Interreg
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ALIENA

INTERREG ITALY-CROATIA PROGRAMME 2021 – 2027

Deliverable 1.1.1 KoM implemented and initial Pilot areas description

Part 2 – Initial pilot areas description (Version 1.0 - August 2024)

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ALIgning Efforts to control Non-indigenous species in the Adriatic sea

WP 1	Review of non-indigenous species and causes determining their invasiveness in Adriatic Sea
Activity 1.1	Identification of non-indigenous species which can pose a threat to ecosystems and human activities in the Adriatic sea
Deliverable 1.1.1	KoM implemented and initial Pilot areas description
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Summary

1. The rocky coast areas of Apulia region

1.1. General geographical context

The general objective of the ALIENA project is to deepen knowledge on invasive alien species (IAS) in the Adriatic Sea, the spread of which can represent a threat to local marine ecosystems, to public health and also affect socioeconomic activities. For this purpose, some IAS species will be the subject of specific monitoring activities to be carried out in 4 pilot areas (2 in Croatia and 2 in Italy); the species will be identified as targets at a local level, chosen on the basis of the knowledge and experience of the respective Project Partners.

This first part of the document describes the pilot area chosen for the Puglia Region, which was identified in the marine-coastal area of the southern Adriatic, on the Italian side between the territory of Molfetta (Bari) and Torchiarolo (Brindisi). The choice of this area was made according to the widespread presence of rocky seabed, with the associated habitats and benthic communities of hard and/or bioconcrete bottoms, which extend from the mid-littoral to the infralittoral zone, as well as the strong anthropization rate due to the presence along the coast of some of the most important cities in Puglia as well as several ports and coastal defense works (hard structures); in Figure 1 the location of the pilot area.

Figure 1: geographical context of the pilot area (indicated by the thick red line).

1.2. Brief description of the main environmental characteristics

1.2.1. Geomorphology

In general terms, the average slope of the Apulian Adriatic continental shelf falls within the range of 1÷2%: the values are on average lower in the north-western part and increase towards the south-east, where the percentages reach values of 4÷5%. As regards the geomorphological aspects that most concern the pilot area located in the middle of the Apulian Adriatic side, this is made up of 73% of a rocky coast, 16% by sandy, pebbly and rias beaches and the remaining 11% of a coast modified by anthropic works and infrastructures (Figure 2).

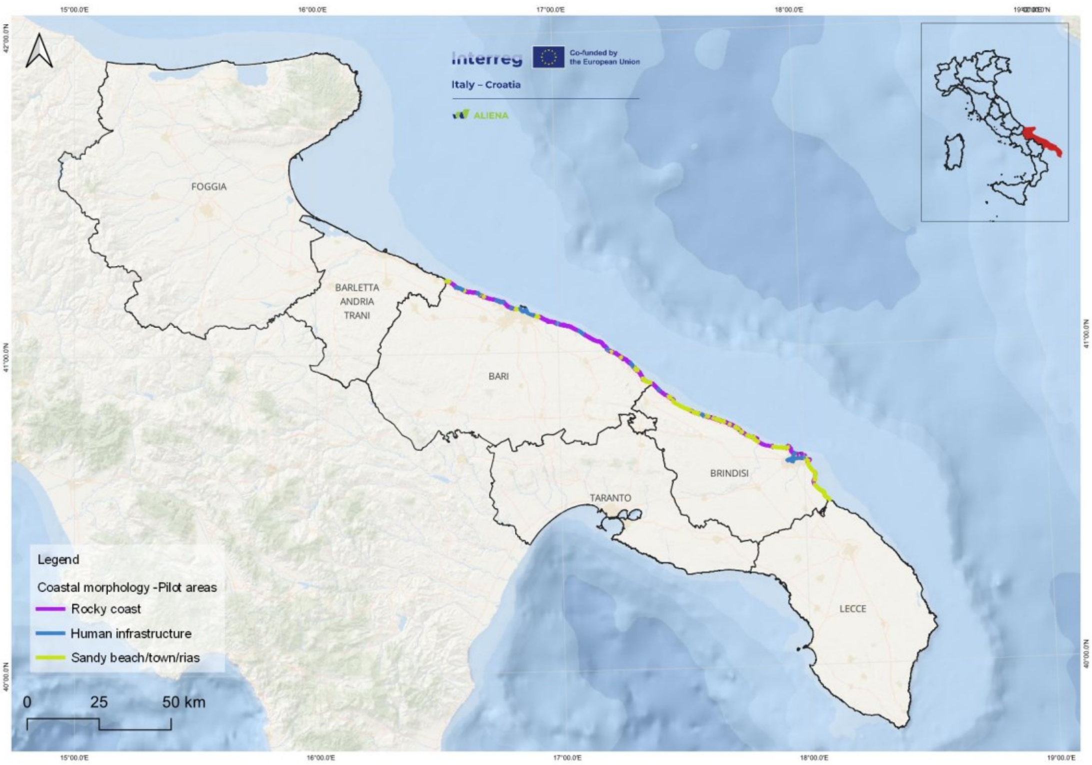

Figure 2: main geomorphological features of the pilot area coastline.

Going into the detail of the coastal morphology, the northernmost trait between Molfetta and Bari takes on the typical characteristics of the low rocky coast, with alternating small headlands and pebbly pocket beaches. Going south, the trait between Bari and Polignano is characterized by a low rocky coast interrupted locally by pocket beaches located in a protected position within coves and natural recesses (rias). The trait between Polignano and Monopoli is characterized by a high rocky coast with alternating vertical walls (cliffs) and more sloping profiles, furthermore there are several submerged and semi-submerged caves of karst origin; moreover, the presence of fresh water inputs coming from the outcropping aquifer is significant. Most of the coast between Monopoli and Brindisi-Punta Penne is low, with a sloping profile and generally ends with small cliffs up to Torre Canne; the coastline has a rather complex layout, and there is an alternation of coves with pocket beaches and not very accentuated promontories. Beyond Torre Canne, still towards the south, the coast is linear, regular, low and sandy, sometimes with marshy areas behind the dunes; worth noting in the area is the presence of the Marine Protected Area and State Nature Reserve of Torre Guaceto, which protects habitats of community importance. Finally, the coastline between Brindisi-Punta Penne and Brindisi-Punta Riso is characterized by a low coast, mainly rocky, and then takes on a rather linear trend up to Torchiarolo, a location at the extreme south of the entire coastline identified as the pilot area; in last trait the coast is generally low, with alternating rock and sand, as well as limited portions of not excessively high cliffs, sometimes even reconstituted by the man to make them safe.

1.2.2. Idrology

The marine waters general circulation affecting the pilot area is conditioned by the specific position and conformation of the entire Apulian regional territory, located at the confluence of the Adriatic and Ionian seas. In particular, as regards the western side of the southern Adriatic, the dominant surface current is the descending one, which runs parallel to the coastline towards the south-east, especially with winds from the 1st to the 4th quadrant; within 40 miles from the coast, this current flow can reach speeds of approximately 1 to 3 knots (between 0.4 and 1.5 m/s).
Within the ALIENA project, for the Southern Adriatic Sea CMCC provided the processing and mapping of the average 2020-2023 surface current velocity field, as it is shown in the Figure 3.

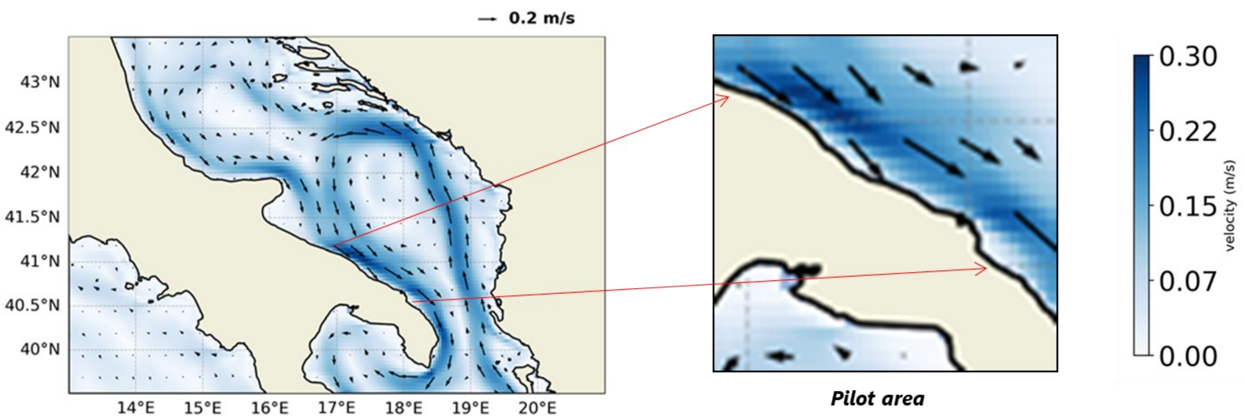

Figure 3: 2020-2023 surface current velocity field in the Southern Adriatic Sea (CMCC data processing and mapping).

1.2.3. Main chemical-physical characteristics of the marine coastal waters

As regards the main thalassographic characteristics, it is worth highlighting that the identified pilot area includes some marine-coastal water bodies (n. 9 CWB) monitored by ARPA Puglia in compliance with the Water Framework Directive (2000/60/EC), as represented in Figure 4.

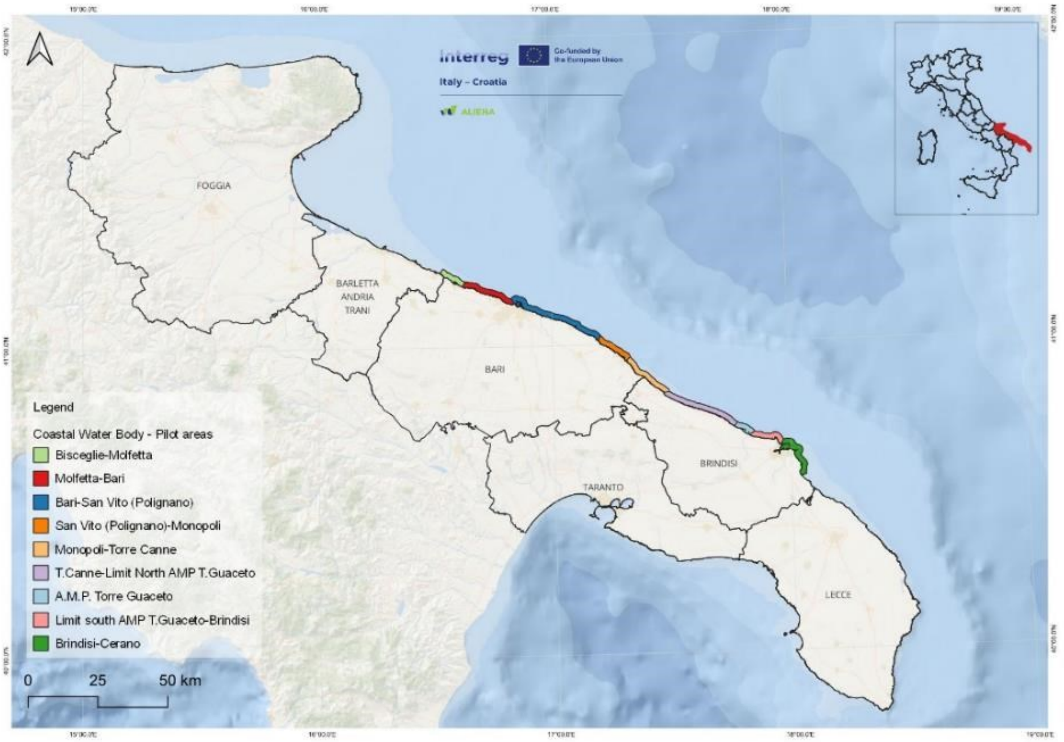

Figure 4: geographical location of the nine marine-coastal surface water bodies falling within the pilot area.

In the nine coastal water bodies, the aforementioned monitoring pursuant to Directive 2000/60/EC acquire data on the main chemical-physical parameters characterizing marine waters, including surface temperature, salinity, dissolved oxygen, concentration of macronutrients (nitrogen and phosphorus compounds) and chlorophyll "a". Thus, given the data availability, in Table 1 the average values referring to the year 2022 are reported for the mentioned parameters, for each of the nine CWB considered.

