulation regime was identified as a major driver of long-term variations of phytoplankton. The information on species succession and phytoplankton dynamics along the eastern coast of the NA was described by Viličić et al. (2009), Godrijan et al. (2010.) and Marić et al. (2010.), but those studies described only recent and short data series.

Long term observations, spanning over several decades, help us to distinguish between regular, recurrent patterns and occasional and exceptional events (Ribera d'Alcalà et al., 2004.). The phytoplankton seasonal cycle in the NA was characterized by spring and fall maxima coinciding with the maximum of the Po River discharge and/or periods of low water column stability and vertical mixing (Revelante and Gilmartin, 1976; Viličić et al., 2007). Diatoms dominate the phytoplankton assemblages over most of the year, while autotrophic and mixotrophic flagellates dominate in oligotrophic conditions in April-July, and dinoflagellates reach their maximum abundance in spring and summer (Bernardi Aubry et al., 2004.).

Trophic status of some area is very well visible through the levels of primary production, with respect to levels of biomass created by process of primary production.

Approximately 520 microphytoplankton species (including several cryptic species complexes) are catalogued for the NA. Their identification relies on light- and electron microscopy. State of the art in microphytoplankton monitoring is the measurement of chlorophyll a concentrations or the light microscopic identification and quantification of groups and several selected species. The exhaustive dataset of the CIM Rovinj for the NA however contains microphytoplankton quantifications at species level and thus already exceeds the state of the art. The further inclusion of molecular taxonomy and ultrastructural analysis allows the identification and quantification of species, subspecies and populations, which spearheads microphytoplankton research and allows resolving uncertainties based on the lack of taxonomic resolution.

Biodiversity analysis will encompass the following activities: Water samples will be analyzed microscopically (LM) to determine microphytoplankton diversity. On the background of the aforementioned microscopical analysis and on the pre-existing long term data, molecular biodiversity analysis (barcoding) will further complete the knowledge on planktonic biodiversity of the NA. DNA-extractions from all samples will be pooled and analysed via next generation sequencing analysis methods. This will dramatically enhance the knowledge on molecular biodiversity of the NA.

The seasonal phytoplankton cycle is also a good indicator for trophic condition of some areas. Seasonal phytoplankton cycle has two maxima, spring maximum, which is more and fall maximum, which is less intense. Both of these maxima are mostly conditioned by quantities of available sunlight and nutrients.

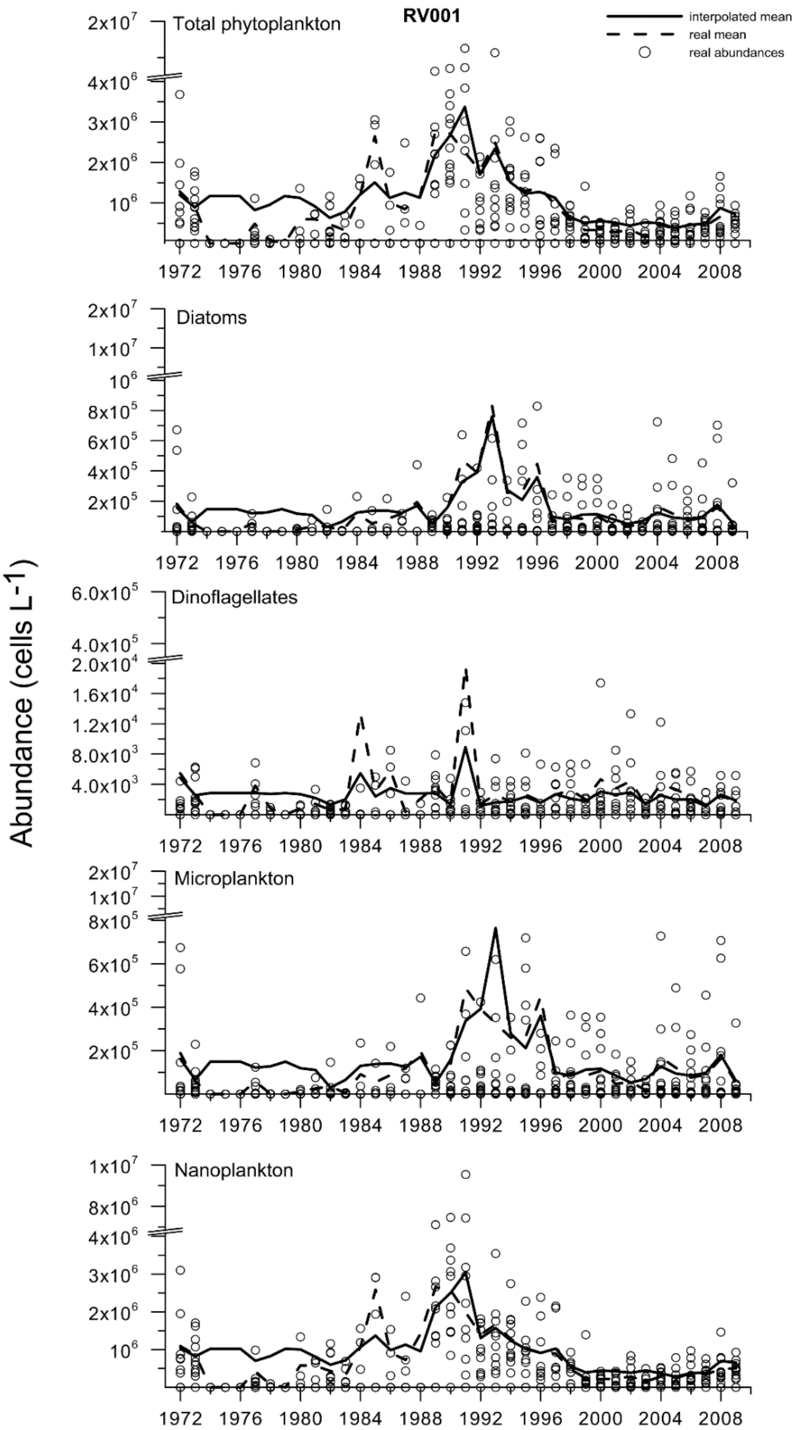


Figure 113. Annual mean of surface values of total phytoplankton, diatoms and dinoflagellates for the whole study period (A) RV001. Dashed lines indicate mean yearly abundances, bold lines stand for interpolated yearly means and circles show real abundances.

In spring days are longer with higher quantities of sun light are available and concentration of nutrient salts after turbulent winter mixing is homogenous distributed in water levels. Summer time is characterized by a significant drop of phytoplankton biomass, caused by insufficient quantities of nutrient salts in surface layer, which are depleted during spring bloom. Additionally, summer stratification precludes mixing and inflow of nutrients in surface layer. In autumn, surface layer is cooling which is causing it to become more dense and heavier than bottom layer and it is falling to the bottom while bottom layer which is rich in nutrients is rising to the surface layer. During autumn there is still enough sunlight to support intense phytoplankton growth. Phytoplankton autumn maximum in the Adriatic Sea is usually prolonged to wintertime depending on climatic and meteorological characteristics of the year. Normal seasonal cycle with intensive spring and less intense autumn maximum is indicating satisfactory trophic status of certain area, while irregular seasonal cycle with higher concentrations of chlorophyll a in summer time is indicating existence of additional source of nutrient salts in surface layer caused by human activities.

The changes in phytoplankton abundance over the seasonal cycle could also be observed. Viličić et al. (2009.) noted a shift in timing of phytoplankton maximal abundances from spring to autumn in the NA (years 2005-2007). As mentioned in Mozetič et al. (2010.) over the last decade that Chl *a* maxima in the late winter–early spring period have shifted from January to February to April to May, especially at stations most affected by reduced Po River discharges. However, some of the previously regular bloom events seem to have virtually disappeared like e.g. the winter/early spring bloom of diatoms and microplankton in the second investigated period on RV001. The station RV001 was characterized by significantly higher abundances of total phytoplankton in the first period (before 2000) while lower abundances were observed in second period (after 2000). A shift in the seasonality of blooms has been recorded as well. In the first period higher abundances were noted from April to July and by second maximum observed in October. The second period higher abundances were recorded from March to April and in October.

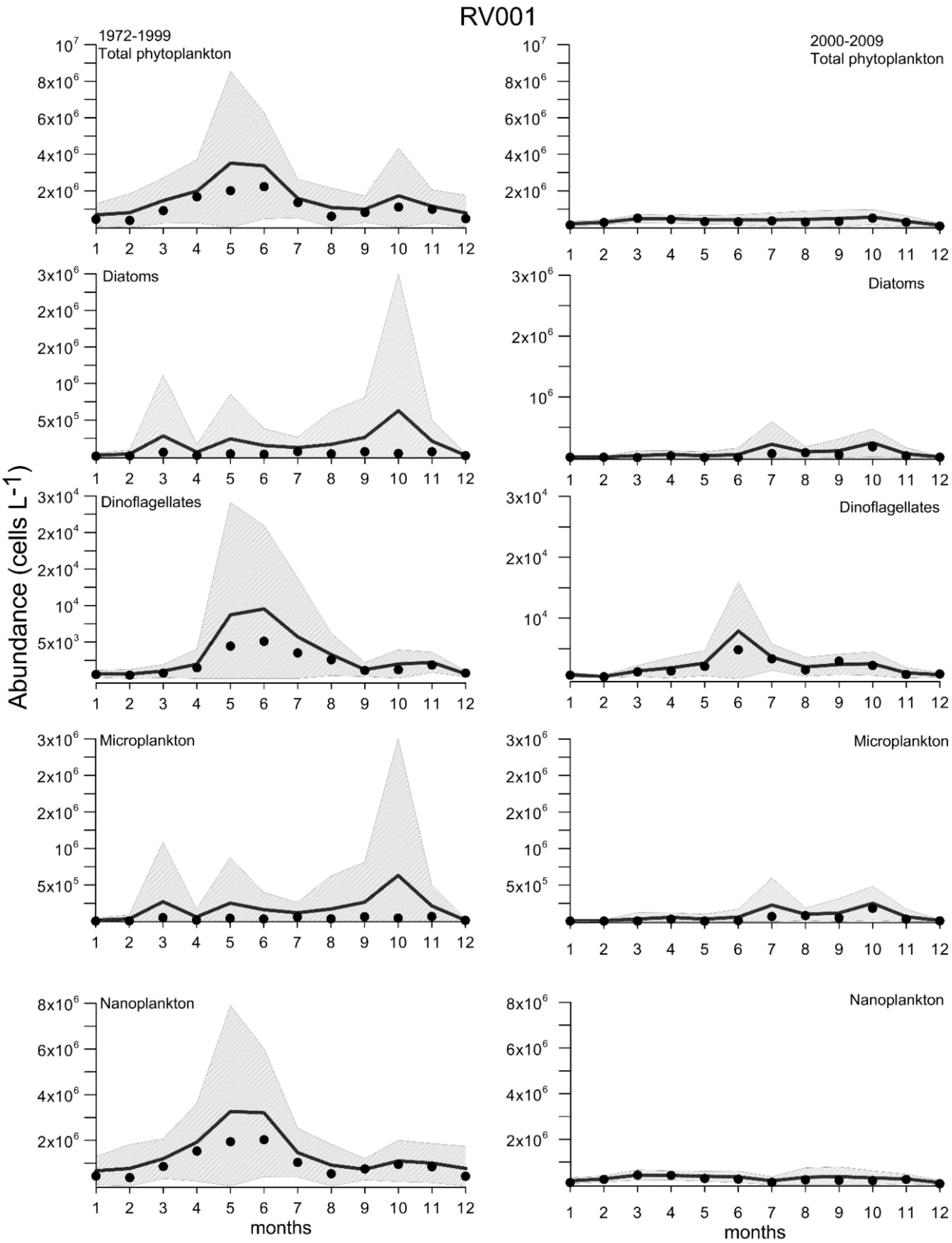


Figure 114. Annual cycle of total phytoplankton, diatoms, dinoflagellates, microplankton and nanoplankton abundances (cell/L) for the station RV001 (A) 1972-1999 and (B) 2000-2009 period. Bold lines represent average abundance while dashed-shaded area represents standard deviation.