Marine-coastal water bodies	Mean surface temperature	Mean salinity (PSU)	Mean O_2 concentration (mg/l)	Mean inorganic Nitrogen - DIN concentration (µg/l)	Mean P-PO_4 concentration (µg/l)	Mean Chlorophyll "a" concentration (µg/l)
Bisceglie Molfetta	18,9	38,4	7,4	40,1	3,7	0,6
Molfetta Bari	18,6	38,5	7,3	57,0	4,8	0,3
Bari S. Vito (Polignano)	18,4	38,6	7,2	40,5	3,8	0,6
S. Vito (Polignano) Monopoli	17,8	38,6	7,2	62,1	2,6	0,5
Monopoli Torre Canne	21,3	38,8	6,8	27,7	3,1	0,2
Torre Canne Limite nord AMP Torre Guaceto	20,8	38,8	6,9	29,6	4,6	0,2
Area Marina Protetta Torre Guaceto	19,9	38,8	6,7	30,1	3,7	0,2
Limite sud AMP Torre Guaceto Brindisi	19,4	38,9	6,8	28,0	3,1	0,3
Brindisi Cerano	18,8	38,9	6,9	25,9	4,6	0,2

Table 1: chemical-physical parameters average values in the marine-coastal water bodies falling within the pilot area

Within the ALIENA project, for the Southern Adriatic Sea the CMCC also provided the processing and mapping of the average 2020-2023 surface temperature and surface salinity fields, as shown in Figure 5 and 6 respectively. To analyse the annual mean of surface temperature and salinity in the Southern Adriatic Sea CMCC used the Mediterranean Forecasting System (MedFS), that was run for the period 2020-2023 in the framework of the Copernicus Marine Service. MedFS is composed of a 2-way coupled ocean-wave modeling system based on NEMO and WaveWatch3 at a resolution of around 4 km and includes. Ocean measurements from satellite altimeters (Sea Level Anomaly) and in situ temperature and salinity vertical profiles are assimilated though the OceanVar (3DVAR) assimilation model. The model is forced with high resolution ECMWF (European Centre for Medium-Range Weather Forecasts) atmospheric fields and is nested in the Atlantic Ocean through the Copernicus global ocean analyses and forecasting system.

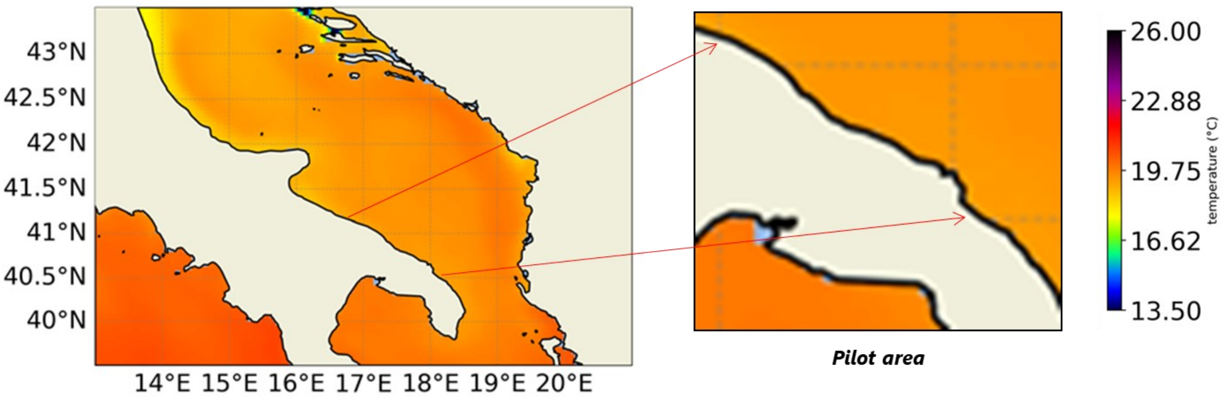

Figure 5: 2020-2023 surface temperature field in the Southern Adriatic Sea (CMCC data processing and mapping).

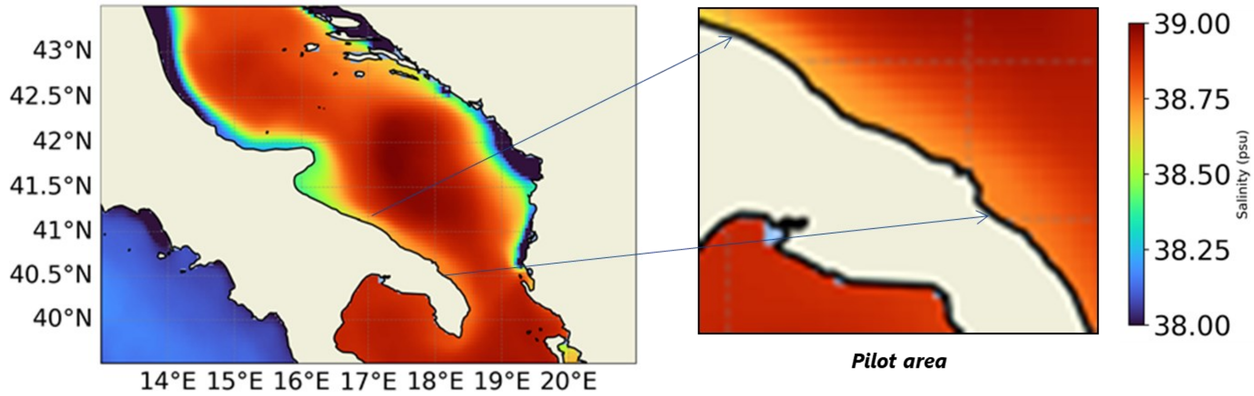

Figure 6: 2020-2023 surface salinity field in the Southern Adriatic Sea (CMCC data processing and mapping).

Looking at Figure 5, in the period 2020-2023 the mean values of sea surface temperature are between 19 and 20°C in the Southern Adriatic. However, in the same period the surface temperature was clearly influenced by seasonality, ranging from a winter minimum of 13 °C to a summer maximum of over 26 °C. As regards sea surface salinity, the 2020-2023 mean values reported in Figure 6 are between 38 and 39 PSU.

1.3. Brief description on the main characteristics of biological communities

1.3.1. Main features

The identified pilot area is characterized by a high variety of hard bottom habitats and benthic communities. These are among the most productive and diverse marine environments, contributing largely to the functioning of marine ecosystems (Boero et al., 2019), particularly coastal ones, proving crucial for the provision of ecosystem goods and services. In these environments there are generally several marine species, most of them sessile ones both erect and encrusting, sciaphilous or photophilous (depending on exposure to light) and more or less resistant to hydrodynamism. In the marine coastal strip identified as a pilot area, associations of photophilous algae are found on the rocky seabed subject to a high hydrodynamic regime, including calcified red algae of the *Corallina* and *Lithophyllum* genera, as well as brown algae; for the latter, in some zones the presence of species of the *Cystoseira* genus is reported, all forming habitats (often in association with red algae of the *Laurencia* genus). Where hydrodynamism is less accentuated, on deeper but well-lit rocks, the benthic communities are characterized by associations of photophilous algae dominated by green algae belonging to the genera *Acetabularia* and *Dasycladus*, by brown algae of the genera *Padina*, *Dictyota*, *Dictyopteris* and by other red algae of different genera. In some zones there are also lenses of soft bottoms which may host seagrasses, including *Cymodocea nodosa* (closest the coastline) and *Posidonia oceanica* (further offshore).

1.3.2. Critical/vulnerable habitats

The entire marine-coastal area identified as the pilot area for Puglia is characterised by the presence of different habitats of environmental-naturalistic high value, many of which are indicated by the Habitats Directive (92/43/EEC), which establishes the Natura 2000 Network, or by other international conventions such as those of Bern and Barcelona, and therefore subject to protection regimes (Figure 7).

Also due to its significant extension, the Special Conservation Area (SAC) named "Posidonieto San Vito – Barletta" (code IT9120009, between Polignano a Mare and Barletta) is particularly important, together with the aforementioned Marine Protected Area (AMP) of Torre Guaceto (BR).

In addition to these, in the pilot area there are other sites subject to protection regimes, as well as it is absolutely not negligible the presence of the coralligenous biocoenosis, more or less developed on the infralittoral bottoms of the entire marine coastal zone from Molfetta to Torchiarolo.

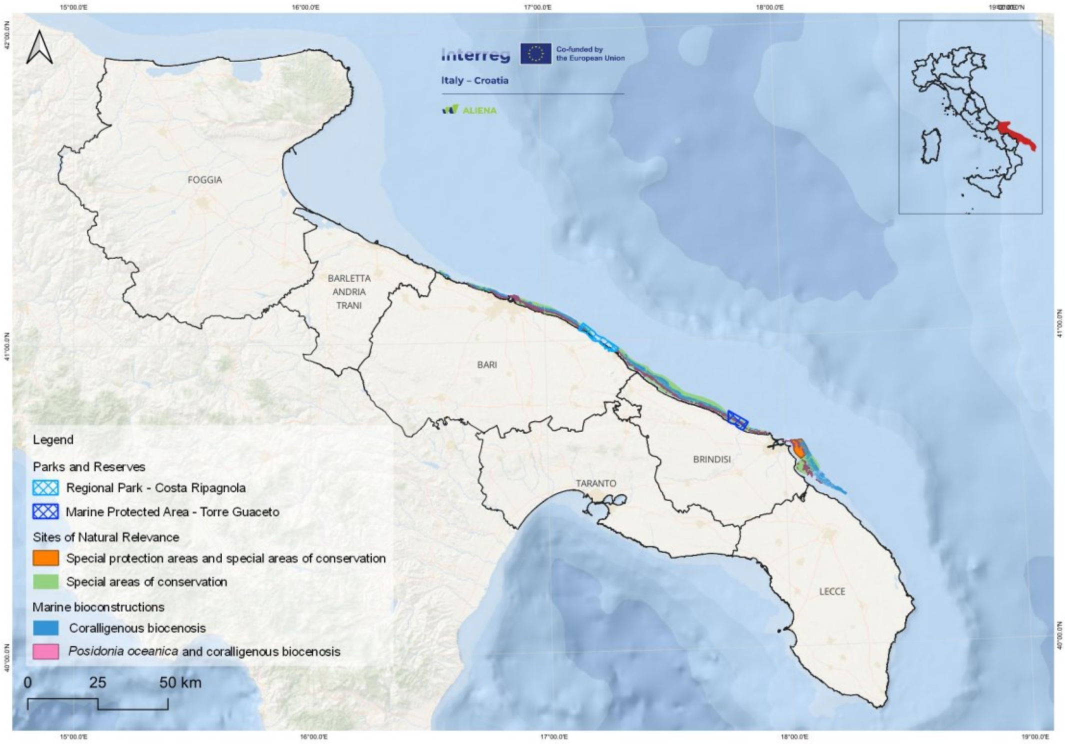

Figure 7: localization of zones of particular naturalistic value and subject to protection in the pilot area.

1.4. Main anthropic pressures

As already mentioned, the choice of the pilot area was also based on the analysis of anthropic pressures; considering the entire Apulian context, the marine coastal area identified can be considered among those most subject to anthropization.
In fact, along the coast there are two of the most important cities of Puglia, Bari (the regional capital city) and Brindisi, as well as other important towns in terms of number of inhabitants (e.g. Molfetta, Mola di Bari, Polignano a Mare, Monopoli), all potentially impacting the marine coastal environment.
Making particular reference to the presence of ports (which can be considered as potential entrance doors for any alien species), going from north to south of the pilot area are located the main ports of: Molfetta (mainly fishing boats, with a part dedicated to pleasure boating), Bari (mainly industrial and commercial/cruise traffic, with smaller spaces dedicated to fishery and pleasure boating), Mola di Bari (mainly fishing, with a part dedicated to pleasure boating), "Cala Ponte" near Polignano a Mare (exclusively dedicated to pleasure boating), Monopoli (dedicated to both commercial and fishing activities as well as pleasure boating), Brindisi (one of the most important in the entire Puglia region, mainly dedicated to commercial and industrial activities). Other small cabotage ports are: Giovinazzo, Santo Spirito, Palese, Torre a Mare, Savelletri, Torre Canne and Villanova.
Along with those mentioned, there are other pressures both point and diffuse which can affect the pilot area; in Figure 8 the mapping of the most important.

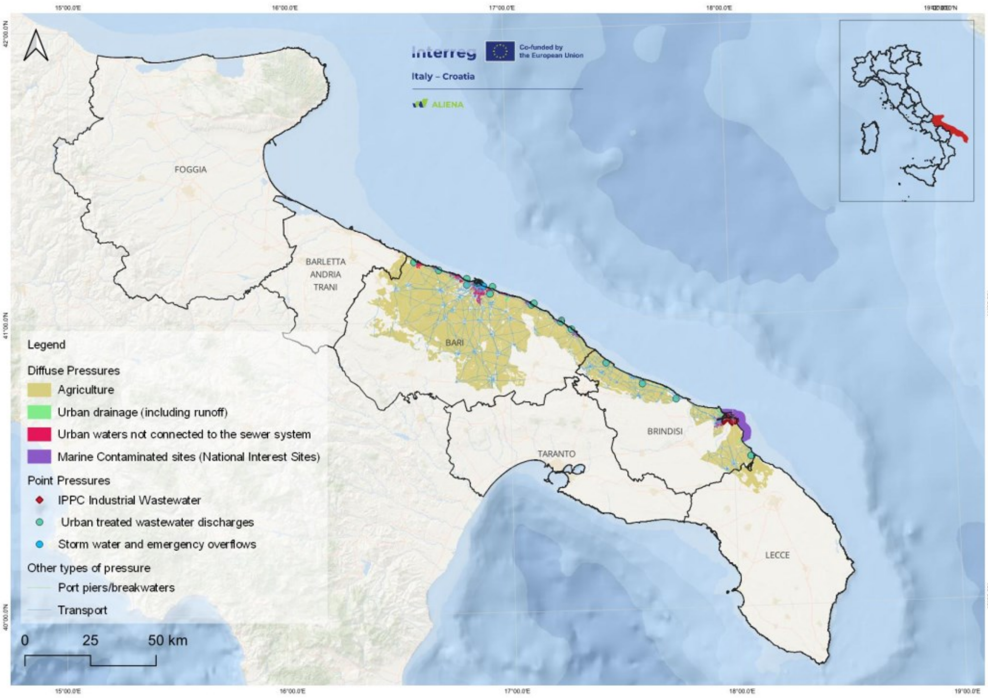

Figure 8: geographical location of the main anthropic pressures falling in the pilot area.

1.5. Possible vulnerability of the area to invasive species

The pilot area was chosen as it was potentially vulnerable to the invasion of alien marine species that can impact on biodiversity, public health, economy and ecosystem services in general.

Among the causes of potential vulnerability, the presence of several ports, including the medium-large ones of Bari and Brindisi, characterized by intense cross-border maritime traffic (which could favor the introduction of alien species also through ballast water), in addition to the general high rate of anthropization and coastal urbanization (with the presence of urban treated wastewater discharges, etc.), which could facilitate the establishment of invasive species in some circumstances.

With regard to the effects of the presence of alien species already recorded in the reference macro-area, it can be cited as an example the massive blooms of the potentially toxic dinoflagellate *Ostreopsis* cf. *ovata* (Ungaro et al., 2010), with inconveniences also for public health (Gallitelli et al, 2005), the massive seasonal presence of the ctenophore *Mnemiopsis leidyi* (sea nut) which caused damage to local fishing (in addition the clogging of the fishing nets when it is very abundant, the sea nut is a voracious predator of plankton and larvae of fish species commercially exploited), or the case of the green alga *Caulerpa racemosa* var. *cylindracea*, which in the pilot area considered, in particular in the coastal area of Brindisi and Torre Guaceto, is massively present (Felline et al., 2014) and contributed to significant alterations of the benthic communities in recent years.

1.6. References

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2. The rocky shore in the Gulf of Trieste and 1 or 2 marine sites

2.1. General geographical context

The “rocky shore in the Gulf of Trieste and 1 or 2 marine sites” will represent the Pilot area 2 of the Project ALIENA. This area is part of the Gulf of Trieste (hereafter Gulf), an epicontinental semi-enclosed shelf basin situated in the northern area of the Adriatic Sea and extended from the Tagliamento river mouth (Italy) to Cape Savudrija (Croatia). Most of the coastline is located in the Italian Region Friuli Venezia Giulia, between the municipalities of Lignano Sabbiadoro (UD) and Muggia (TS) (Figure 1). The eastern side of the Gulf is bordered by rocky coasts and dominated by plunging cliffs and shore platforms (Biolchi et al. 2016 and references therein), whereas the western one is an alluvial plan characterized by low and sandy coasts.

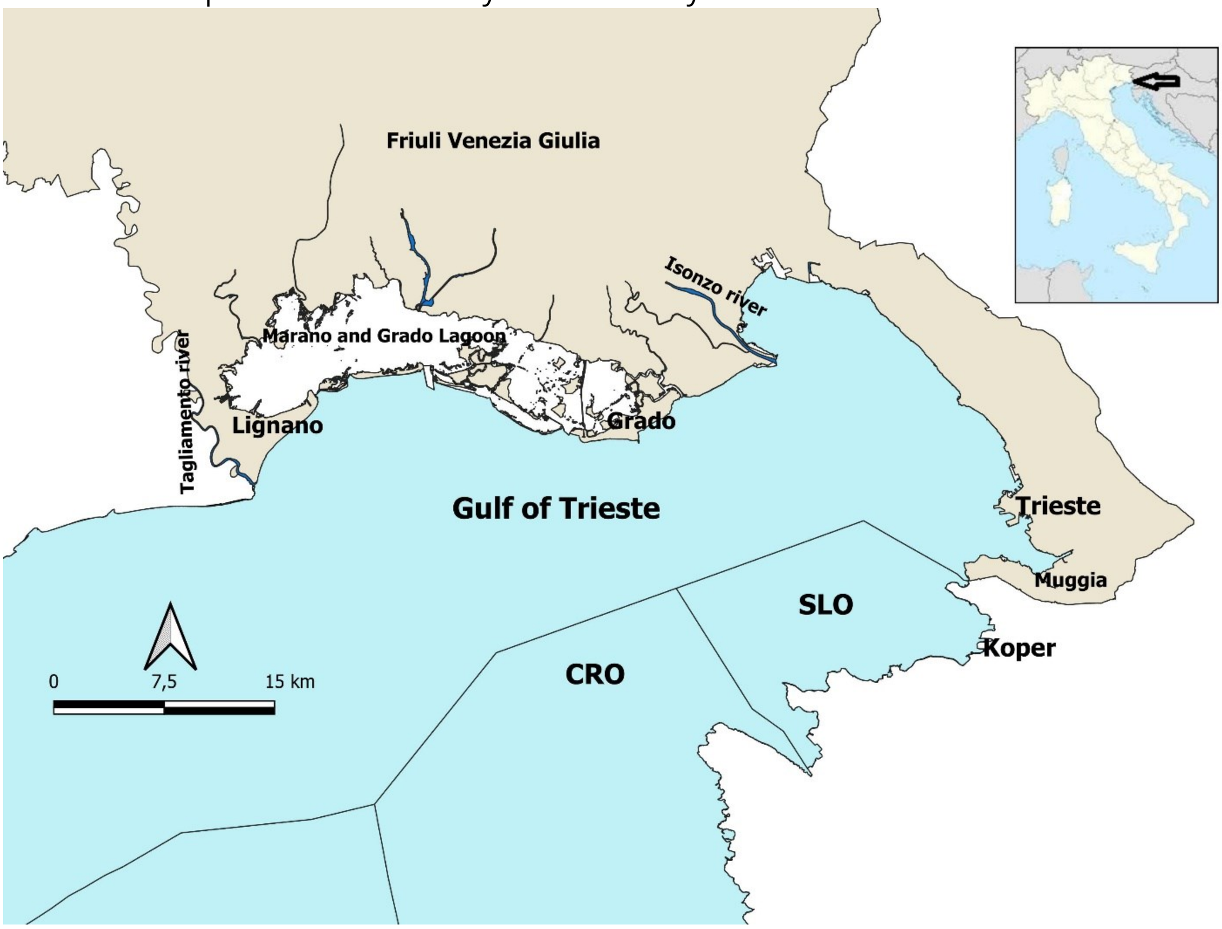

Figure 1: Map of the Gulf of Trieste showing Pilot Area 2 of the ALIENA Project, between the Tagliamento river mouth and Cape Savudrija.

2.2. Brief description of the main environmental characteristics

2.2.1. Geomorphology

The Gulf covers an area of approximately 600 km^2 with the Lagoon of Marano and Grado (160 km^2) facing the coastline between Lignano Sabbiadoro and Grado. The Gulf is characterized by relatively shallow depths compared to the Central and Southern Adriatic with a maximum water depth of 25 m (average depth 17 m) and a very low bathymetric gradient. Despite its limited size, 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.

2.2.2. Main chemical-physical characteristics of the marine coastal waters

The oceanographic characteristics of the Gulf are very variable due to a marked seasonality, with temperatures that can reach minimum values of 5 °C in particularly cold winters, driven by strong Bora winds, to summer maxima of over 26 °C. The salinity gradient is also highly variable between 25 and 38 PSU (Malačič and Petelin, 2001) due to the fluvial inputs of the Isonzo/Soča River, Tagliamento and other minor river inputs, as well as the presence of interchanges of water masses with the lagoon.

2.2.3. Idrology and dynamics of nutrients

The circulation is predominantly cyclonic and influenced by the Istrian-Dalmatian current flowing up the eastern Adriatic, which conveys warmer, saltier and more oligotrophic water masses (Poulain and Cushman-Roisin, 2001).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, 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^{-1}, 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 also the productivity is mostly influenced by river inputs. The main source of freshwater is represented by the Isonzo/Soča River, which flows for approximately 135 km (from the Julian Alps to the northern Adriatic and covers a basin of 3430 km^2). The hydrological regime of this river is torrential with a narrow flow range (31-2253 m^3/sec at the Solkan station),

averaging 82 m³/sec (data from the period 1998-2008) (Comici and Bussani, 2007; Cozzi and Giani, 2011). Minor contributions come from the Timavo and Rosandra rivers (Italian sector), Rižana, Badaševica, Drnica and Dragonja rivers (Slovenian sector; Malej et al., 1995; Turk et al., 2007). In the westernmost part of the Gulf, the Tagliamento River, on the other hand, is characterized by rather variable flows (Spaliviero, 2003).

The dynamics of nutrients, organic matter and production processes are determined in this area by riverine inputs (Malej et al., 1995) , emission from waste water plants (Mozetič et al., 2008), atmospheric deposition and benthic fluxes. The trophic state of the Gulf 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 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 has been affected by hypoxia/anoxia and/or mucilage "phenomena", dinoflagellate and jellyfish blooms (Turk et al., 2008). 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 (Malej et al., 1995).

2.3. Brief description on the main characteristics of biological communities

The rocky substratum of the eastern coast of the Gulf 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 (Orlando-Bonaca et al., 2013). The sediments are quite varied, ranging from sands with patches of beachrocks to muds, predominantly detritic, so that the associated biocoenoses of the Gulf traditionally belong roughly to the DC (Détritique Côtier), DE (Détritique Envasé) and VTC (Vases Terrigènes Côtières) biocoenoses (Bettoso et al., 2023 and references therein).The marine community of living organisms of the coastal and marine areas of Friuli Venezia Giulia Region (FVG) is regularly monitored by ARPA FVG, according to the Water Framework Directive (WFD-2000/60/EC) and the Marine Strategy Framework Directive (MSFD-2008/56/EC). In particular, sensu WFD monitoring has been carried out for over a decade in FVG's coastal waters, analysing the biological quality elements macrozoobenthos and phytoplankton. Despite the persistent anthropic pressures on the Gulf and its extremely variable environmental frame, the analyses on the soft bottom macrozoobenthos have resulted in the achievement of the High and Good ecological status (Bettoso et al., 2022), as well as for the phytoplankton, the latter classified on the basis of chlorophyll a concentration (ARPA FVG, 2023).

2.4. Main anthropic pressures

The marine environment of the Gulf 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.

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 has been heavily modified by human activity, and these modifications become more evident while approaching the city of Trieste (Brambati and Catani, 1998). In detail, from Miramare (Trieste) to Punta Sottile (Muggia) the coastal urbanization, tourism, the presence of harbors and other human pressures generated pollution (*i.e.*, aromatic hydrocarbon, tributyltin and heavy metals) and the original littoral morphology is not easily found. The habitats are strongly modified by the wide tidal range and the unceasing changes of the coastal morphology, which now appears different compared to the past.

2.5. Possible vulnerability of the area to non-indigenous species

The Gulf is characterized by an intense maritime traffic due to the presence of two big harbors in the coastal area of the Region Friuli Venezia Giulia (Trieste and Monfalcone) and the port of Koper in the Slovenian territory. The port of Trieste is one of the most important commercial port in Italy and in the Mediterranean Sea where numerous transoceanic cargoes and oil tankers dock connecting the Adriatic Sea with the Indian Ocean, China Sea and Japan (Occhipinti-Ambrogi, 2002), and therefore it could represents a gateway for the non-indigenous species (NIS).