Diatoms have been dominant phytoplankton group during autumn blooms in the Northern Adriatic as well as during spring bloom in the Middle and Southern Adriatic implying diatoms as the most important group, which are responsible for seasonal phytoplankton bloom.

4.9. P9 – Cetina river mouth

4.9.1. Load / load input by rivers (WEU7)

Since the Republic of Croatia is a signatory to the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources, Croatian Waters is monitoring Load/ load input by rivers at the mouths of watercourses into the sea. For the Cetina River, monitoring is carried out on the inland waters of the downstream Hydro power plant Zakučac. The condition of a surface water body is assessed on the basis of the ecological or chemical condition, whichever is worse. Ecological status is assessed on the basis of biological, hydromorphological, basic physicochemical elements (temperature, oxygen regime, ion content, pH, alkalinity and nutrients in watercourses and in transitional waters: transparency, temperature, oxygen regime, nutrients, salinity) and chemical elements that accompany biological elements (specific pollutants: arsenic, copper, zinc, chromium, fluorides, AOX and PCB in watercourses, and copper and zinc in transitional waters). Based on the results of the assessment of quality elements, five categories of ecological status are distinguished: very good, good, moderate, bad and very bad. The chemical status of surface waters is determined on the basis of the measured concentration of priority and priority hazardous substances (cadmium, lead, mercury, nickel, polyaromatic hydrocarbons, organochlorine pesticides, volatile chlorinated hydrocarbons), and is assessed on the basis of poorer chemical status indicators. There are two classes of chemical status assessment: good chemical status and no good chemical status achieved.

Annual quantities of nutrients and hazardous substances introduced by Cetina river into the coastal part of the sea are monitored yearly from 2007. Number of the station is 340110.

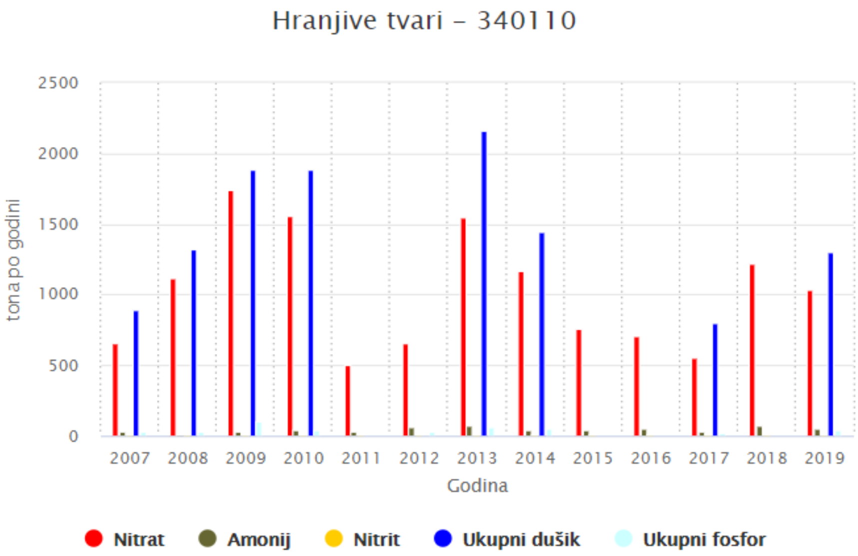


Figure 115. Annual quantities of nutrients introduced by watercourses into the coastal part of the sea (in order of appearance: nitrate, ammonium, nitrite, total nitrogen, total phosphor).

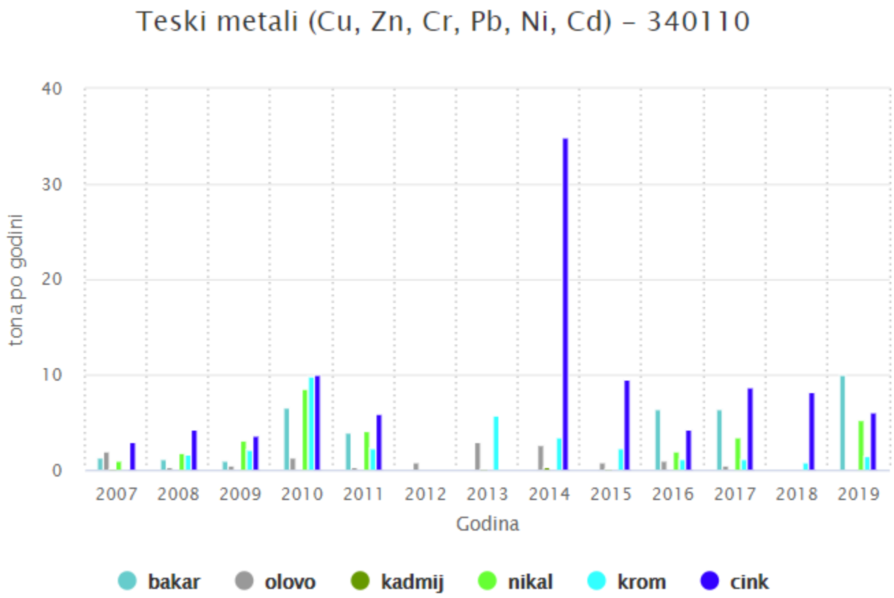


Figure 116. Annual quantities of hazardous substances introduced by watercourses into the coastal part of the sea (in order of appearance: copper, lead, cadmium, nickel, chromium, zinc).

The results of surface water quality monitoring were processed according to the Regulation on water quality standards. Despite fluctuations mainly due to water flow, values show that the ecological condition of the body of surface waters of the Cetina estuary is good. The assessment of the chemical state of watercourses is the same.

4.9.2. Biological quality of transitional waters - phytoplankton WEC1a

Assessment of water quality based on biomass, and the number and composition of the phytoplankton community is a very good indicator since any disturbance of the balance in the marine ecosystem is first reflected in this first trophic stage. Institute of Oceanography and Fisheries is monitoring phytoplankton biomass since 1998 which results will be discussed under chapter 2.7.3 – State of eutrophication. Phytoplankton community composition was measured in 2014 and 2016 only. Microscopic analysis of phytoplankton is performed by the Utermohl method. The Biological quality of transitional waters is assessed on the basis of number and composition of the phytoplankton community. Number of the station for Cetina estuary is OC04.

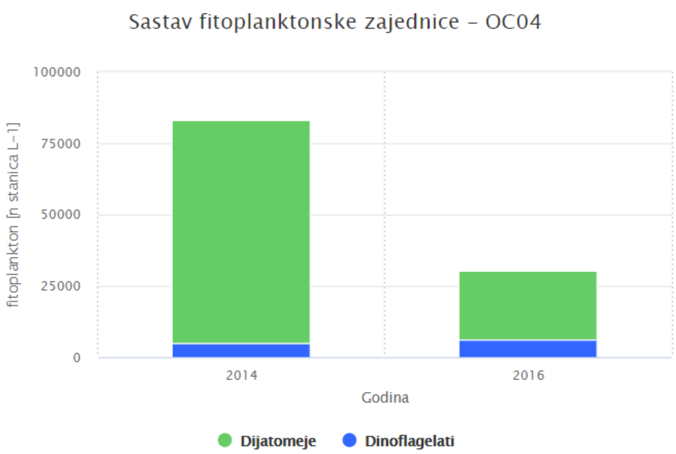


Figure 117. Composition of the phytoplankton community in Cetina estuary (in order of appearance: diatoms, dinoflagellates).

The average annual number of phytoplankton cells in Cetina estuary in 2014 was higher, which was mostly due to summer phytoplankton blooms caused by extremely high rainfall in the warmer period of the year, i.e. increased input of the river Cetina. Due to the very low content of dinoflagellates in the phytoplankton community, these blooms took place without consequences for the environment. Based on the number and composition of the phytoplankton community, the ecological status of the Cetina estuary can be described as very good.

4.9.3. Biological quality of transitional waters - fish WEC1d

Biological quality of transitional waters based on fish community has been monitored for Cetina estuary by the Institute of Oceanography and Fisheries since 2007 (station is RR09). Spatial and temporal distribution of ichthyofauna composition in the marine ecosystem in transitional waters with regard to the number of species, representation of dominant families, their trophic composition, resident status and indicators of habitat changes such as the number of diadromous, new and indicator fish species is monitored. Given the above distribution, EFI (Estuarine Fish Index) index is calculated which ranges from 1 to 5, 5 being the highest. Sampling is performed with small special tugs for catching juvenile fish, triple standing nets - poponica, trawl and a special fishing technique - tramat (ludar). The results are supplemented by data obtained by diving because the majority of small cryptobenthic species cannot be sampled with the above fishing gears.

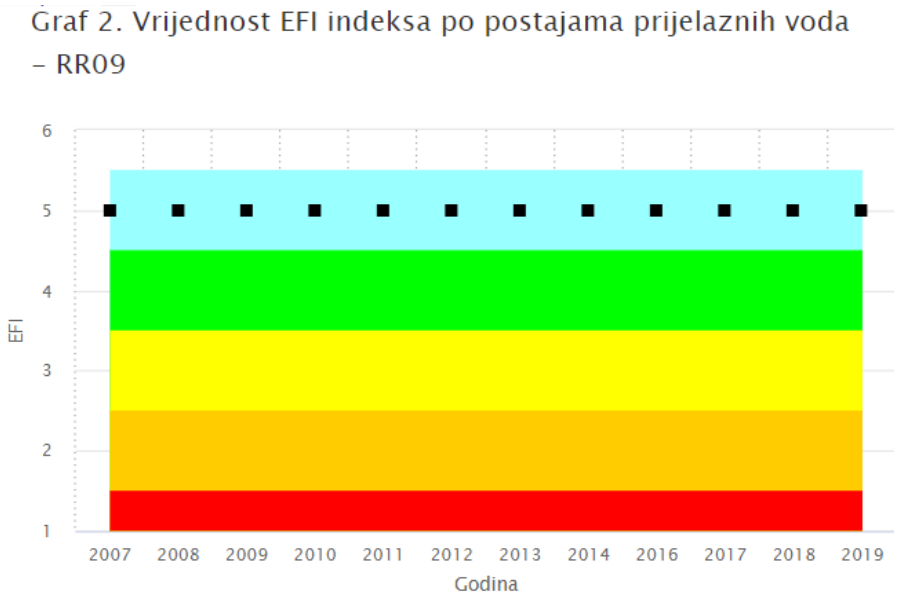


Figure 118. Values of the EFI index for the Cetina estuary (station RR09).