In addition to that, aquaculture is a very common activity along the coasts of the northern Adriatic Sea representing an important economic sector (mainly mussel farming). Shipping and aquaculture industry are considered the most likely pathways of introduction of NIS worldwide and especially the Adriatic Sea (Slišković et al., 2021), making the Gulf an area particularly at risk for this kind of threat.

Arpa FVG is involved in regular monitoring activities of NIS in the waters of the Gulf according to the Descriptor 2 (D2) of the MSFD. The goal of D2 is to avoid new introductions and monitor the established allochthonous species.

In the recent period, the Gulf has experienced some particularly significant invasions of NIS. *Mnemiopsis leidyi* A. Agassiz, 1865 is an invasive ctenophore arrived in Europe through ballast waters loaded in the Atlantic coast of South America, with initial invasion into the Black Sea and secondary spread into the other Mediterranean areas. In 2005, the species was recorded for the first time in the Gulf, but it was only 10 years later that blooms of *M. leidyi* were observed in different localities in the northern Adriatic (Malej et al., 2017 and references therein). It has been reported that blooms of this invasive species in these areas can have massive consequences on fish stocks and marine food webs (Budiša et al., 2021) making *M. leidyi* of particular environmental and economic interest.

The most recent invasion concerns the Lagoon of Marano and Grado due to the spreading of the blue crab *Callinectes sapidus* Rathbun, 1896. This species is native of the western North Atlantic Ocean and it reached the Mediterranean basin in ships' ballast tanks. In the Adriatic Sea, *C. sapidus* was first recorded in 1949 from Grado Lagoon and more recently, many records have been reported along both the western and eastern coasts of the Adriatic Sea (Manfrin et al., 2016 and reference therein). The blue crab seems to be capable of causing considerable damage to fisheries by consuming netted fish and by cutting nets. It has excellent swimming abilities, opportunistic feeding behaviour, broad habitat preferences, high fecundity, and wide larval distribution. These characteristics have led to its recognition as one of the 100 worst invasive alien species in the Mediterranean (Streftaris and Zenetos, 2006; Glamuzina et al., 2023 and reference therein).

An allochthonous organism of particular sanitary interest in the Gulf of Trieste is *Ostreopsis ovata* Fukuyo, 1981, an epiphytic dinoflagellate. This unicellular alga can produce toxins and blooms which have been related to human health problems. *O. ovata* was observed in the coastal waters of Friuli Venezia Giulia for the first time in 2006 and the first report of a noticeable bloom dates back to 2009. Starting from 2011, blooms have been observed along the bathing coasts of the gulf appearing periodically in the second half of August and in September after a long period of stable weather, with high air temperatures and intense solar radiation. ARPA FVG has developed a monitoring network to guarantee bathing safety which includes monthly sampling of coastal waters from May to September with an increase in frequency in case of alert situations (further information are available in: https://www.arpa.fvg.it/temi/temi/acqua/sezioni-principali/balneazione/ostreopsis-ovata/).

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3. The western Istrian coast & Northern Adriatic: several sites on rocky shore and RV001 station 1 nm from coast

3.1. General geographic context

The coastal area of the eastern northern Adriatic could be divided in two zones considering thermohaline properties: the western Istrian coast and Rijeka Bay including the adjacent inter island area. Western Istrian Coast waters are quite shallow (30-40 m) and under a significant inflow from eastern Adriatic current coming from south and occasionally by the Po River waters. They are considerably influenced by atmospheric forcing as well. 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 2; 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 3 will take place the sampling stations can be seen in figure 1.

Figure 1. P3 Pilot Area is depicted in blue.

3.2. Brief description of the main environmental characteristics

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, 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 2; seasonal changes in sea temperature (t), salinity (s) and reduced density (γ) for some depths during the period 1972-2006).

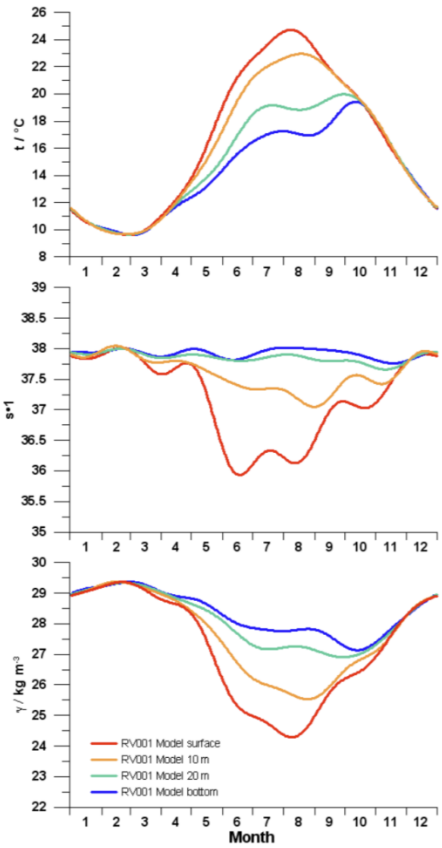

Figure 2. Average seasonal variation of sea temperature (t), salinity (s) and density (γ) for distinct layers in the period 1972-2006. A function with three harmonics was fit to the data.

Seasonal average temperature values on RV001 vary between 10-24°C, salinity 35.5-38.2, and reduced density 24.3-29.5 kg m3. 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, 2002; 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 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, Marić et al. 2012). Over the past decade, and corresponding to the decrease in Po River discharge, 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 (Mozetič et al., 2010, Marić et al.2012) as well as a general shift toward smaller plankton size classes (Bernardi-Aubry et al., 2006; 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. 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.).

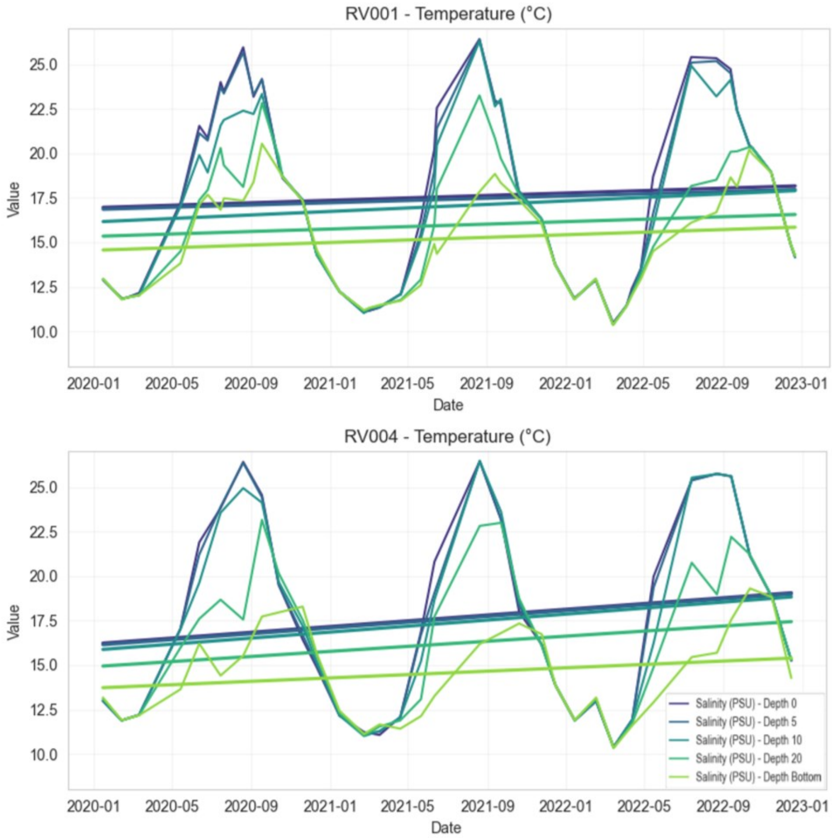

Figure 3a: Time series for temperature for stations RV001 and RV004.

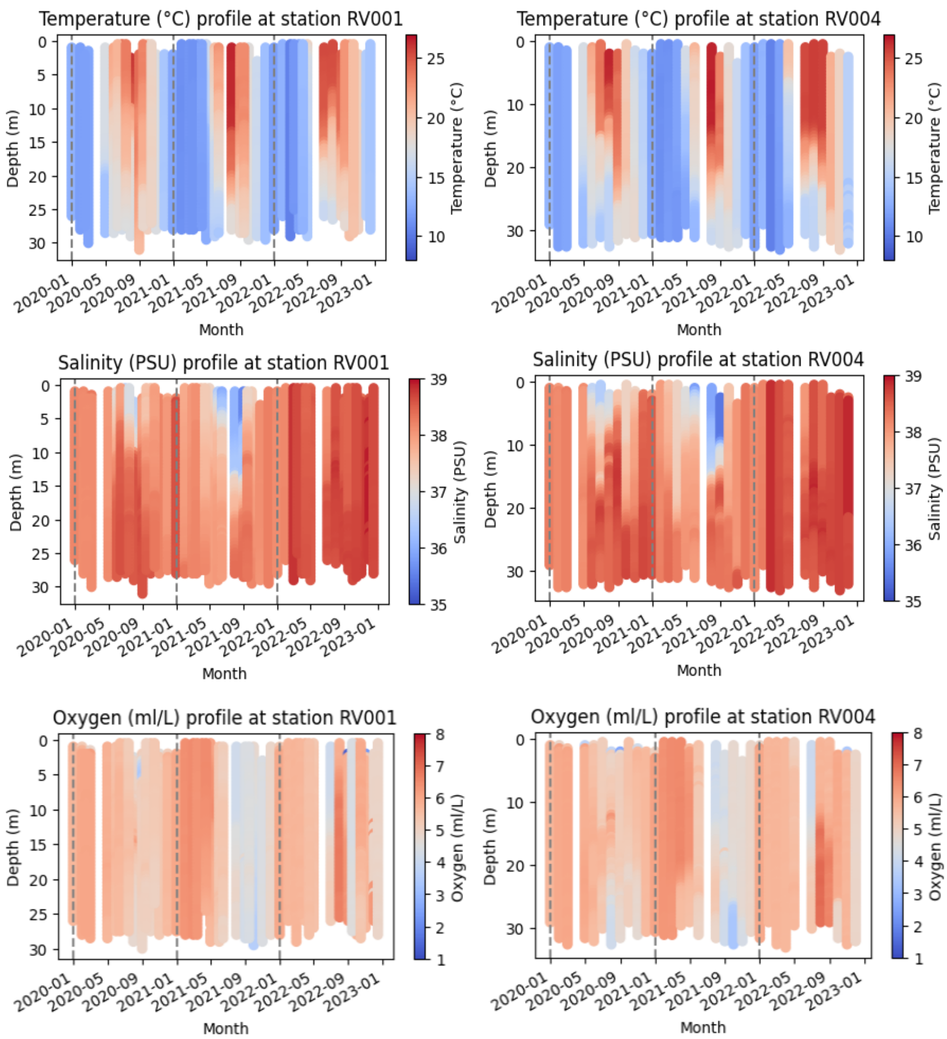

Figure 4: Depth profiles of temperature, salinity and oxygen collected by the CTD probe for stations RV001 and RV004. Profile is made up of data measured every 0.5 meters.

Data showed before where the averages and typical values calculated on long time data series and on models. Here we show data measured on two stations in the pilot area 3 in last 3 years. Temperature, salinity and oxygen concentration at the sea surface show a seasonal cycle (Figure 3). Winter (December-February) is characterized by good mixing of the water column (Figure 3) with temperatures between 11.09°C and 15.25°C. Lower winter temperatures were measured on eastern coastal stations. Surface salinity varied between 33.94 PSU near the Po Delta and 38.94 PSU near Rovinj, and there was a clear (stable) spatial pattern with an area of high salinity along the

Croatian coast (Figure 2 RV001 and Figures 3-4). The distribution of temperature and salinity is followed by the concentration of dissolved oxygen. Spring (March-May) is still characterized by homogeneity of the water column, which begins to stratify in May. Surface temperatures range between 10.07°C and 19.98°C and are higher on the coastal than on the central northern Adriatic stations. Surface spring salinity values were higher compared to winter (37.64±0.84 PSU), but the spatial distribution is similar. Oxygen concentrations, compared to the winter season, also show higher values and a wider range with average value of 5.95±0.62 ml/L (Figure 4). The concentration of dissolved oxygen followed for the surface layers a clear east-west gradient. During the summer (June-August), the stratification of the water column was clearly visible for all oceanographic parameters (Figure 4). The seasonal warming of the surface up to 26.45°C (station RV004 in summer 2021) coincides with a reduction of the salinity to 35.70±1.85 PSU and oxygen concentrations (4.95±0.55 ml/L). Surface seasonal pattern was in accordance with previous studies for the area but the measured maximum exceeded the mean values. In summer, northern Adriatic is usually occupied by lower salinity water due to the influence of eddy winds that create a double circulation system which, combined with strong stratification, facilitates the accumulation of lower salinity water on the surface (Poulain, 2001). The oxygen concentration in spring is further reduced compared to the spring season. Autumn (September-November) was characterized by cooling throughout the northern Adriatic and by mixing of the water column. As in winter, the lowest temperatures were found on stations closer to the eastern coast. During autumn, salinity and oxygen concentrations increased to 38.69 PSU and 6.88 ml/L, respectively. In addition, in autumn (September and November) 2022, two measurements at the coastal station (RV001) showed a significant decrease in oxygen to values below 2.5 ml/L, which is indicative of respiratory processes dominating the system in this situation (del Giorgio and Duarte, 2002; Robinson, 2019). Such trends are worthwhile monitoring, as changes in plankton ecology are recognized drivers in the role of the Sea in the global carbon cycling, and trends towards lower oxygen production lead to significant declines in the role of marine ecosystems as CO_2 sinks. During summer, the intrusion of low salinity water from the Po Delta towards the eastern coast was pronounced (Figure 3b). The seasonal pattern of oxygen concentration at the surface was also strongly influenced by temperature trends. The highest oxygen values were recorded in spring when the surface temperatures were the lowest. Similarly, oxygen lowest values were recorded in summer, when the temperatures were the highest. In addition, the highest dissolved oxygen concentration was measured in the areas with the lowest salinity. This can be linked to primary production, which is boosted by nutrient-rich water from the Po (Penna et al., 2004; Kraus and Supić, 2011).

3.3. Brief description on the main characteristics of biological communities

The rocky substratum of the eastern Istrian coast consists mainly of limestone, while the deeper 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 rocks. The sediments are quite varied, ranging from sands with patches of beachrocks to muds, predominantly detritic, so that the associated biocoenoses belong roughly to the DC (Détritique Côtier), DE (Détritique Envasé) and VTC (Vases Terrigènes Côtières) biocoenoses (Bettoso et al., 2023 and references therein). The marine community of living organisms of the coastal and marine areas in our Pilot area is regularly monitored by Center for marine research, Ruđer Bosković Institute, according to the Water Framework Directive (WFD-2000/60/EC) and the Marine Strategy Framework Directive (MSFD-2008/56/EC). In particular, sensu WFD monitoring has been carried out for over a decade in Istrian coastal waters, analysing the biological quality elements macrozoobenthos and phytoplankton. Despite the persistent anthropic pressures and its extremely variable environmental frame, the analyses on the soft bottom macrozoobenthos have resulted in the achievement of the High and Good ecological status Figure 5, as well as for the phytoplankton, the latter classified on the basis of chlorophyll a concentration.

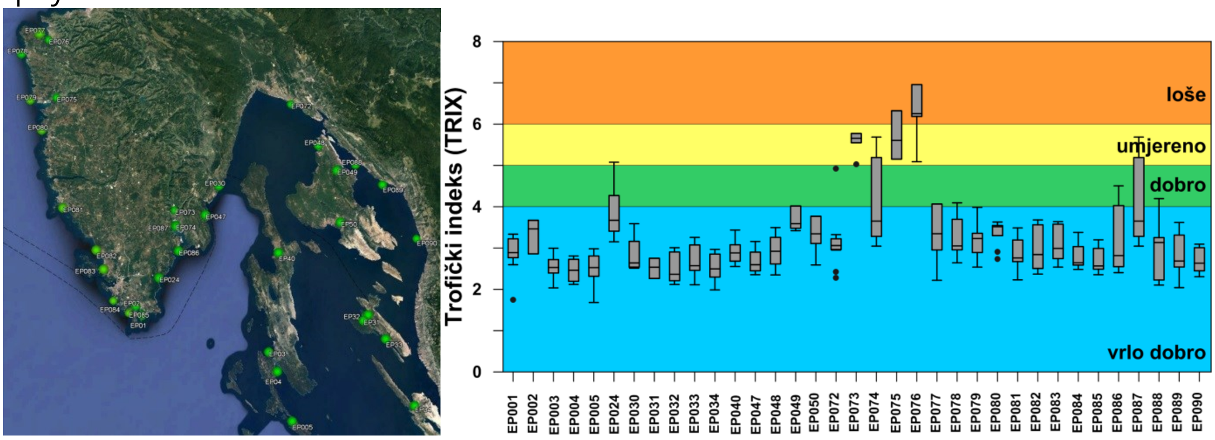

Figure 5. Positions used in MFSD and Marine strategy in the northern Adriatic b) Box i Whisker display of data for every station for trophic index (TRIX).

3.4. Main anthropic pressures

The marine environment of the western Istrian Coast 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 city of Pula.

The Istrian province has 195237 inhabitants and those numbers multiply several times during the touristic season. Istrian peninsula has a low density of inhabitants (69.41 inh. Km^2). The coastal morphology of the Istrian peninsula has been modified by human activity. The habitats are strongly modified by the wide tidal range and the unceasing changes of the coastal morphology, which now appear different compared to the past which results in lost of habitats and species of macroalgae.

Figure 6. Destruction of natural cost along western Istrian coast.

In the last four years, as part of the project "Responses of brown algae of the genus *Cystoseira*, which form habitats, to local and global stressors; HabCYS" from the Croatian Science Foundation, led by dr.sc. Ljiljana Iveša macroalgae were mapped along the west coast of Istria, from Umag to Medulin. The focus of the research was on the presence of large brown macroalgae, which are an indicator of good seawater quality. However, their disappearance in the last ten years has been noticed throughout the Mediterranean Sea. Unfortunately, along the Istrian coast, the disappearance of these species is directly related to the modification of the coast, a practice that has become common in recent years. This process disrupts the natural shoreline, replacing the limestone shoreline with materials such as river gravel or sand (Figure 6).

As a consequence, there is permanent and irreversible destruction of habitats and biodiversity. Field observations have shown that the destruction is present not only in the tidal zone, but also in deeper shallow areas, because over time the artificial material sinks into the depths, covering the seabed and thus disrupting the flora and fauna, including strictly protected species, whose habitats are crucial for the health and functioning of the marine environment.

Currently, the damage is significant, and the return from the "damped coast" to a natural coast again is not possible. Local self-government and large tourist companies should focus more attention on preserving the remaining natural coastline and minimize interventions to the smallest possible extent.

Our research and monitoring of important habitats such as sea grass meadows show that we have lost most of the meadows and the remaining settlements have significantly decreased in size in the last 100 years in the northern Adriatic. First, we lost the habitats of *Posidonia oceanica*, an endemic seagrass of the Mediterranean at the end of the 60-70s of the last century, and the remaining meadows of *Cymodocea nodosa* and other smaller species are under great pressure and have been decreasing year by year for the last 20 years. In Figure 7, the red color on the right shows where and in what proportion the habitat has been lost. Such loss significantly reduces the stability and resilience of the coastal belt. Meadows of sea flowers are very important for the spawning of the flounder stock and the preservation of biodiversity.

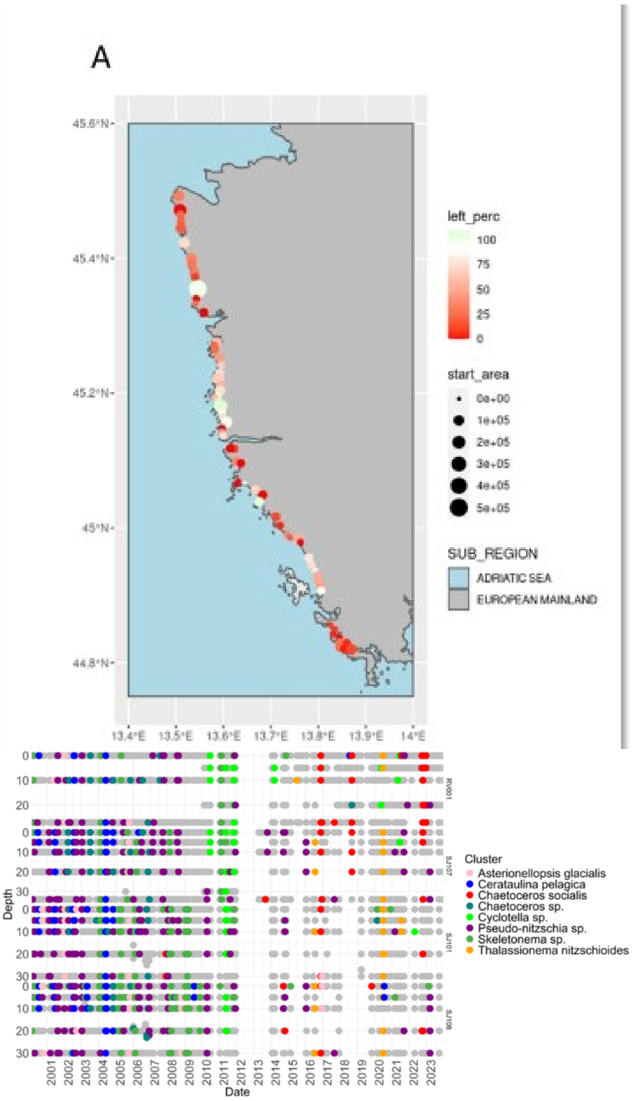

Figure 7. distribution of seegrass medows along Istrian peninsula.

Phytoplankton consists of single-celled organisms (algae) which are the first link and base of the entire marine food web and the base of the entire marine ecosystem. The Center for Sea Research has been systematically and continuously monitoring and researching the phytoplankton of the northern Adriatic for over 40 years. As part of systematic monitoring (EU marine strategy and MFSD), phytoplankton is prescribed as an indicator for the state of the environment. Our research shows significant changes in the succession (time sequence) and composition of the phytoplankton community in the last 20 years. Such changes at the very beginning of marine food webs have a significant impact on the stability, resilience, and productivity of the entire ecosystem of the northern Adriatic.

3.5. Possible vulnerability of the area to invasive species

The northern Adriatic is characterized by an intense maritime traffic due to the presence of two big harbors in the coastal area of Italian and Slovenian Coast (Trieste and Koper). The port of Trieste is one of the most important commercial port in Italy and in the Mediterranean Sea where numerous transoceanic cargoes and oil tankers dock connecting the Adriatic Sea with the Indian Ocean, China Sea and Japan (Occhipinti-Ambrogi, 2002), and therefore it could represents a gateway for the non-indigenous species (NIS).

In addition to that, aquaculture is a very common activity along the coasts of the North Adriatic Sea representing an important economic sector (mainly mussel farming). Shipping and aquaculture industry are considered the most likely pathways of introduction of NIS worldwide and especially the Adriatic Sea (Slišković et al., 2021), making the area particularly at risk for this kind of threat.

Majnarić et al. (2022) observed intensive ascidian invasion in the summer of 2020 at shellfish farms on the western and eastern coasts of Istria, Northern Adriatic, Croatia. Based on collected specimens examined, it was confirmed that the present species is already known as invasive ascidian *Clavelina oblonga*, previously recorded in Savudrija in 2015 (Miokovic, 2016, 2018.). *Clavelina oblonga* (Ascidiacea, Aplousobranchia, Clavelinidae) is a distinctive and conspicuous epi/benthic colonial tunicate naturally distributed in the Gulf of Mexico´s tropical area, on the west coast of the Atlantic, in the Azores and the west coast of Spain (Scarponi et al., 2018). *Clavelina oblonga* specimens have caused big damages to the shellfish farm in the region and it should be monitored.

In the recent period, the area has experienced some particularly significant invasions of NIS. *Mnemiopsis leidyi* A. Agassiz, 1865 is an invasive ctenophore arrived in Europe through ballast waters loaded in the Atlantic coast of South America, with initial invasion into the Black Sea and secondary spread into the other Mediterranean areas. In 2005, the species was recorded for the first time in the Gulf, but it was only 10 years later that blooms of *M. leidyi* were observed in different localities in the northern Adriatic (Malej et al., 2017 and references therein). It has been reported that blooms of this invasive species in these areas can have massive consequences on fish stocks and marine food webs (Budiša et al., 2021) making *M. leidyi* of particular environmental and economic interest.