The area of Cetina estuary is characterized by an important biodiversity of fish species. The ichthyofauna of the wider area of the Cetina estuary is closely related with the habitats that form sandy-rocky bottoms overgrown with brown photophilous algae and seagrass meadows, especially *Cymodocea nodosa*. Such habitats provide a whole range of benefits, especially for juvenile fish stages, and more than 40 species of fish have been identified, while more than 70 have been recorded in the surrounding coastal waters. The following eurythermal and euryhaline marine fish species dominate in the fish community: Big-scale sand smelt, *Atherina boyeri* and Mediterranean sand smelt, *Atherina hepsetus*. Sparid species of fish such as Salema, *Sarpa salpa*, Annular sea bream, *Diplodus annularis*, Common two-banded sea bream, *D. vulgaris* and batoglavac, *Pagellus acarne* should certainly be singled out in terms of number and abundance. The Striped sea bream, *Lithognathus mormyrus*, for which this area is an essential feeding ground and habitat, is very numerous. In addition to sheep with seasonal fluctuations depending on spawning, there are also scales (8): Gilt-head sea bream, *Sparus aurata*; Common two-banded sea bream, *Diplodus vulgaris*; Annular sea bream *D. annularis*; White sea bream, *D. sargus*; Common pandora, *P. erythrinus*, Axillary sea bream, *P. acarne*, Common dentex, *Dentex dentex* and Salema, *Sarpa salpa*. This area is clearly profiled as an important feeding and habitat for a number of species from the family of sea breams (Sparidae) which are among the leading target species of coastal and demersal fisheries on the eastern Adriatic coast. This area is a recruiting center for livestock of these species in the canals of central and southern Dalmatia. The juvenile Red mullet, *Mullus barbatus*, is very common. The following species are always present: Golden grey mullet, *Liza aurata*; Thin lip grey mullet, *Liza ramada*; Thicklip grey mullet, *Chelon labrosus* and Leaping mullet, *Liza saliens*. A whole range of species of genus gobius, parablennius, syngnathus and of family labridae have been identified, as well as species typical of sandy sediment: weevers and flat fishes. European eel, *Anguilla anguilla*, is frequent and common species in this area. The number and diversity of species show seasonal variability: the highest values are recorded in spring (the period when after spawning in open waters the largest number of juveniles of different fish species enters this area), and the lowest in winter (large number of species before winter leaves these shallow bays). So far, no newly introduced fish species have been identified, but since 2014, the Bluefish, *Pomatomus saltatrix* and European barracuda, *Sphyraena sphyraena*, have been identified, which may be a hint of trophic gloss changes due to the aggressive predatory characteristics of these two species. In 2013 an increased number of blue crabs *Callinectes sapidus* has been found, which was not previously caught in the area of the mouth of the river Cetina. Since then, it is regularly fished at the mouth of the Cetina and in 2021 it was recorded in significant numbers upstream within the boundaries of the significant landscape. Cetina estuary has a constant EFI (Estuarine Fish Index) of 5. Results of monitoring activities carried out by the Institute of Oceanography and Fisheries from 2007 till now show that very good biological quality of this transitional water body.

4.9.4. Hazardous substances in marine organisms WHS6

The indicator monitors the spatial and temporal distribution of concentrations of heavy metals and organic pollutants in marine organisms. Hazardous substances enter the sea in different ways: wastewater from industry and public sewerage systems, leaching from agricultural areas and roads, such as municipal waste, dry and wet deposition from the atmosphere, ship waste, combustion of marine fuel, etc. shellfish indiscriminately feed on plankton filtering particles of a certain size from seawater so that dangerous substances accumulate in their body and the concentrations of dangerous substances are higher in shellfish than in the surrounding sea, therefore they pose a danger to human health. Organotin compounds (OTC - organotin compounds) are present in the environment as a consequence of their use in various industrial applications and agriculture, and are a special problem in the marine environment where they are present due to their use in antifouling paints for ships. Croatia is a signatory to the AFS (Anti-fouling system) convention, and the use of antifouling paints for OTC-based ships has been banned in Croatia since 2008. However, control of TBT use and the state of TBT pollution in the coastal area is not carried out, because until 2009 no laboratory in Croatia was qualified to measure OTC in marine samples.

Samples of mussels (*Mytilus galloprovincialis*) for the analysis of chlorinated hydrocarbons are taken under the condition that at the time of sampling at the station there is a colony of mussels of satisfactory size (4-5 cm) and quantity (20-25 individuals). According to the recommendations of the international organization MED-POL (UNEP), shellfish are sampled once a year, in the period before the sexual maturation of individuals (in the Adriatic no later than the end of March), to avoid the effects of biological factors on the content of contaminants in the tissue. The mass fractions of lindane, DDT compounds and PCB compounds were determined by gas chromatography with an electron capture detector (GC- ECD) on the capillary column.

Hazardous substances in marine organisms of Cetina estuary have been monitored by the Institute of Oceanography and Fisheries only for certain year or periods depending of the substance. Pesticides were measured during two periods, from 2005 to 2008 and from 2009 to 2012. Among metals, only mercury was measured in 2009 and 2011, while organic-tin compounds were measured in 2010 and 2011. Number of Cetina estuary station is OT07.

Pesticides (DDTx, Lindan, PCBx)

Based on the collected data, the average annual values of DDTx in marine organisms were calculated for years 2005, 2006 and 2008. For the period 2005-2008, DDTx mean value was calculated. Results are shown in the following graphs.

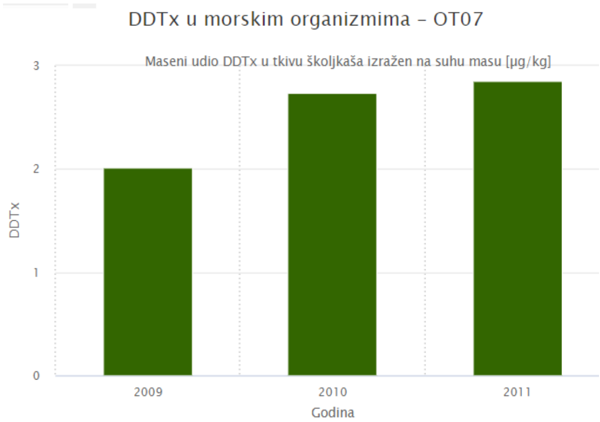


Figure 119. Average annual values of DDTx in marine organisms of Cetina estuary (station OT07, values from 2,01 to 2,855 microg/kg).

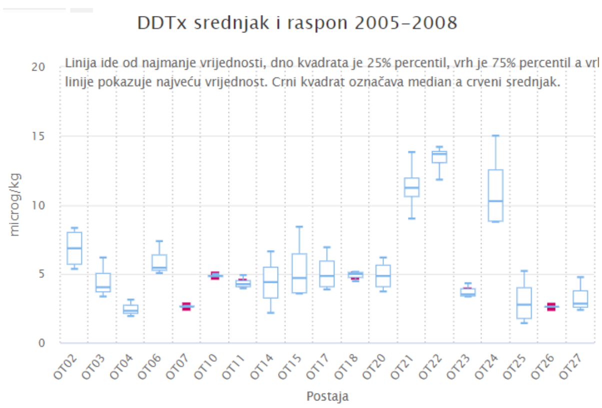


Figure 120. Mean value of DDTx in marine organisms of Cetina estuary (station OT07, value: 2,61 microg/kg) and other stations in Croatian part of the Adriatic Sea.

Lindan and PCBx were measured during the period 2005-2008. Their mean values were calculated. Results are shown in the following graphs.

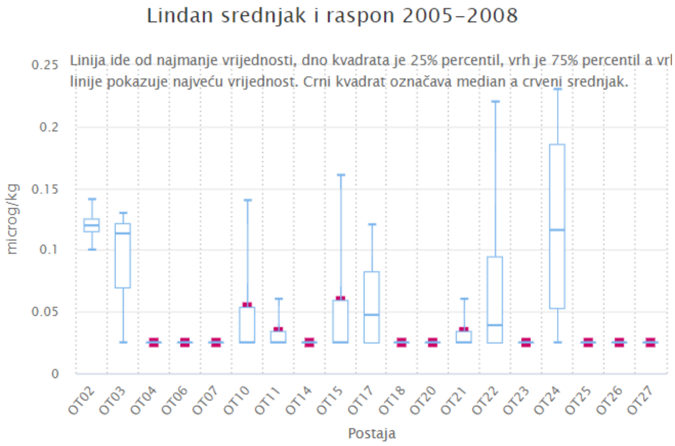


Figure 121. Mean value of Lindan in marine organisms of Cetina estuary (station OT07, value: 0,025 microg/kg) and other stations in Croatian part of the Adriatic Sea.

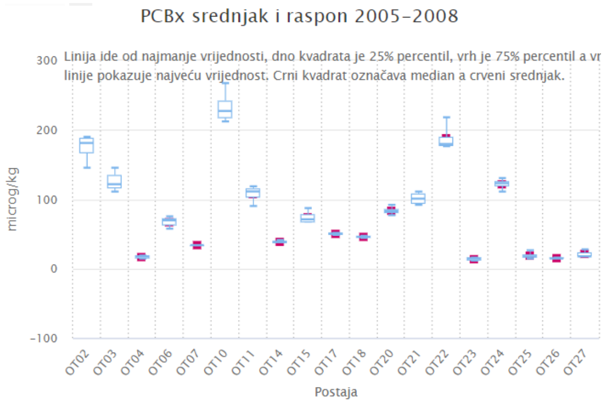


Figure 122. Mean value of PCBx in marine organisms of Cetina estuary (station OT07, value: 33,7 microg/kg) and other stations in Croatian part of the Adriatic Sea.

From the results is visible that Cetina estuary is among the areas that have the lowest concentrations of pesticides in marine organisms. The obtained results were compared with the proposed values for hazardous substances in the marine environment of Europe (Legal Framework - EEA 2003). Values do not exceed the suggested lower value.

Metals (Hg - mercury)

Based on the collected data, the average annual values of mercury in marine organisms were calculated for years 2009 and 2011.

Figure 123. Average annual values of mercury in marine organisms of Cetina estuary (station OT07, values: 349 microg/kg d.w. for 2009 and 390 microg/kg d.w. for 2011).

At the stations of the Croatian part of the Adriatic, in 2009 the content of total mercury in the soft tissue of shellfish ranged in a wide range from 164 - 4118 µg/kg d.w, and in 2011 from 116 and 2378 µg/kg d.w. For mercury, the legally prescribed value is 500 microg/kg d.w. The values for the Cetina estuary are below the legally prescribed value.

<u>Organic tin compounds</u>

Based on the collected data, the average annual concentration of organotin compounds in mussels of Cetina estuary were calculated for years 2010 and 2011.

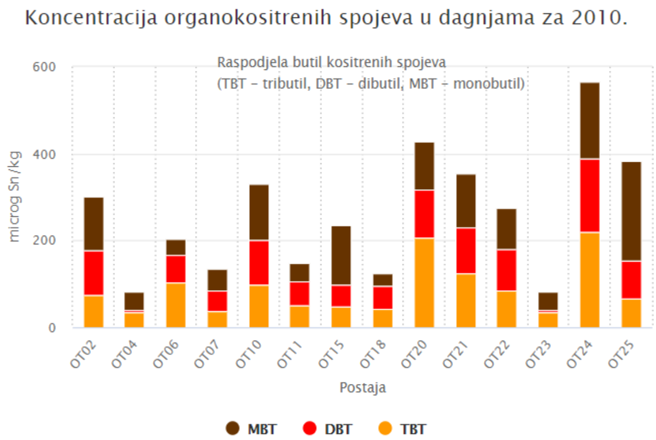


Figure 124. Average annual concentration of organotin compounds in mussels of Cetina estuary (station OT07, values: TBT = 35,8 microg/kg, DBT = 48,2 microg/kg, MBT = 50,2 microg/kg).