The most recent invasion concerns the spreading of the blue crab *Callinectes sapidus* Rathbun, 1896. This species is native of the western North Atlantic Ocean and it reached the Mediterranean basin in ships' ballast tanks. In the Adriatic Sea, *C. sapidus* was first recorded in 1949 from Grado Lagoon and more recently, many records have been reported along both the western and eastern coasts of the Adriatic Sea (Manfrin et al., 2016 and reference therein). The blue crab seems to be capable of causing considerable damage to fisheries by consuming netted fish and by cutting nets. It has excellent swimming abilities, opportunistic feeding behavior, broad habitat preferences, high fecundity, and wide larval distribution. These characteristics have led to its recognition as one of the 100 worst invasive alien species in the Mediterranean (Streftaris and Zenetos, 2006; Glamuzina et al., 2023 and reference therein). Even though has been found in the region around Rovinj the results are not jet published. Personal observation by M. Pfannkuchen and N. Iveša.

An allochthonous organism of particular interest in the area is *Ostreopsis ovata* Fukuyo, 1981, an epiphytic dinoflagellate. This unicellular alga can produce toxins and blooms which have been related to human health problems. *Ostreopsis ovata* was first time observed in the coastal waters of Istria in 2006 by Monti et al. and the blooms have been recorded in 2009 by Pfannkuchen et al with toxin profile examination (Pfannkuchen et al. 2012). Later on also molecular barcode for *Ostreopsis ovata* from our pilot *area* was made by Kužat et al 2021.

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4. Several sites in Kaštela Bay and Šibenik Bay area

4.1. ŠIBENIK BAY

4.1.1. General geographical context

Šibenik Bay is part of the highly stratified Krka estuary in the central part of the eastern Adriatic coast (Figure 1). The Krka River is one of the most pristine rivers in Europe and is 49 km long before it flows into the estuary. The 2450 km2 karstic watershed is scarcely inhabited and is partly located in the Krka National Park. The estuary is mainly fed with fresh water by Lake Visovac, followed by a series of travertine barriers and waterfalls. The estuary is 23.5 km long and relatively narrow, with the exception of Lake Prokljan and Šibenik Bay. Lake Prokljan looks like a lake in terms of landscape, but corresponds to the physical and chemical characteristics of the sea. In the lower part of the estuary lies the town of Šibenik (Šibenik Bay), which is separated from the open sea by a narrow, steep canyon (St. Ante Channel). This channel is a natural phenomenon with numerous capes and bays, about 2.5 km long and 140 m to 220 m wide. The water depth gradually increases from 2 m below the waterfalls to 43 m near the mouth. Most of the river is a pristine environment within the boundaries of a national park, but the middle reaches of the estuary near the town of Šibenik are affected by anthropogenic eutrophication (Gržetić et al., 1991; Legović et al., 1994).

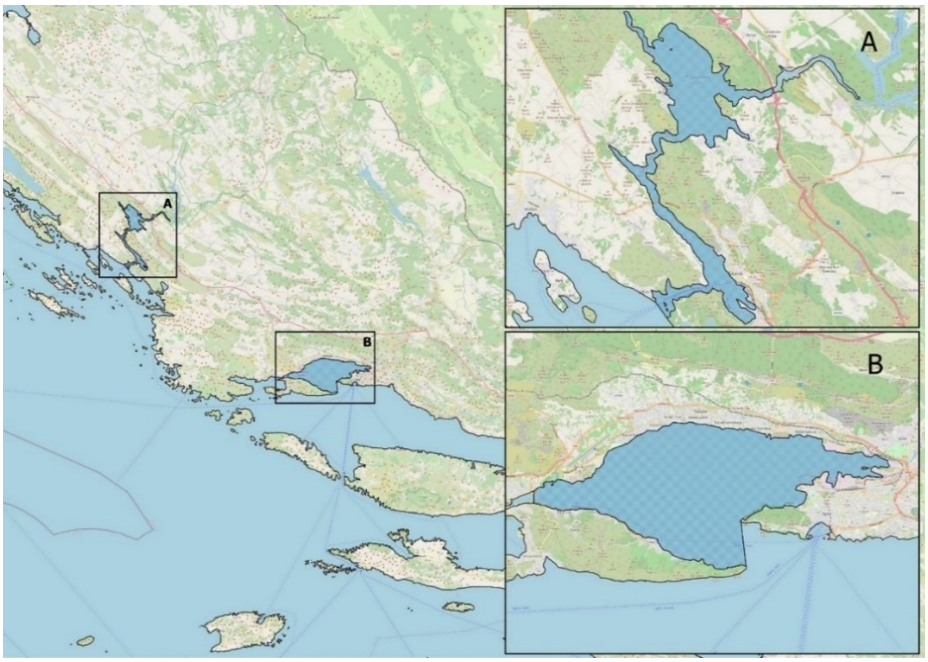

Figure 1. Central part of the eastern Adriatic Sea with two marked pilot areas: Šibenik Bay (A) and Kaštela Bay (B).

4.1.2. Brief description of the main environmental characteristics

This region has attracted the interest of researchers for more than a century because of its geomorphological features, its hydrological and hydrographic regime, its chemical and biological characteristics, its anthropogenic pressures and its protection. However, the natural features have not yet been fully described and assessed (Kršinić, 2010a).

The area from Skradinski Buk to the outer regions of the St. Ante Channel is considered a Mediterranean estuary, characterized by relatively low tidal fluctuations and strong vertical stratification. The seawater flows in the bottom layer as far as Skradin, while the surface layer of fresh water (which extends to different depths) reaches the outermost part of the estuary as far as the island of Zlarin. The average flow of the Krka at the Skradinski Buk waterfalls has fluctuated between 40 and 60 m3 s-1 over the last 50 years, with a minimum flow of 5 m3 s-1 and a maximum of 565 m3 s-1 (Bonacci et al., 2006). Significant tributaries of the Krka are Krčić, Kosovčica, Orašnica, Butišnica and Čikola. The maximum tidal range is ~40 cm at the mouth and ~30 cm at the head of the estuary. In late summer, when the freshwater discharge is very low, the salinity at the mouth can rise to 30 PSU in the upper layer (Legović, 1999). The maximum is located at the lower edge of a steep halocline and reaches the highest temperature in the Adriatic Sea. Temperatures of up to 31°C have been measured (Legović et al., 1991). The formation of the maximum and its persistence during the summer is the result of a relatively thin and transparent layer of brackish water, while the inflow of seawater is weak. The maximum can be measured from mid-April to the end of October. In the bay, salinity values fluctuate between 4.42 PSU and 38.74 PSU (Bužančić et al, 2012). The exchange time of freshwater and seawater in the Krka estuary is shorter in winter than in summer. In winter, the exchange time of freshwater is 6-20 days, while in summer it is up to 80 days. The exchange time of seawater is 50-100 days in winter, while in summer it is extended to 250 days (Legović, 1999).

This salt wedge estuary is heavily stratified all year round due to the low tidal range (20-40 cm on average). Due to the strong stratification (salinity gradient of 30 PSU per 20 cm), the river/seawater boundary was reduced to a clearly defined visible region (Žutić and Legović, 1987). The upper freshwater and lower saltwater layers are separated by a narrow interface at the halocline, which contains an organic film formed mainly by the accumulation of organic material from planktonic organisms (Žutić and Legović, 1987). Both layers are characterized by suspended matter concentrations that do not exceed 10 mg L-1 (Legović et al., 1994). The differences in suspended biomass concentrations above and below the halocline indicate that phytoplankton and organic matter are produced at the freshwater-seawater interface. In addition, the high sedimentation rates of organic detritus in this area could be the result of decomposition of plankton at the interface, which then settles to the bottom. However, low sedimentation rates in the channel area suggest oligotrophic conditions outside the estuary (Svensen et al., 2007).

The main sources of nutrients in this estuary are the Krka River, the city of Šibenik (Gržetić et al., 1991) and numerous submarine groundwater discharges connected to the karst aquifer (Liu et al., 2019). Nutrient concentrations vary in ranges of 0-59.2 μmol L-1 NO3-, 0-1.1 μmol L-1 NO2-, 0-13.2 μmol L-1 NH4+, 0-1.73 μmol L-1 PO43- and 0-65.8 μmol L-1 SiO44- (Gržetić et al., 1991). The fluctuation ranges of nutrient concentrations in the Šibenik Channel were of lower intensity, as it is less influenced by land and the freshwater inflow of the Krka River (Bužančić et al., 2012). According to these estimates, the main source of nitrogen and silica is the Krka River, while the main source of total phosphorus is of anthropogenic origin and comes from the Šibenik area. Most of the primary production takes place in the shallow brackish water layer up to 4 m above the sharp halocline (Svensen et al., 2007). The in situ measurement of this parameter varies between 1 mgC m-3 h-1 and 30 mgC m-3 h-1 (Gržetić et al., 1991). On the other hand, the seasonal distribution of phytoplankton biomass is characterised by highest values in spring and autumn-winter, and lowest values during summer stratification with chlorophyll a in the range of 0.07-4.73 μg L-1 (Bužančić et al., 2012). High concentrations of POC, Chl a and phytoplankton are measured in the lower part of the estuary (Šibenik Bay and Šibenik Channel) where the town of Šibenik is an important source of anthropogenic nutrient enrichment (Svensen et al., 2007). Trophic status of the investigated area, as a result of long term monitoring activities, is presented in more details in the State of the marine environment, aquaculture and fisheries indicators database (https://vrtlac.izor.hr/ords/bazapokpub/bindex).

Terrigenous and biogenic particles are deposited throughout the Krka estuary. The river flows through the karst area and carries very small amounts of particles that settle in front of the Krka waterfall and therefore have no influence on the deposition of terrigenous material in the estuary. However, a larger amount of terrigenous fine-grained particles, formed by the weathering of marl in the hinterland, reaches the northern part of Lake Prokljan via the Guduča River. The biogenic particles contained in the sediment are predominantly of marine origin and indicate a shelf environment with the salinity typical of marine ecosystems (Bogner, 2001).

4.1.3. Brief description of the main characteristics of biological communities

Apart from its aesthetic value and uniqueness, the Krka estuary is also valuable for its biological diversity, a large number of fish, invertebrates, algae and flowering plants. It is precisely for this reason that the entire Krka estuary was included in the Republic of Croatia's ecological network for the protection of estuaries and sandy bottoms. The estuary as a protected part of nature includes two important landscapes, the lower reaches of the Krka River and the Channel-harbour, as well as the Natura 2000 Krka Estuary ecological network HR3000171. Upstream of this area lies the Krka National Park.

Due to its geomorphologic features and the pronounced stratification of nutrient-rich water, this area is a biologically very productive ecosystem and an important breeding, rearing and feeding area for numerous organisms, especially fish (PU 6077, 2023). It is an important habitat for many species, and the Canestrini's goby (*Pomatoschistus canestrini*), an endemic and endangered species of the Adriatic basin, has been found in the estuary. Of the strictly protected bivalves, the date mussel (*Lithophaga lithophaga*) lives in this area, and it is also a suitable habitat for the endangered noble pen shell (*Pinna nobilis*) (PU 6077, 2023). Ciliates *Favella taraikaenzis* is a summer estuarine tintinnid species, that has only been recorded in two locations in the Adriatic (Krka and Neretva estuaries) (Kršinić, 2010b). A loggerhead sea turtle (*Caretta caretta*), a strictly protected species in Croatia, has been observed in this area, as well as the bottlenose dolphin (*Tursiops truncates*) (PU 6077, 2023). As this is an area with a high trophic level, some populations are very numerous. Due to the high abundance of planktonic organisms, there is a high proportion of filter feeders in the ecosystem. These natural conditions make the entire area suitable for mussel farming.

A total of 121 species of benthic invertebrates were found in the studied area of the Krka estuary, especially sponges in the lower part of the estuary, which is most strongly influenced by the sea (IOR, 2019). The detected organisms form communities that differ significantly from the communities they inhabit in the coastal waters of the central Adriatic. The benthic communities contain dense populations of organisms that feed on suspended organic matter and plankton. Sponges, bivalves and molluscs dominate in the studied area. In the near-surface part, in the area of water with reduced salinity, the number of species is low, but their biomass is high in some places (e.g. the mussel *Mytilus galloprovincialis* and the crab *Amphibalanus eburneus*). During the surveys in 2019, a non-native polychaete species *Ficopomatus enigmatic*us was found in a large area of the estuary directly on the surface. In the deeper layers with seawater, the ecological conditions are more stable and the species diversity is significantly higher than on the surface.

In the Krka estuary a total of 130 species of algae and three seagrasses were found: the marine species dwarf eelgrass *Zostera noltei*, the freshwater species beaked tasselweed *Ruppia maritima* and the marsh plant of the genus *Carex*. Most algal taxa were found at the stations in the lower part of the estuary, and the depth distribution of benthic vegetation decreases from the entrance to the St. Ante channel upstream.