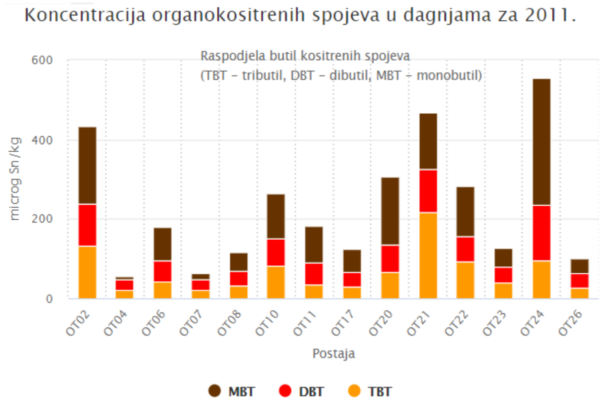


Figure 125. Average annual concentration of organotin compounds in mussels of Cetina estuary (station OT07, values: TBT = 21,2 microg/kg, DBT = 26,4 microg/kg, MBT = 15,4 microg/kg).

Tributyl tin (TBT) is degraded in the environment into dibutyl (DBT) and monobutyl (MBT) tin, so the total concentration of butyltin compounds (ΣBuT = TBT + DBT + MBT) indicates how much TB is introduced into the environment, and the proportion of TBT in the concentration of butyltin compounds (TBT / ΣBuT) indicates how much time has elapsed since the introduction of TBT into the environment. The presence of TBT and its degradation products (DBT and MBT) was determined in all samples along the Adriatic coast, which indicates that the pollution with organotin compounds is spread along the entire Adriatic coast. In 2010, the average concentration of ΣBuT along the Adriatic coast was 270.4 µg/kg, while in 2011, the average concentration of ΣBuT along the Adriatic coast was 232.5 µg/kg. A significant share of TBT in total butyl tin compounds indicates that TBT was recently introduced into the marine environment, which means that dyes based on organotin compounds are still used in our country, despite the fact that they are banned.

The values for the Cetina estuary are below average, and there is a decrease in concentrations in 2011 compared to 2010. According to OSPAR, the degree of pollution of the marine environment (i.e. the expected degree of imposex) can be estimated from the concentration in mussels. The concentrations measured at the mouth of the Cetina correspond to the average level of pollution (category C according to OSPAR, on a scale from A to F), which indicates that toxic effects (imposex) of TBT on marine organisms can be expected.

4.9.5. State of eutrophication

Eutrophication is a change in the ecosystem caused by the excessive rate of formation of organic matter. Eutrophication can occur by natural mechanisms, but also by human influence (anthropogenic influence). As a consequence of the increased intake of nutrients in the sea-eutrophication, phytoplankton algae grow and multiply, which increases the concentration of chlorophyll a in the sea and reduces transparency. When nutrients are consumed, mass starvation occurs and phytoplankton algae die off and fall to the bottom where oxygen is consumed due to bacterial decomposition. Therefore, the state of eutrophication is assessed based on the following parameters:

- transparency of the sea
- oxygen saturation
- concentrations of nutrient salts of nitrogen and phosphorus
- chlorophyll a concentration

The state of eutrophication in Cetina estuary has been monitored by the Institute of Oceanography and Fisheries since 2002, including oxygen saturation, concentration of nutrient salts of nitrogen and phosphorus, and since 1998 for concentration of chlorophyll a. For Cetina estuary monitoring stopped in 2016. Number of the station is OC04. Based on the collected data, the average annual values of the surface layer (0-10m) are calculated, which are shown in the following graphs.

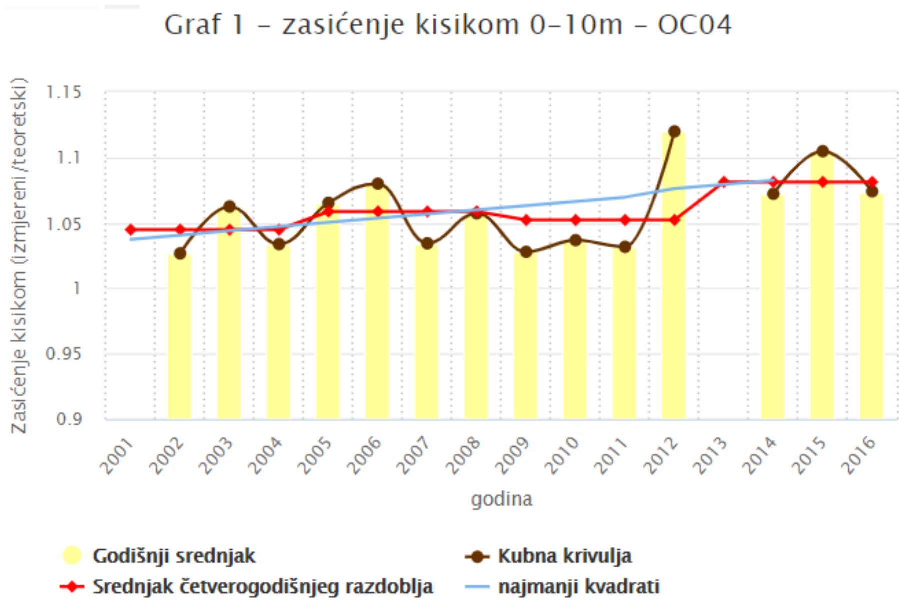


Figure 126. Average annual values of oxygen saturation in the surface layer of Cetina estuary (in order of appearance: annual mean, the mean of a four-year period, cubic curve, smallest squares).

By the end of 2011, the mean values of oxygen saturation in the surface layer of the Cetina estuary were close to the average of all Adriatic stations (1.047 ± 0.068 for the period from 2005 to 2008; 1.050 ± 0.063 for the period from 2009 to 2012). In 2012, there was an increase compared to previous years. Although the Adriatic recorded a declining trend of oxygen saturation, the Cetina estuary recorded a slight increase but within the average (the average of all Adriatic stations was 1.035 ± 0.086 for the period from 2013 to 2016). Like the rest of the Adriatic, the surface layer of the Cetina estuary is slightly supersaturated with oxygen.

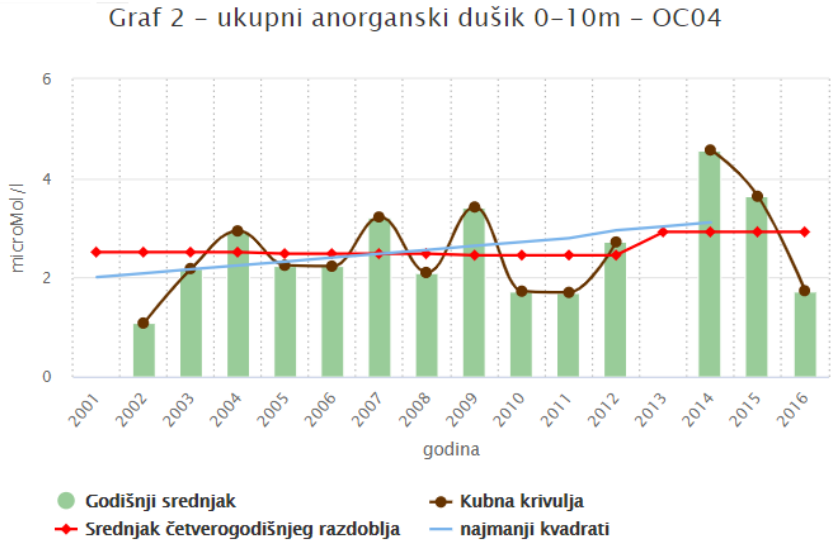


Figure 127. Average annual values of inorganic nitrogen concentration in the surface layer of Cetina estuary (in order of appearance: annual mean, the mean of a four-year period, cubic curve, smallest squares).

The Cetina estuary has low inorganic nitrogen concentrations in the surface layer that are on average of all Adriatic stations (2,393 ± 3,664 for the period from 2005 to 2008; 2,618 ± 4,062 for the period from 2009 to 2012, and 2,268 ± 3,350 for the period from 2013 to 2016). The increase was recorded in 2014 as a result of freshwater inflows (which naturally contain higher levels of nitrogen salts compared to seawater) but these values are still low.

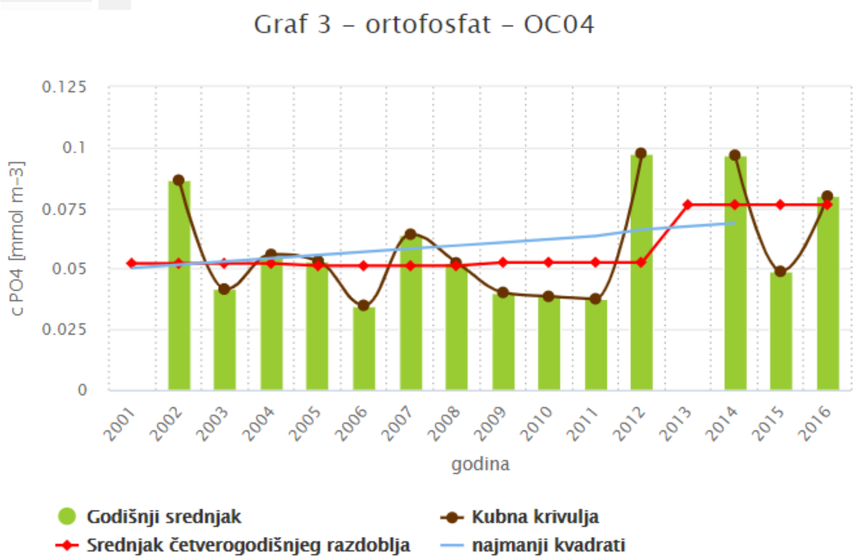


Figure 128. Average annual values of orthophosphate concentration in the surface layer of Cetina estuary (in order of appearance: annual mean, the mean of a four-year period, cubic curve, smallest squares).

The Cetina estuary has low orthophosphate concentrations in the surface layer that are on average of all Adriatic stations (0.042 ± 0.062 for the period from 2005 to 2008; 0.045 ± 0.040 for the period from 2009 to 2012, and 0.058 ± 0.050 for the period from 2013 to 2016). In certain years, higher mean values were recorded (2012, 2014), which indicates a stronger anthropogenic impact, although these values are still low.

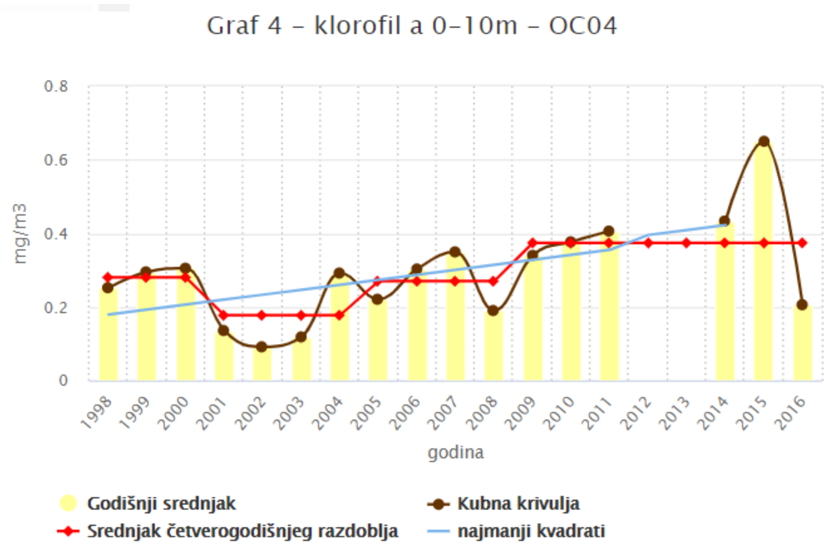


Figure 129. Average annual values of chlorophyll a concentration in the surface layer of Cetina estuary (in order of appearance: annual mean, the mean of a four-year period, cubic curve, smallest squares).