Since 1994, extensive oceanographic studies of estuarine plankton communities have been carried out as part of various national and regional projects within a multi-year program to monitor the quality of coastal and transitional waters.

In the phytoplankton community, 221 species were taxonomically identified. The composition of the community is typical for coastal waters and estuaries. The community is dominated by diatoms and small flagellates. Dinoflagellates are most abundant in summer, especially the unarmored dinoflagellates *Gymnodinium spp*. The community composition in this area also indicates a healthy and diverse community. Species characteristic of eutrophic areas were not present in large numbers (IOR, 2019).

The zooplankton community in the Krka River estuary shows relationships between groups characteristic of semi-enclosed coastal ecosystems under freshwater influence (IOR, 2019). The micro-fraction is dominated by non-loricate ciliates, while other groups are quantitatively less represented. Occasionally this area is under the very strong influence of the open sea, which allows open sea species to enter the estuary in the layer below 20 m depth. Some ciliate species quickly adapt to the new habitat, reproduce and reach a significantly higher abundance than in their original habitats. These are the tintinnid species *Xystonella lohmanni* and *Eutintinnus tubulosus* (Kršinić, 2010a). On the other hand, the mesozooplankton community is characterized by the dominance of copepods and developmental stages of benthic invertebrates, while other mesozooplankton groups are quantitatively less represented (Appendicularia, Chaetognatha, Thaliacea) or show a pronounced seasonality of occurrence and reproduction (Cladocera) (IOR, 2019). The taxonomic composition and abundances within the overall zooplankton communities indicate the good preservation of this pelagic habitat and the undisturbed functioning of the pelagic food web at the primary consumer level.

4.1.4. Main anthropogenic pressures

Potential sources of threats in the estuary are mainly related to anthropogenic pressures that affect estuaries as habitat types and ultimately lead to a reduction in the quality of the habitat and a resulting deterioration in the conditions for the development of biota. This mainly concerns excessive nutrient enrichment (nutrient salts), which can lead to uncontrolled and/or toxic phytoplankton blooms, the occurrence of pathogenic microorganisms, changes in the taxonomic and size structure of prey for zooplankton organisms and a decrease in oxygen concentration in the water column.

According to the latest census from 2021, a total of 38,473 inhabitants live in the entire area of the Krka estuary, while the town of Šibenik itself has 31,085 inhabitants. Systematic work on Šibenik's wastewater disposal began in early 2007. The city's wastewater is pumped to the central wastewater treatment plant, from where the treated water is discharged into the sea near the southern cape of the island of Zlarin. In this way, all discharges into the city harbour were collected and diverted into collection sewers, thus meeting the prescribed wastewater quality criteria and improving overall environmental standards. However, an anthropogenic influence was detected in the uppermost segment of the freshwater part, due to the discharge of untreated municipal and/or industrial wastewater around the town of Knin (Filipović Marijić et al., 2018). On the other hand, nautical tourism has been associated with increased concentrations of some trace elements in the estuary during the summer season (Cindrić et al., 2015). Harbour activities and an old ferromanganese smelter have been identified as current and historical sources of pollution of elements in sediment at selected sites in the estuary (Cukrov et al., 2008a, 2020). In addition, climate change undeniably has a significant impact on biota by altering environmental temperature conditions, promoting opportunistic development of thermophilic populations and influencing salinity gradients in the estuary through sea level rise. Some low-lying coastal areas, e.g. Dolac in

Šibenik, are frequently flooded. This area, as well as the rest of the Šibenik coast, is affected by rising sea water, which is a vivid example of what coastal regions could look like in the future if sea levels rise.
The maritime economy (shipbuilding, construction of coastal zones, ports and other technical facilities at and on the sea), fishing, shellfish farming and Mediterranean aquaculture are also significant sources of pressure, as are tourism, hotels and restaurants in coastal towns and communities, whose growth has been particularly evident in recent years. All this potentially favours the introduction of alien (allochthonous, non-native) species, especially invasive species, which, due to their highly adaptable life strategies, gain a competitive advantage over native (autochthonous, native) species and dominate the community, which can affect the functioning of the trophic network.

4.1.5. Possible vulnerability of the area to invasive species

All the anthropogenic influences mentioned above, especially those of the maritime economy (ballast water management), aquaculture (fish and shellfish farming) and nautical tourism, as well as changing climatic conditions, favour the introduction of non-native species into this ecosystem. Due to its hydrographic characteristics, geomorphology and high natural productivity, it is an ideal habitat for the introduction, survival and development of populations of these species.
As mentioned above, Šibenik Bay is regularly monitored under various national programmes, with a particular focus on alien species according to the MSFD under descriptor D2. Decision EC 848/2017 defines the primary and secondary criteria and the initial methodological standards for assessing the pressure on the introduction or spread of alien species. We would like to mention some of them that have been detected so far in this estuary.
In December 2014, adult females and copepodites of the alien paracalanid *copepod Parvocalanus crassirostris* were recorded for the first time in the Adriatic Sea in the port of Šibenik (Vidjak et al., 2016). The species is characterised by a circumglobal distribution with a preference for tropical/subtropical regions. The most likely transmission vector for this small copepod is ballast water from cargo ships, which is regularly discharged at this location (Vidjak et al., 2016). Another non-indigenous calanoid copepod, *Pseudodiaptomus marinus* (originating from the Northwest Pacific), was also detected in Šibenik Bay in November 2015 near the harbour, presumably the place of origin, and in the deeper central part of the bay (Uttieri et al., 2020). The specimens were usually collected either at sunset or at night at a surface salinity of 5.0-32.2 and a surface temperature between 9.4°C and 22.8°C. In the last 15 years, *P. marinus* has been increasingly detected in European waters and is spreading unexpectedly fast. This species has specific biological and behavioural characteristics that make it particularly interesting for ecological and applied research topics. In January 2018, 29 scientists from nine European countries founded the EUROBUS (Towards a EURopean OBservatory of the non-indigenous calanoid copepod *Pseudodiaptomus*

marinus) working group (https://eurobuswg.wordpress.com) with the aim of creating a European network dealing with the various aspects of the biology and ecology of *P. marinus* and to create an open forum in which the working group participants can share their experiences and knowledge.
The tubeworm *Ficopomatus enigmaticus* is a cryptic species that probably entered in the Mediterranean as ship fouling or via ships transporting stones from Dalmatia (Mikuš et al., 2007; Cukrov et al., 2010). In the Adriatic, it was first recorded in 1934 in the lagoon of Venice and forms huge "reefs" in the Po Delta (Bianchi and Morri, 1996). According to these authors, *Ficopomatus* reefs can influence the brackish water ecosystem in many ways: they form the main hard substrate, are an important source of sediment, help regulate the trophic state of the lagoon and serve as a refuge for numerous invertebrate species. In Croatian waters, it was first recorded in Šibenik Bay in 2006 (Mikuš et al., 2007). On the coast of the lower part of the Krka estuary, *F. enigmaticus* occurred as single tubes or as large, dense aggregations of fouling, mainly on artificial substrates such as cement (embankments) and iron structures at a depth of 0.5-3 metres (Cukrov et al., 2010). The incrustations can be up to 25 cm thick and reach the water surface at low tide. The species forms fouling in combination with *Mytilus galloprovincialis* and bryozoans. Dead fragments of fouling aggregates from *F. enigmaticus* tubes indicate that fouling aggregates perish in winter due to overweight or mortality as they are exposed to low temperatures, low salinity and/or high current intensity over long periods of time.

4.2. KAŠTELA BAY

4.2.1. General geographical context

Kaštela Bay is the largest bay in the coastal area of the central Adriatic. It is a semi-enclosed bay bordered by the island of Čiovo, the slopes of Mount Kozjak and the Split peninsula. It has a total area of 61 km2, a volume of 1.4 km3 and an average depth of 23 m and is connected to the adjacent channel by a 1.8 km wide and 40 m deep inlet (Figure 1). The main freshwater source is the Jadro River, which flows into the eastern part of the bay (Vranjic Basin), with an average annual inflow of ~10 m3 s-1 (Orlić et al., 2007). A small part of the fresh and brackish water reaches the sea via the Pantan and Slanac streams in the northwestern part of the bay and via numerous underwater springs along the northern coast of Marjan hill and in the northwestern part of the bay.

4.2.2. Brief description of the main environmental characteristics

The geographical characteristics of the bay, its proximity to the mainland and anthropogenic influences have a major impact on the hydrographic parameters of the bay. Water circulation in the bay is mainly generated by the local wind, which is related to the passage of mid-latitude cyclones over the area (Gačić et al., 1987; Beg Paklar et al., 2002). The average water renewal time is about one month, while it can be up to five days during strong winds (Zore-Armanda, 1980), which is more frequent in winter when the water enters the bay in the surface layer, sinks and leaves the bay in the bottom layer. In the warm season (July to September), the wind is relatively weak and the freshwater inflow is low, so the water renewal period is relatively long. In summer, the situation is reversed, i.e. the water leaves the bay in the surface layer with the occurrence of upwelling and enters the bay in the bottom layer (Zore-Armanda, 1980). The tides in bay are of mixed type. The average daily amplitude is about 28 cm. The maximum amplitude in 1955 was 118.5 cm, while the average annual sea level fluctuated by 6.5 cm (Kraus and Starčević Stančić, 2023). Within Kaštela Bay, its eastern part is interesting due to its natural features and the concentration of industrial facilities and the cargo port. The peculiarity of this area is determined by the stationary eddy current of seawater in calm weather in summer, which completely isolates this part of the bay from the rest of the basin (Zore-Armanda, 1980).

The most important hydrographic parameters are known from long-term studies (Zore-Armanda, 1980, Buljan and Zore-Armanda, 1971). The annual temperature fluctuations in Kaštela Bay are most pronounced in the surface layer, which is coldest in February (~10°C), while in summer the highest values of over 27°C were measured in the eastern part of the bay. The winter period is characterized by a temperature inversion and a minimum of the column mean. The warming of the surface layer at the end of winter and the beginning of spring led to temperature stratification and the formation of a thermocline in May. The maximum column mean of over 24°C was measured in July in the eastern part of the bay. The vertical temperature gradient was greatest in July and August at depths between 10 and 20 m. The thermocline disappeared as early as September and all layers cooled at roughly the same rate (Bojanić et al., 2012, Bužančić et al., 2012). The salinity in the bay ranges from 28.17 PSU in December and January to 38.19 PSU in August (Buljan and Zore-Armanda, 1971). Average monthly salinity values fluctuated between 37.0 PSU and 37.5 PSU for most of the year. The largest fluctuations were observed in the surface layer, with a particularly strong gradient from the eastern to the central part of the bay, which is directly influenced by the Jadro River.

In the central Adriatic, thermohaline changes in the water column are influenced by atmospheric disturbances and advective oceanographic processes. During a series of very to extremely warm years (such as 2022, 2023) with significant heat waves and a lack of precipitation during the summer, the vertical structure of temperature and salinity reflected the atmospheric stress, especially in the surface layer down to the depth of the thermocline (Grbec et al., 2023). Salinity showed very high values, which is a continuation of the increase in salinity that started in 2015 on the eastern coast of the Adriatic Sea (Beg Paklar et al., 2020, Mihanović et al., 2021). These high values of surface salinity (above 39.3) are attributed to increased evaporation in a very dry and extremely warm atmosphere, as well as to the specific dynamics of the Adriatic Sea and the exchange of water masses between the Adriatic and the eastern Mediterranean.

An upward sea surface temperature (SST) trend is observed along the eastern Adriatic coast after 1979. SST increased by an average of 1.03°C during the period from 1979 to 2015 (Šolić et al, 2018).

This is in accordance with the sea surface warming trend observed in other Mediterranean areas (Shaltout and Omstedt, 2014). In the recent period, starting from 2008, a strong upward almost linear trend of 0.013°C per month was noted and SST increased by 1.25°C (Šolić et al, 2018).