The mouth of the Cetina has low chlorophyll a concentration in the surface layer that are on average of all stations in the Adriatic. As at other Adriatic stations, the highest values were in 2015. This difference is most pronounced in the winter. The reason for this may be the different meteorological conditions that prevailed in the analyzed years in the winter, but also throughout the year. Comparing the established concentrations with the limit values proposed by the MED GIG group for water quality, phytoplankton biomass can be considered relatively low and the area can be described as a high-quality area.

Despite the year-on-year variability caused by natural oscillations of hydrological conditions, current field, etc., the overall state of eutrophication of the Cetina estuary is very good. The proportions of water column oxygen saturation are within the usual limits for the investigated area, and the concentrations of nutrient salts and chlorophyll a are in the range's characteristic of the oligotrophic coastal sea. Despite the influence of the river Cetina, the mouth of the Cetina belongs to oligotrophic areas.

4.9.6. Frequency of low oxygen concentrations in the bottom layer Oxygen saturation WEU15

A condition of low oxygen concentrations (below 2 mg/L in the bottom layer) is called hypoxia. In areas where the oxygen concentration is low, the so-called "suffocation" of sensitive species of organisms on the seabed but also migrations of other species. The dissolved oxygen content in seawater (mg/L) was determined by the classical Winkler method (iodometric titration with sodium thiosulfate), and the proportion of oxygen saturation was calculated from the ratio of established and theoretical oxygen content at a given temperature and salinity.

The oxygen saturation in Cetina estuary bottom layer has been monitored by the Institute of Oceanography and Fisheries since 2002. Monitoring stopped in 2016. Number of the station is OC04. Based on the collected data, the temporal distribution of minimum concentrations and proportions of oxygen saturations in the bottom layer of Cetina estuary are calculated, which are shown in the following graphs.

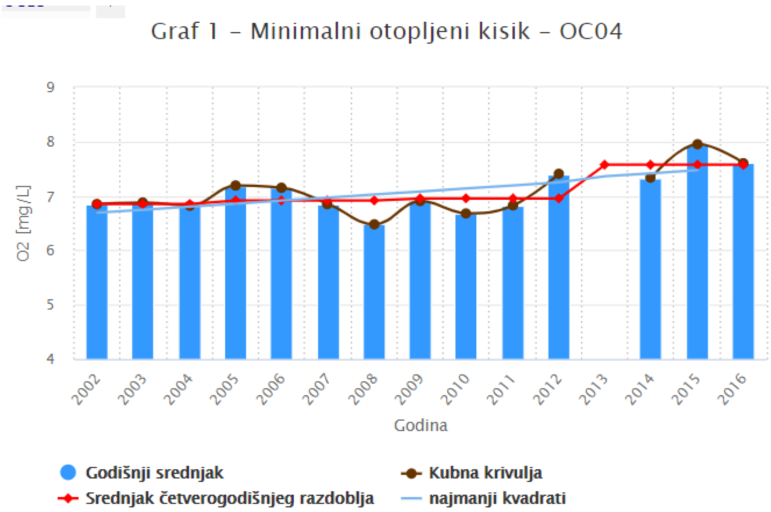


Figure 130. Minimum concentrations of oxygen in the bottom layer of Cetina estuary (in order of appearance: annual mean, the mean of a four-year period, cubic curve, smallest squares).

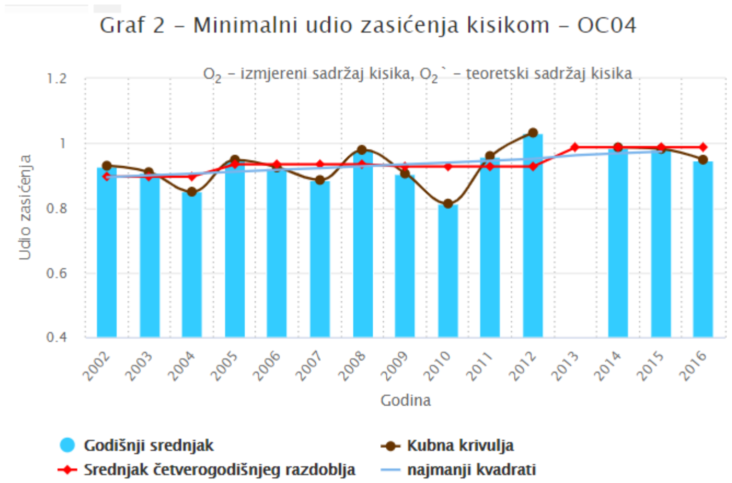


Figure 131. Minimum proportions of oxygen saturations in the bottom layer of Cetina estuary (in order of appearance: annual mean, the mean of a four-year period, cubic curve, smallest squares).

The Cetina estuary has satisfactory concentrations of oxygen in the bottom layer which values are among highest of all Adriatic stations (from 4,79 to 7,57 mg/L for concentrations of oxygen and from ρ = 0,62 to ρ = 1,00 for proportions of oxygen saturations in the period 2013. – 2016.). In the last measured period, as for most stations in the Adriatic, the situation for Cetina estuary is unchanged or only partially better. In conclusion, the condition of Cetina estuary is very good with values exceeding those of other stations monitored in Croatian part of the Adriatic. Conditions for development of organisms on the seabed are very good.

4.9.7. Monitoring of Pinna nobilis population in Natura 2000 site Cetina Estuary

Research was conducted on November 7, 2019 near the port of Omiš. A total of 57 specimens of noble pen shell (*Pinna nobilis*) were recorded in the area of 100m^2, which was characterized as area of high-density population. Among recorded specimens, 34 were living and 23 dead individuals. For each individual, the measures of the narrowest (w) and widest (W) part of the body were taken, the height of the individual from the seabed (UL) with accuracy of 1 mm, and also the orientation of the shell opening in relation to the side of the world were taken with accuracy of 10 degrees. Based on individuals that were out of sediment, the most accurate results for the total length were obtained through the formula from Garcia - March & Ferrera from 2006. The largest individual according to the formula for calculating the total length was 56.82 cm while the smallest individual was with a total length (TL) of 9.34 cm. Most individuals were recorded that were larger than 40 cm and less than 50 cm in total length, which is 53% of the individuals in the observed area. Furthermore, when we compare the representation by length classes of dead and live individuals, we get similar results, which means that mortality evenly affected the entire population regardless of size. The number of dead and the number of living comes mostly from the most represented length class of the population (individuals larger than 40 cm and less than 50 cm TL). At the end of December 2019, all recorded individuals of the pen shell (*Pinna nobilis*) died.

4.9.8. Bathing sea quality assessments

Sea quality for bathing on the sea beach is monitored by the Ministry of economy and sustainable development from 2009. It determines the limit values of microbiological indicators and other characteristics of the sea. Microbiological indicators monitored in the sea are intestinal enterococci and Escherichia coli. Other features of sea quality that are monitored are meteorological conditions, sea temperature and salinity, and visible pollution. For Cetina estuary measurements are performed at 12 stations distributed from far west to far east of the pilot site. Based on measurements sea quality is ranked from excellent, well, satisfactory to unsatisfactory. At all 12 stations prevails excellent yearly sea quality from 2009 till 2020. Only for 1 station sea quality for bathing was rated satisfactory in 2010. Two stations had good sea quality in 2011 and one in 2017. In conclusion, sea beaches of Cetina estuary have excellent sea quality for bathing.

4.9.9. Natura 2000 habitat types and species

<u>Habitat types</u>

Within the area, 3 habitat types are protected: 1130 - estuaries (677 ha), 1110 - sandbanks which are slightly covered by sea water all the time (135 ha), and 1140 - mudflats and sandflats not covered by seawater at low tide (1 ha). Actual data quality for estuaries is good, for sandbanks poor and mudflats and sandflats moderate. In terms of site assessment, estuarian habitat has good representativity, represents 2-15% of the total area covered by that natural habitat type within the national territory, however his conservation status is reduced due to its location in the heart of the town of Omiš. Sandbanks have also good representativity, represents less than 2% of the total area covered by that natural habitat type within the national territory and their conservation status is also reduced due to same reasons. In shallow areas from 20cm to 6m depth the marine Angiosperm *Cymodocea nodosa* is well spread, creating at eastern side of the river Cetina dense settlements. Another marine Angiosperm *Zostera noltei* is present only at micro location, small bay at western side of river Cetina. Mudflats and sandflats not covered by seawater at low tide have significant representativity, represents 2-15% of the total area covered by that natural habitat type within the national territory but as other habitats, their conservation status is reduced. This is due to beach replenishment activities that impact both habitat types (1140 and 1110) as well as construction of artificial rocky dikes, moorings, piers etc.

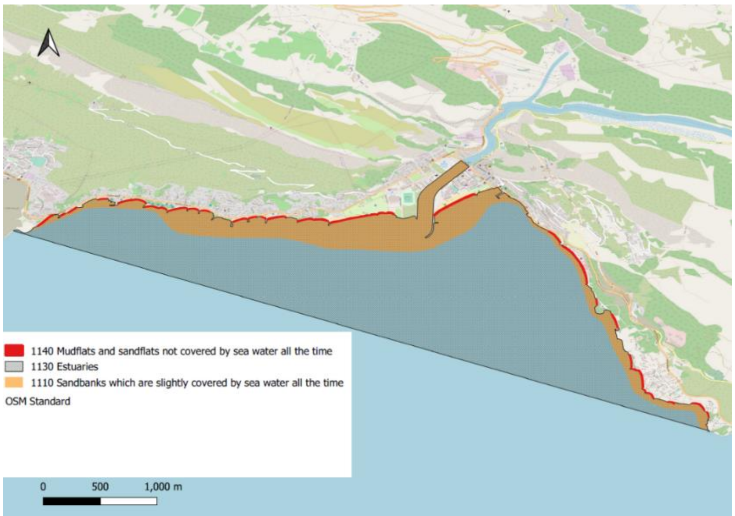


Figure 132. Habitat types zonation in Natura 2000 site Cetina estuary.

<u>Species</u>

Only one species is listed as priority for conservation, sea lamprey *Petromyzon marinus.* Sea lamprey lives in the mouths of Adriatic rivers and in the sea. Adults live in the sea for 2 to 3 years, and then they return to the rivers to spawn. The larvae live in rivers for 6 to 8 years where they feed on microorganisms and detritus. Adults are very rare in rivers and feed on blood and muscle disabled fish or fish caught in the net. They breed between March and May. Spawning in the rivers of the Croatian Adriatic is questionable, including the river Cetina, for which there is very little data.

4.9.10. Transitional water biological quality - classification of transitional waters (ecological status) WEC1e

Biological quality is, according to the Water Framework Directive (2000/60/EC), in addition to chemical and hydromorphological status, the basic criterion for assessing the ecological and overall status of transitional and coastal waters. In the case of transitional waters, biological quality (status) is determined by the status of the biological quality elements: phytoplankton, marine flowering plants, benthic invertebrates and fish (including their supporting physicochemical parameters). The minimum frequency of research of certain biological quality elements in transitional waters within the six-year cycle in the Republic of Croatia is for phytoplankton 4 times a year, for marine flowering plants and fish once in three years, for benthic invertebrates once in six years. The basic units for determining the chemical, biological, ecological and overall status are water bodies.

Transitional water biological quality for Cetina estuary has been monitored by the Institute of Oceanography and Fisheries since 2012, however the methodology is developing over years. Based on the collected data, biological quality of transitional water bodies (P1_2-CEP, P2_2-CE and P2_3-CE) is assessed, which is shown in the following table. The established condition can be VG = Very good, G = Good, M = Moderate, P = Poor, VP = Very poor, - = Not determined or NR = Not rated.

Year or period	Transitional water body	phytoplankton	marine flowering plants	benthic invertebrates	fish
2019	P1_2-CEP	VG	-	-	-
	P2_2-CE	VG	-	-	-
	P2_3-CE	-	-	-	-
2018	P1_2-CEP	VG	-	-	VG
	P2_2-CE	VG	-	-	G
	P2_3-CE	VG	-	-	G
2017	P1_2-CEP	-	-	-	-
	P2_2-CE	-	G	-	-
	P2_3-CE	-	-	-	-
2016	P1_2-CEP	G	-	-	G
	P2_2-CE	VG	-	-	G
	P2_3-CE	VG	-	-	G
2014-2015	P1_2-CEP	G	-	-	G
	P2_2-CE	VG	G	-	G
	P2_3-CE	VG	-	G	G
2012-2013	P1_2-CEP	G	-	-	G
	P2_2-CE	VG	G	-	G
	P2_3-CE	VG	-	-	G

Table 19. Biological quality of transitional water bodies of Cetina estuary (P1_2-CEP, P2_2-CE and P2_3-CE).

Compared to the transitional waters of other rivers of the Adriatic basin, the transitional waters of the Cetina estuary are among the ones with the highest values of biological quality, which are generally very good for phytoplankton, and good for other parameters: marine flowering plants, benthic invertebrates and fish. Transitional water biological quality of Cetina estuary is good. The results also show that during years the trend remains unchanged.

4.10. P10 - Torre Cerrano Marine Protected area

4.10.1. Coastal dune and psamophile ecosystems

In Torre del Cerrano coastal dunes, several monitoring approaches for monitoring natural ecosystems have been implemented. In this example, we will mainly consider vascular plants and vegetation as bioindicators. We adopted a re-visitation procedure consisting on the periodical sampling of permanent random plots distributed across the coastal dune sea-inland gradient (study area figure red dots). Vegetation was sampled for the first time in the 2014 and was partially re-sampled periodically until today.

In this example, we focus on diversity patterns of some species guilds (e.g. focal, ruderals and aliens' species) in relation to anthropic disturbance (trampling) on coastal dune herbaceous vegetation.

4.10.2. **Plant species. Bioindicators of ecosystem functioning and health**

Among the different threats to coastal ecosystems, the spread of alien plants, trampling, and beach litter have been claimed to produce a wide range of impacts, including changes in community structure and diversity as well as alterations of ecosystem processes. Still, it is possible that biodiversity threats and anthropogenic disturbances could have specific impacts on certain native plant groups, while not on others, or could promote the growth of non-natural ones.

In this context, the EC Directive 92/43/EEC (Habitats Directive) (EEC 1992), one of the major steps towards a European strategy for nature conservation, lists a series of "diagnostic species" for the habitats of conservation interest. Diagnostic species are those taxa that play a major role in determining the structure and functioning of these ecosystems as, directly or indirectly, they control the availability of resources for other species. In these context, we consider as focal species the diagnostic native plant species mentioned in the Interpretation Manual of habitats.

Focal species, being strictly adapted to coastal environments (Santoro et al., 2012.), should be particularly sensitive to disturbance. On the other hand, other opportunist taxa as ruderals should be favored by human pressure. Furthermore, some life growths are a good proxy of ecosystem response in vegetation structure to cope with human pressure.

4.10.3. Sample collection and preparation

Each vegetation plot is georeferenced in a GIS environment. Plots are 16 m^2 wide (4m x 4m) and are carried out during the vegetative period (April – June). In this example, we describe the effects of human trampling on herbaceous dune habitats of European conservation concern. We considered two sets of vegetation plots: the first collected on well preserved herbaceous habitats, and the second occurring in trampled ones. In each plot, the complete list of vascular plants is reported along with their cover percentage, using the Braun-Blanquet scale of abundance/dominance (Braun-Blanquet 1964). Vascular plants taxonomy conforms Conti et al. (2005) for natives and Celesti-Grapow et al. (2009) for exotic species.

4.10.4. Data analysis

We compare species richness in natural and disturbed plots through rarefaction curves (Gotelli and Colwell 2001). This method, based on a data interpolation process, is widely used in ecological studies for estimating, comparing and describing species diversity. Using the sample-based method, rarefaction curves compute the cumulative number of species deriving from the sequential input of the sampled plots. The curves were computed with a 95% confidence interval that makes it possible to determine whether species richness was significantly different among the groups analyzed (Colwell et al., 2004.).

We also compute rarefaction curves considering the following plant functional groups:

a) Focal species: a pool of native and characteristic species that identify the habitats (diagnostic species), determine the structure and guarantee the functionality of the habitats. We identify focal species of each macro-habitat among those indicated by the Italian Interpretation Manual of Habitats Directive (Biondi et al., 2009.; Angelini et al., 2016.).

b) Ruderal and exotic species: ruderals are opportunist species well adapted to live in different habitats. They are usually associated with disturbance and a poor conservation state, causing changes in plant community structure (Biondi et al. 2015). Exotic (or alien) species are introduced accidentally or intentionally from another geographical area for ornamental or commercial purposes and they may become invasive: the spread of exotic species causes a change in biotic interactions and community functioning, as well as the alteration of ecosystem services (Lazzaro et al., 2020.).

4.10.5. Research results

Total richness of not disturbed (i.e. not-trampled) plots tends to be higher than in disturbed ones (trampled; Figure 133), and curves tends to be separated with not overlapping confidence intervals.

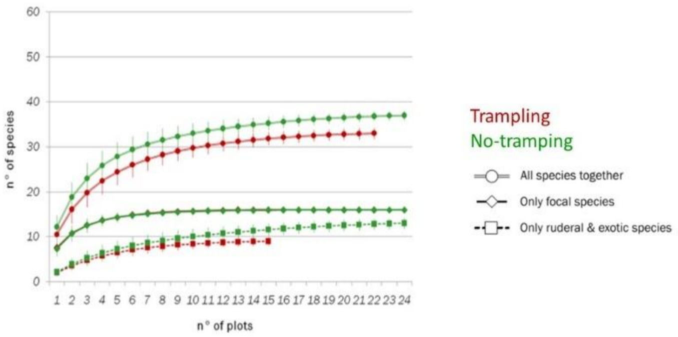


Figure 133. Example of one representative set of rarefaction curves used to compare species richness between plots with trampling pressure and without trampling considering all species (circle with double lines), focal species (diamond with bold lines) and ruderal/exotic species (square with dotted lines).

Some focal species clearly tend to occur in a higher number of well-preserved habitats, where they tend to present higher cover values. We also explored the set of species that contribute to differentiate disturbed form non-disturbed plots and we present an example in Figure 134. In this case, some focal species clearly showed a cover difference between the two groups of plots. The presented example underlined higher cover values in well preserved plots of *Lotus cytisoides, Cakile maritima and Medicago marina, Vulpia fasciculate, Silene colorata and Pistacia lentiscus*. On the other hand, we found a decrease of *Elymus farctus subsp. farctus, Ammophila arenaria subsp. australis*, *Calystegia soldanella, Rostraria litorea, Rosmarinus officinalis* and *Rhamnus alaternus*.

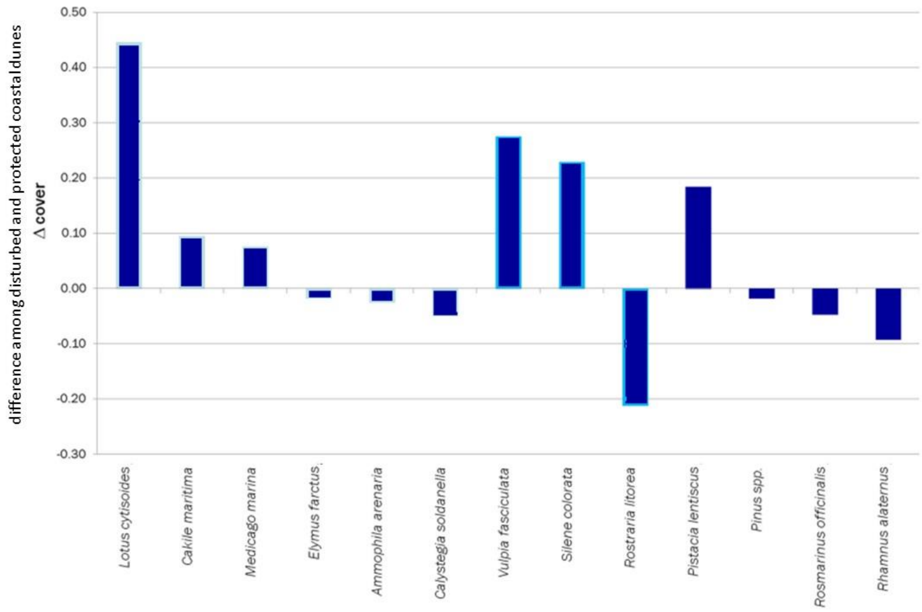


Figure 134. Differences in the cover of some species with the major cover alteration in disturbed plots. Positive values indicate a cover increase in protected (well preserved) plots.

4.11. P11 - Marche coastal area (IT)*

4.11.1. Historical and current evolution of the Marche coastline

The historical and recent evolution of both the river mouths and the shoreline along the Marche Region was investigated on the basis of both historical data and maps, taking into account effects of natural events and anthropogenic activities. The research started with searching, acquiring, georeferencing and digitizing available maps realized from 1835 up to the present, integrated by the study of the existing bibliography. The above data were used to implement a vector geodatabase containing the successive position and shape of shorelines, with special attention to the river mouths. The results obtained were correlated with the main natural events and human activities occurred in different time intervals. The results of such comparison highlighted that in the study area the progradation of river mouths continued after the end of the Little Ice Age (around the

1850). Successively, the evolution of both the river mouths and the neighboring beaches mostly depended both on the deforestation and the anthropization and agricultural development carried out in the hydrographic basins during the first decades of the last century. During the following decades, further interventions (such as construction of dams and check dams, extraction of sediments from thalwegs, modifications of stream paths, abandonment of agricultural practices, etc.), contributed to a severe reduction of the river solid load, causing a subsequent retreat of both river mouths and beaches, thus making it necessary to carry out artificial coastal defense works; the latter, anyhow, often simply displaced the problem or demonstrated a low effectiveness. (PP 13-28 “Historical and current evaluation of the coast of Marche region”-Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Federico Spagnoli 2,4, Mario Tramontana 2,3,5).

**based on the research “Coastal studies n.30/2021 – The Marche Region coastal line and the new ICZM Plan ISSN 1129-8588 which is under approval of Marche Regional Government”*

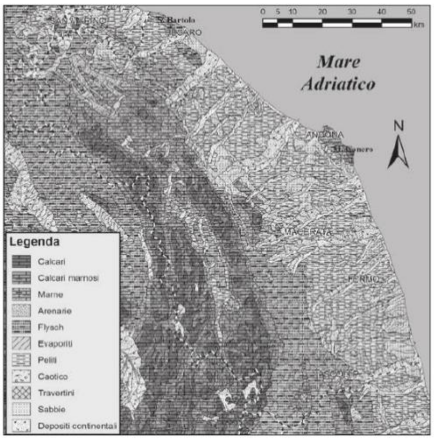


Figure 135. Orographic diagram of the region.

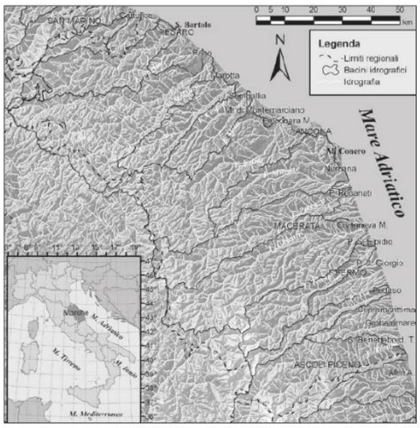


Figure 136. Lithological scheme of the region.

4.11.2. Balneability of the coastal waters of Marche

This chapter examines the quality of bathing water along Marche coast and illustrates the results of the monitoring activities carried out by ARPA Marche (ARPAM) during the bathing season (2017). In general emerges a high level of quality of the bathing areas of the Marche, with 80.08% of bathing waters classified as “excellent” and 11.16% classified as “good”. From the comparison with previous years, 14 bathing waters have improved the classification compared to the class attributed in 2016 and 12 bathing waters have worsened the classification previously attributed to it. The 14 bathing waters (of which 6 located in the province of Pesaro and Urbino, 4 in the province of Ancona, 2 in the province of Macerata and 2 in the province of Fermo) have improved their classification compared to the class assigned in 2016, four of them reached the “excellent” class. At the same time, however, 12 bathing waters (2 in the province of Ancona, 3 in the province of Macerata including an inland water in the lake of Fiastra, 3 in the province of Fermo and 2 in the province of Ascoli Piceno) worsened their previous classification, with ten waters downgraded from “excellent” to “good” and two waters downgraded from “good” to “sufficient”.

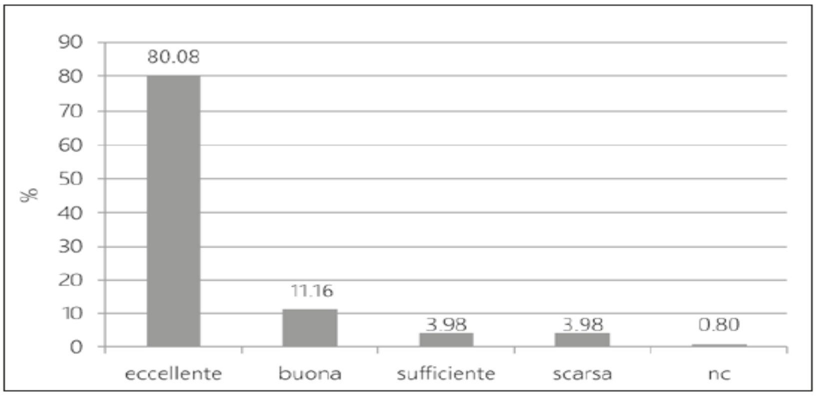


Figure 137. Quality of bathing water of the Marche coast (2017 ARPAM data).

Finally, the results of the Algale Surveillance Programme reported no particular critical issues regarding the proliferation of marine phytoplankton and the monitoring of the toxic alga Ostreopsis cf. ovata, which only in one case led to a trade union order of temporary clothing to bathing. By comparing the findings of the ARPAM 2017 results with those of the previous “GIZC 2003 Report”, the following can be summarised: all in all, there is a positive settings, which demonstrates the good quality of bathing waters along the Marche coast; as already highlighted in 2003, the Macerata province remains the most critical one, with the highest percentage of “poor” quality waters (corresponding, it should be emphasised, to the areas located near the mouths of the Musone and Potenza rivers); the analysis of the most recent trends shows situations of improved water quality, but at the same time there are some situations of deteriorated quality in some coastal areas (mostly located near ditches, drains or river mouths), which require attention.

In some areas there are situations of short-term pollution, leading to the issuing of a temporary bathing closure order. These evidence, therefore, outline a growing risk, recalling the urgent need to pay attention to the proper management of wastewater, sewerage systems and spillways, also in the light of ongoing climate change and its consequences on the flow of pathogenic microorganisms toward the coastal strip.

Based also on data from analyses of the backshore sediments, the Marche coast often shows faecal contamination not only in the water column, but also in sediments close to the coast. This phenomenon can also be observed in areas whose waters are considered as swimmable according to the analyses of Directive 2006/7/CE which regulates bathing (Vignaroli et al., 2013).

These results suggest that, although no foreseen by the current Directive, it would be useful to start thinking about an intensive, rigorous and systematic control of the microbiological status of sediments of the Marche coast, with reference also to the issue of the quality of bathing water and the safety of the various activities of human use of the coast. (PP 65-72 "Bathing of the coastal waters of the Marche - Gian Marco Luna 4, Elena Manini 4)

4.11.3. Marine-coastal biocenosis of Marche

Since no new samplings of the benthic community were carried out in order to evaluate the biocenosis present along the Marche coast, in this report the results of the analysis carried out by ARPA MARCHE in 2003 have been compared with the most recent bibliography. (PP 83-78 „Marine-coastal biocoenosis in the Marche region" - Elisa Punzo 4, Alessandra Spagnolo 4)

The main findings of the Biocenosis of Marche Region coastal area are illustrated: Sabellaria spinulosa and Pholas dactylus have been found for the first time in an area close to the artificial reefs of Gabicce Mare (Cerrano et al., 2014a). These organisms have ecological interest as they increase the heterogeneity and complexity of the habitat; the Bivalve P. dactylus is also protected by national and international legislation.

Three non-indigenous species of algae, Antithamnion hubbsii, Grateloupia turuturu and Sargassum muticum, have been found for the first time in Ancona harbour (Falace et al, 2010.). They have probably been introduced by ship hulls or ballast waters.

In the Conero area two species of Community interest were recorded: Pinna nobilis and Lithophaga lithophaga (Cerrano et al., 2014a; Punzo et al., 2017.). In addition, the presence of many colonies of Cladocora caespitosa, is reported.

Finally, the coastal area between Chienti river and Tronto river is characterized by the presence of mussels, Anemonia viridis and S. spinulosa settled on coastal defense artificial reefs.

4.11.4. Quality of sediments located at the back of breakwaters

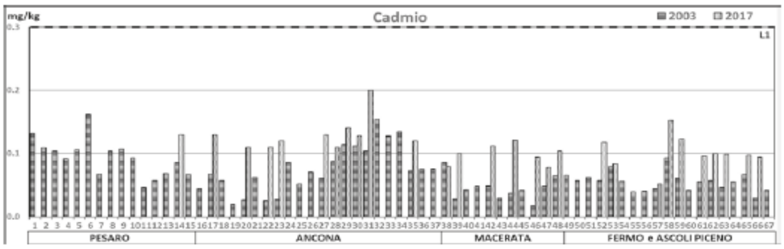
The main features determining the quality of the sediments located at the back of breakwaters along the Marche coast are illustrated. The above considerations derive from the results of analyses carried out by the ARPA Marche on samples taken by the Service for the Territorial Protection, Management and Planning of the Marche Region in 2003 and 2016-17. (PP 31-64 „Quality of sediments located at the back of breakwaters" - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Gian Marco Luna 4,5, Elena Manini 4,5, Emanuela Frapiccini 4,5, Federico Spagnoli 1,2,4,5, Mario

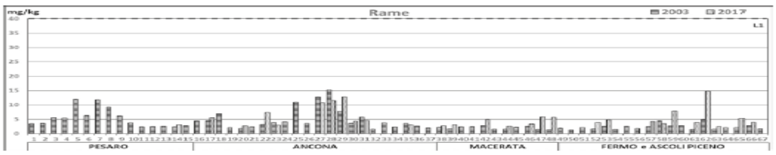
Tramontana 3,6, Gianni Scalella 7, Stefano Parlani 7, Mauro Sinigaglia 7, Giordano Forchielli 7, Fabrizio Mazzoli 7, Diego Magnoni 7, Carmine Bellino 7, Daniele Pernini 7)

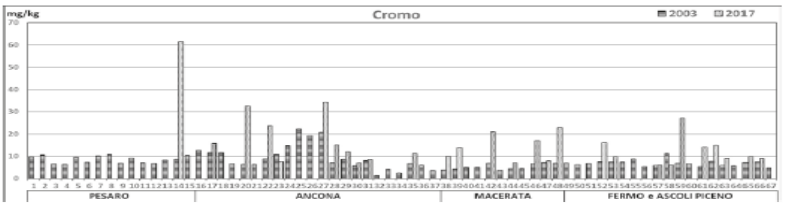
Particularly, the following features are synthetically analysed and discussed:

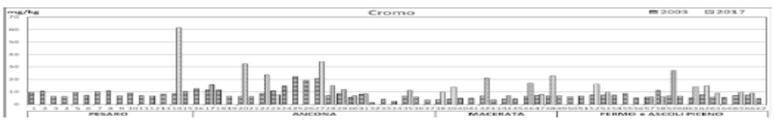
- Texture,
- Concentration of metals;
- Biological toxicity;
- Presence of organic pollutants;
- Microbiological pollution;
- Presence of organic matter.

The statistical validity of the results is strongly affected by the reduced number of analyses carried out in 2017 with respect to the previous ones. Further problems derive, besides the excessively long timespan intercurred between the two sampling campaigns, from the lack of homogeneity of the sapling periods (therefore potentially resulting in samples representing different conditions of meteo-marine features and littoral dynamics) and from the uncertainty on a precise coincidence of the sampling sites.









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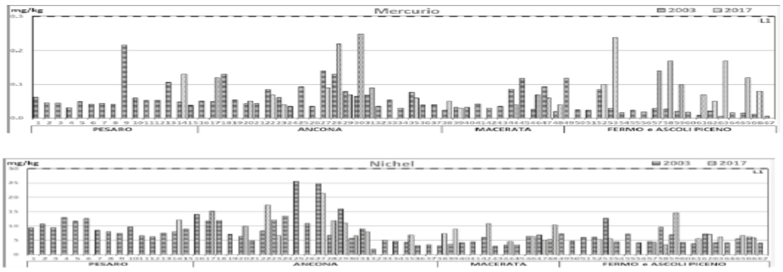

Figure 138. Contents of Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni)in the samples taken in 2003 and in 2016-17. The dotted lines represent the limit of the D.M. 173/16.

4.11.5. Protection and enhancement of the residual coastal dunes in the Marche Region

In the dynamics of natural evolution of the presence and development of fine sediment accumulations making up the coastal dunes is physiological, behind the emerging beach. The coastal dunes represent a very important element for the protection of the areas behind them, also constituting a fundamental factor in mitigating the risk associated with the rising of the saline wedge in the freshwater aquifers near the coasts.

The coastal dunes basically originate from the accumulation of clastic material (fine sands), mostly coming from the emerged beach, carried by the wind and deposited in correspondence with natural (vegetation, boulders, debris stranded by wave motion, etc.) or artificial (fences, walls, embankments etc.) mechanical obstacles, which is finally fixed by the vegetation. The vegetation is very differentiated according to its position - even at very short distances within the same deposit - and it is an important indicator of conservation status and stability of dunes themselves.

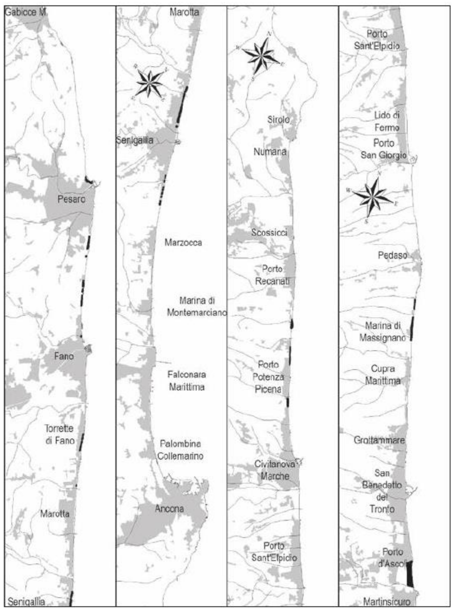

Figure 139. Distribution of residual dunes along the Marche coast.

The results of a critical analysis carried out on the main morphometric, sedimentological and morpho-evolutionary features of both the relict coastal dunes and the areas poorly modified by human intervention located along the Marche littoral belt. The above areas, surveyed by technicians of the Marche Regional Government to make an inventory, were integrated, where possible, highlighting further areas potentially evolving into coastal dunes, based on the knowledge of the Universities and CNR working group.

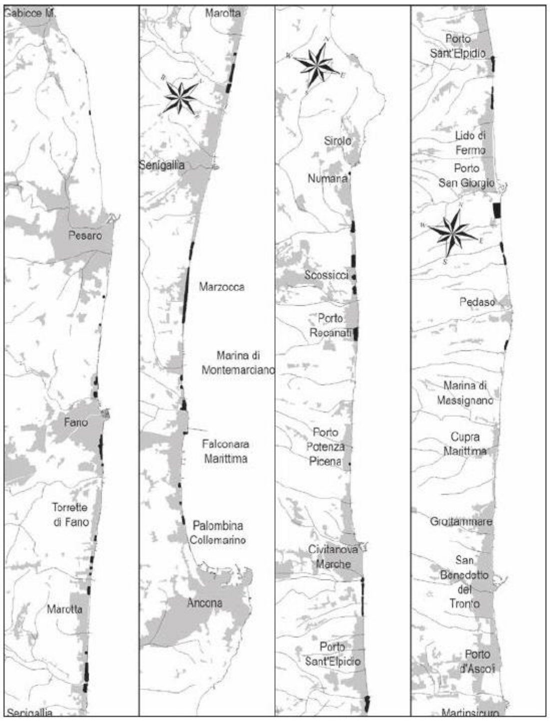

Figure 140. Distribution of the less populated areas along the Marche coast.

For each of the above areas, the main botanical-vegetational, florist and ecological aspects are briefly illustrated too. (Protection and enhancement of the residual coastal dunes in the Marche Region - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Simona Casavecchia 4, Roberta Gasparri 4, Simone Pesaresi 4, Federico Spagnoli 2,5, Gianni Scalella 7, Mario Tramontana 3,8, Silvia Zitti 4, Stefano Parlani 7, Mauro Sinigaglia 7, Giordano Forchielli 7, Fabrizio Mazzoli 7, Diego Magnoni 7, Carmine Bellino 7, Daniele Pernini 7)

Then, final considerations are reported on the state of conservation and the spatial distribution of coastal dunes in the Region, as well as of the vegetational associations and Habitats characterizing them.

Chapter 5 Conclusion

Given the complex context of the P1 pilot area, its pressures and the aim of this project to identify and improve new tools for monitoring, protecting and managing the coastal and lagoon marine ecosystem. As the activities are still ongoing, it is planned to collect further environmental information that can contribute to the understanding of the functioning of the ecosystem, also taking into account climate change and socio-economic impacts.

In addition, all the environments considered as part of the P2 pilot area, are regularly monitored by the Emilia-Romagna Region, together with other basins that are also included in the region's transitional water system. This report mainly takes into account the results of the recent studies carried out in WP3 of the project CASCADE by different partners. The parameters measured and the timing of annual sampling are not the same as those used in routine monitoring. Therefore, the combined information and the ongoing investigations, including the experimental set-up, will provide data to find new tools for monitoring, protection and management of the coastal and lagoon marine ecosystem.

All measured parameters of the processed hazardous substances were below the maximum allowable values set in the WFD, European Commission, 2006. CommissionRegulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, EFSA J 2006; L 364:5-24 and other guidelines.

Considering the complex context of the P3 pilot area, its pressures and the purpose of this project, the main objective of this study is to identify and improve new tools for monitoring, protection and management of the coastal marine ecosystem. As the activities are still in full swing, it is planned to collect further environmental information that can contribute to the understanding of the functioning of the ecosystem, also taking into account climate change and socio-economic impacts.

As regards the chemical status, the review of the available assessments shows the importance of increasing the sampling points at the outlet of the Canale Reale and the Salina Punta della Contessa, as several priority substances have exceeded the thresholds: The possibility of establishing 5 new sampling points along two transects will make it possible to establish a chemical trend for heavy metals and chlorinated pesticides in the water column and sediments.

The situation is different on the coast of the municipality of Melendugno, where a gas pipeline was recently constructed by Trans Adriatic Pipeline AG. Therefore, as part of the P3 pilot project and with the consent of the Municipality of Melendugno, a transect was established parallel to the coast and north of the pipeline to assess the chemical status of the water column and sediments. ARPA monitoring will be conducted in the transitional waters of Cesine and Alimini Grande, while coastal waters will be monitored at a point in between. The same priority substances will be monitored, i.e., heavy metals and chlorinated pesticides.

In addition, an accurate characterization of microplastics in all water column and sediment samples will be conducted to address the lack of information on this topic.

Indeed, monitoring under the Marine Strategy provides information on microplastics up to 300 µm in size at only one point along the coast between Torre Guaceto and Melendugno, namely Brindisi Capo Bianco, located next to the port of Brindisi.

A more efficient monitoring system is important to learn more about the presence and impact of microplastics in the P3 pilot area. Therefore, samples collected for chemical analysis will be analyzed for microplastics down to the low micron range. The determination of microplastics in the same samples used for chemical analysis will also make it possible to demonstrate any correlation between microplastic pollution and the chemical status of water bodies.

In terms of concentrations of measured parameters, the pollution of the P4 - Neretva River is similar to other European rivers with good ecological status. The data from the present report are useful for water management authorities and can contribute to sustainable use, management and protection of water resources of the Neretva River.

The example presented in chapter 4.6.1. Coastal dune and halopsamophilous vegetation in P7 show how the composition and structure of vegetation differs significantly between the natural Mediterranean dune macchia and the forest formations invaded by *A. saligna*. According to our results, the invasion of species seems to favor the occurrence of ruderals and other non-native taxa. We observed that invasion of Cisto-Lavanduletalia maquis (EU 2260) on the Adriatic coast promotes flora ruderalization, a decline in focal species and a drastic change in vegetation structure. In contrast, Juniperus macrocarpa scrub (EU habitat 2250*) seems to be more resistant to *A. saligna* invasion.

The community structure (e.g., frequency of growth forms) of Mediterranean coastal scrub is greatly altered by A. saligna invasion, with a drastic decrease in the shrub layer and the addition of a new, taller layer. The invasion and dominance of the alien tree species reduced the presence of native species and led to a simplification of the vegetation structure. The change in vegetation structure and architecture directly affects ecosystem functioning (e.g., water and nutrient uptake, pollination, seed dispersal, abundance of specialized small mammals, predator density). Our results confirm the negative impact of A. saligna on natural ecosystems, promoting severe structural and functional changes in Mediterranean scrub.

Long-term observations on the studied coasts provide an optimal platform for bio-monitoring the ecological impact of A. saligna on native vegetation and provide a solid basis for identifying preventive measures and effective strategies for its control and eradication (as required by EU Regulation No. 1143/2014 of the European Parliament and of the Council). The chosen sampling and monitoring method, as well as the use of ecological guilds and life forms as surrogate variables to investigate possible changes in vegetation structure, could be used as a rapid and standardized assessment survey to monitor the impact of the spread of alien species on the conservation status of Mediterranean coastal dune habitats of concern to the European Union.

Also, the results presented in chapter 4.9.1 Coastal dunes and psamophilous ecosystems underline how disturbances (e.g. trampling by bathing tourism) alter psamophilous vegetation, having different impacts on different species groups (e.g. diagnostic, ruderal, invasive).

In recent years, management plans for Natura 2000 sites have regularly included soft management infrastructure such as boardwalks and fenced areas to reduce the impact of trampling and pressure on coastal dunes (Prisco et al. 2021). Unfortunately, knowledge of the benefits of preserving natural vegetation that can stabilize dunes is generally lacking among residents and policy makers. Nevertheless, new efforts must be made to reduce trampling through integrated management initiatives such as walkways, dune fencing, and planting campaigns.

Environmental researchers have a great responsibility to publicize the importance of conservation through targeted dissemination in line with socio-economic needs. Only through dialog and knowledge sharing can we strive for integrated environmental management that empowers citizens to shape their own future.

All parameters measured and monitored in the seawater, sediments and marine organisms of the P9 - Cetina estuary show values that prove that the state of eutrophication of the Cetina estuary is very good, that the biological quality of transitional waters is good, that the seabed is enriched with oxygen, which provides very good conditions for the development of organisms on the seabed. The ecological status of the surface waters of the Cetina estuary is also good. The sea beaches of the Cetina estuary also have excellent water quality for bathing. All hazardous substances, with the exception of TBT, are also below the levels required by law. Despite the influence of the Cetina River, the Cetina estuary belongs to the oligotrophic areas.

Like other marine ecosystems, the Cetina estuary is under pressure from climate change, which brings changes in the food chain, the appearance of invasive species and the occurrence of mass mortalities. There are probably changes in the usual patterns of salinity and temperature of the water column, but there is no systematic monitoring. There is also a need to introduce additional monitoring of the state of the ecosystem, particularly in relation to the EU Biodiversity Directives. In conclusion, any human intervention, whether physical, chemical or biological, should consider the resilience of the Cetina estuary to climate change.

Moreover, Regione Marche has compared the results of the analyzes carried out by ARPA Marche in 2003 and 2017 on sediment samples taken from the backside of the breakwaters, making some interesting observations. However, they are not statistically meaningful due to several factors. These include the large time gap between the two samplings, the availability of only two sets of samples, the reduction in the number of samples collected during the second campaign (a particularly limiting factor, especially for the province of Pesaro-Urbino), the variations in the classification criteria and indicators analyzed, the uncertainty about the coincidence of the seasonal dates for the two samplings and the lack of knowledge about the exact location of the sites. Similar limitations affected most of the other study strands.

The survey of habitats along the underwater beach identified notable protected species; exotic species were also found in the port of Ancona.

The bathing quality of the marine waters is more than good on average for all the sites surveyed, with a tendency towards improvement, although with local cases of a reverse trend. (PP 143 - 148 "Final reflection" Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Federico Spagnoli 1,2,4,5, Mario Tramontana 3,5).

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198. PP 65-72 “Bathing of the coastal waters of the Marche - Gian Marco Luna 4, Elena Manini 4

199. PP 83-78 „Marine-coastal biocoenosis in the Marche region“ - Elisa Punzo 4, Alessandra Spagnolo 4

200. PP 31-64 „Quality of sediments located at the back of breakwaters“ - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Gian Marco Luna 4,5, Elena Manini 4,5, Emanuela Frapiccini 4,5, Federico Spagnoli 1,2,4,5, Mario Tramontana 3,6, Gianni Scalella 7, Stefano Parlani 7, Mauro Sinigaglia

7, Giordano Forchielli 7, Fabrizio Mazzoli 7, Diego Magnoni 7, Carmine Bellino 7, Daniele Pernini 7

201. Protection and enhancement of the residual coastal dunes in the Marche Region - Carlo Bisci 1,2,3, Gino Cantalamessa 1,2,3, Simona Casavecchia 4, Roberta Gasparri 4, Simone Pesaresi 4, Federico Spagnoli 2,5, Gianni Scalella 7, Mario Tramontana 3,8, Silvia Zitti 4, Stefano Parlani 7, Mauro Sinigaglia 7, Giordano Forchielli 7, Fabrizio Mazzoli 7, Diego Magnoni 7, Carmine Bellino 7, Daniele Pernini 7

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