Until the modern wastewater treatment unit was completed in 2005, agricultural waste and polluted wastewater from the numerous industrial plants along the coast were discharged into the Vranjic Basin, as were large quantities of organic matter and nutrients from the discharge of untreated wastewater. The last part of the purification system, the Kaštela – Trogir sewage system, was put into operation in 2014, creating the conditions for the elimination of one of the most important conditions for the existing marine pollution in the bay. Anthropogenic influences in Kaštela Bay have led to an increase in phosphorus and nitrogen, resulting in the formation of a trophic gradient from the open sea to the coastal area. The highest levels of orthosilicate and nitrate were measured in the eastern part of the bay, which is under the direct influence of the Jadro River. The highest value of orthosilicates (12.32 mmol m-3) was measured at the surface in December, and the highest nitrate value was measured in August at a depth of 5 m (TIN 24.90 mmol m-3). These results confirm the influence of the freshwater inflow from the Jadro River. The orthophosphate concentration reached the maximum value of 1.5 mmol m-3 in this area, as it is closest to the land and is under the direct influence of municipal wastewater. Oxygen saturation in the Kaštela Bay area was between 84% and 133% (Bužančić et al., 2016). Trophic status of the investigated area, as a result of long term monitoring activities, is presented in more details in the State of the marine environment, aquaculture and fisheries indicators database (https://vrtlac.izor.hr/ords/bazapokpub/bindex).

In the Kaštela Bay area, the phytoplankton biomass fluctuated between 0.01 mg m-3 and 2.79 mg m-3, and the Chlorophyll a maximum was recorded in the eastern part of the bay in September (Bužančić, 2016). The seasonal distribution of Chl *a* in this area followed the distribution of nutrients with a considerable increase in autumn and winter. Higher values at the surface compared to the bottom and the vertical distribution of biomass during the summer indicate an inflow of nutrients into the surface layer due to the anthropogenic influence in the bay (Bužančić et al., 2016).

The eastern coast of the Adriatic represents a submerged karst relief, and in areas with a larger supply of material from the mainland, more intensive sedimentation takes place. Kaštela Bay has different bathymetric and morphometric features, so that two separate parts of the bay can be clearly distinguished: the deeper central part and the shallower eastern part. The seabed is covered by different sediment types, which differ in their mechanical composition and follow the bathymetric distribution. These different sediment types are mainly distributed by regular granulometric selection (Alfirević, 1980, Crmarić et al., 1998). Both terrigenous and biogenic components are involved in sediment formation. Terrigenous elements are mainly transported from the adjacent Eocene strata and partly from the submarine springs. Biogenic components include the mass fraction of foraminifera shells as well as special types of “bruchschill” and “schill” sediments which originate from the fragments or whole shells of various organisms deposited on the sea floor. The submarine springs in the bay are among the most productive in the Adriatic.

4.2.3. Brief description of the main characteristics of biological communities

Taxonomic composition and population dynamics of various biological communities in the Kaštela bay and adjacent area are regularly monitored within the Croatian Reference centre for the sea, according to Water Framework Directive (WFD-2000/60/EC) and the Marine Strategy Framework Directive (MSFD-2008/56/EC). In this area, there is a tradition of monitoring plankton and benthic communities and fish since the very foundation of the IOR in 1930.
Based on the latest data on the phytoplankton community, we can say that the abundance ranges of phytoplankton organisms in the coastal areas of the central Adriatic are consistent with the values previously recorded for the area mentioned. The recorded proportions of the most numerous groups (diatoms, dinoflagellates, coccolithophores and nanoflagellates) also correspond to the environmental conditions of the studied areas. Relatively high biodiversity indices and their even distribution indicate a diverse community and the absence of blooms or dominant species. All this indicates that the phytoplankton community is not under the harmful influence of anthropogenic impacts that could affect the numbers, proportion of each group in the population or biodiversity. No particularly sensitive phytoplankton species were identified that play a key role in the functioning of the community and whose absence would affect the functioning of the system (Skejić et al., 2023).
The community composition and abundance of micro- and mesozooplankton organisms in the bay do not differ from those observed in previous multi-year surveys. Fluctuations in abundance can be seen as a consequence of the natural dynamics of the populations of zooplankton groups. For most of the observed zooplankton parameters, a clear gradient towards the coast - open sea can be seen, and the eastern part of the bay is characterised by higher abundances and lower species diversity. Based on the biodiversity indicators used, no significant change in species diversity was observed, and the variability of the diversity index and evenness is consistent with the characteristics of the habitat types and their trophic state. The absence of important zooplankton species, which would have a negative impact on the functioning of the ecosystem, was not observed. The numerical ratio between juvenile and adult copepods indicates an unhindered reproduction of copepods and the expected dominance of juveniles in the community. The meso fraction is dominated by holoplankton groups (Skejić et al., 2023).

4.2.4. Main anthropogenic pressures

The Kaštela Bay area is exposed to anthropogenic pollution, e.g. from municipal sewage (organic substances, nutrient salts, faecal and pathogenic organisms), industrial effluents (organic substances, heavy metals and other toxic and persistent substances), rivers and underwater sources, leaching from agricultural land and atmospheric deposition. The main sources of marine pollution are maritime traffic (pollution from ballast water, oil and oil by-products), nautical tourism, mariculture and fishing, which affect the quality of the biological elements of marine ecosystems. The sudden development of tourism, urbanisation and the degradation of coastal areas are affecting flora and fauna and leading to permanent devastation of the coastal area.

The area of Kaštela town covers 482.98 km2, with a population density of 610.32 persons km-2, the main centre of which is the town of Split. Within this area are the towns of Trogir, Kaštela, Solin and Omiš. The agglomeration is economically oriented towards a variety of different activities, the most important of which are industry, construction, transportation, tourism, trade and crafts. In addition, Split is an important university, banking, health and sports centre of regional and macro-regional importance. There are five ports in the area that are open to public traffic and are classified as Category III ports of local importance. In terms of cargo traffic, the Bay of Kaštela is divided into three port areas: (I) Pool A - complex ex Adriavinil, (II) Pool B - coast of TC "St. Juraj I"; coast TC "St. Juraj II", liquefied gases and (III) Pool C - coast TC "St. Kajo"; coast of Brižina; INA tanker terminal and Mala obala Solin. All ports are under the jurisdiction of the Split-Dalmatia County Port Authority. Detailed information can be found in the document Strategy for the Development of the City of Kaštela 2016-2020 by Lučić et al., 2016.

In the last decades of the last century, the former PVC factory polluted the environment with large quantities of elemental mercury during the production of NaOH and chlorine. For decades, mercury-containing wastewater was discharged into the sea in front of the factory, traces of which can still be found in the marine sediments. After the plant was shut down, the mercury concentration decreased significantly (Mikac et al., 2006). According to these authors, the sediments of Kaštela Bay are contaminated with uranium and its decay series of radionuclides in addition to mercury. Another cause of coastal pollution is organochlorine pesticides and, even more frequently, polychlorinated biphenyls (PCBs), which have toxic effects on birds, fish and mammals (Ngoubeyou et al., 2022).

Recently, more and more attention has been drawn to solid waste and microplastics in the sea, which pose a significant risk to marine organisms and ultimately to human health due to their long retention time in the environment. As Kaštela Bay is a fishing area protected by the Marine Fisheries Act, pollution leads to quantitative and qualitative changes, especially in the ichthyobiotopes on the seabed. The flora and fauna communities of the seabed include a large number of species that have low tolerance to organic and inorganic pollution, and the effects of pollution have a negative impact on the number and structure of these communities. This is compounded by extensive and inadequate development which, in addition to visual pollution, leads to a decline in biodiversity and loss of habitat. Important guidelines for conservation and management are set out in the Protocol on Integrated Coastal Zone Management in the Mediterranean on the PAP/RAC website http://www.pap-thecoastcentre.org

According to the indicators of the state of the marine environment, mariculture and fisheries (Kušpilić et al., 2022), the ecological status at the station in the central part of Kaštela Bay is classified as very good, which is increasingly common over the multi-year study period and indicates an acceptable level of anthropogenic pressure on this area. The ecological status of the station in the Vranjic Basin (eastern part of Kaštela Bay, semi-enclosed area of the mouth of the Jadro River) was very good in 2022, with a visible maximum in the "good status" category, which is again consistent with the previous results of monitoring this parameter. The very good status of this station is generally a significant improvement compared to the period of the 1990s, when the ecological status in this area was at times very bad. Further information can be found under the link https://vrtlac.izor.hr/ords/bazapokpub/bindex

4.2.5. Possible vulnerability of the area to invasive species

Relatively shallow, semi-enclosed coastal areas, especially near urban settlements, are particularly sensitive to changes in environmental factors. In addition to natural changes in the ecological conditions in the water column, that occur seasonally or over the long term, various man-made influences make the marine environment even more sensitive and therefore more vulnerable.

For most of the introduced species, the vector of introduction could be related to climate change, spatial expansion and changes in sea circulation that cause Aegean and Levantine waters to flow into the Adriatic Sea. In addition to climate change, other important vectors such as mariculture, balast water and ballast sediment have also play a significant role in introducing NIS. Unintentional transport on a towed oil platform, the phenomenon of Lessespisan migration, aquarium related introductions, fouling and the anchoring of ships are alongside other possibilities for the introduction of non-native species into an area (Zenetos et al., 2012; Slišković et al., 2021; Glamuzina et al., 2024).

The Kaštela Bay area is regularly monitored under various national programs, with a particular focus on alien species according to the MSFD under descriptor D2. Decision EC 848/2017 defines the primary and secondary criteria as well as the initial methodological standards for the assessment of pressure on the introduction or spread of non-indigenous species. In accordance with the implementation of the Marine Directive in Croatian waters, the number of newly introduced species in the following categories is to be monitored: macroalgae, benthic invertebrates (crabs), fish and phyto- and zooplankton species, as well as the abundance and distribution of already established NIS in the same biological categories.

Climate change, i.e. the rise in sea temperature, is leading to the appearance of new non-indigenous species in the Adriatic, many of which are becoming invasive (Glamuzina et al., 2024). In 2013, 12 fish species were identified as NIS and potentially invasive in the greater Split area. It is assumed that the dwarf flathead (*Elates ransonnettii*) was introduced by shipping activities (Dulčić and Kovačić, 2020). The species is originally distributed from the western Central Pacific to Papua New Guinea and Australia. In 2017, a specimen was caught in the Gulf of Tarano (southern Italy),

which is the first record for the Mediterranean. In March 2010, it was caught in Kaštela Bay at a depth of 15 m with a fish trap on a muddy bottom. This is the first reported observation for the Adriatic Sea. The species is of low commercial importance (Dulčić and Dragičević, 2023).
Three invasive algae species have been detected in the Kaštela Bay area: *Caulerpa cylindracea*, which is mainly spread by sea currents and has now been detected at more than 100 sites in the Adriatic, *Caulerpa taxifolia*, which is introduced by anchors and fishing gear and *Womersleyella setacea*, which occurs throughout the Adriatic and is spread by sea currents. Due to its negative impacts, this macrophyte alga has the potential to become the most invasive non-native macrophyte alga in the Adriatic (Nikolić et al., 2010; IOR, 2016).
Among the alien invasive invertebrate species, the blue crab *Callinectes sapidus* has spread very rapidly and successfully, probably through maritime activities (Dulčić and Dragičević, 2023). Originally from the western Atlantic, the species has spread throughout the eastern Adriatic since it was first recorded in the Venice lagoon in 1949 and is particularly common in the Neretva estuary (Croatian coast). It has also colonised the Albanian coast and the entire west coast of the Adriatic. This species can be harmful to local fisheries and ecosystems as it competes with and displaces native species, alters food webs and modifying habitats, but is a valuable resource for the fishing industry.
In addition, two NIS, scleractinian coral *Oculina patagonica* was recorded in fouling community on concrete vertical wall (Cvitković et al., 2013), and warm water gastropod *Siphonaria pectinate* and possible vector of introduction is shipping.
Ostreopsis species are usually found in tropical waters, but nowadays they are distributed worldwide, including the Mediterranean (Ninčević Gladan, 2019). Dinoflagellates of the genus Ostreopsis are benthic species that live mainly as epiphytes on macroalgae and in the Adriatic Sea mainly on brown algae of the genus *Cystoseira*. Recently they have been intensively studied because they can produce the very strong palytoxin. Blooms of this species have been found near Genoa (Italy) and Rovinj (Croatia, northern Adriatic). *Ostreopsis* blooms are often associated with respiratory symptoms and skin irritation in humans exposed to marine aerosol containing algal toxins and/or cellular debris and seawater (Berdalet et al., 2022; Paradis et al., 2024). The species *Ostreopsis* cf. *ovata* was found in Kaštela Bay in the early 1980s, but no bloom of this species was recorded until September 2015 (Ninčević Gladan et al., 2019).

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5. Supplementary Material for the description of the pilot area of Apulia region

5.1. Introduction

This report provides a description of the main hydrological and physico-chemical features of the Southern Adriatic Sea. It consists of a supplementary analysis conducted by CMCC to support the description of the pilot areas presented in the Southern Adriatic Sea, with particular reference to the Puglia region.
The three-dimensional thermoaline circulation in the Southern Adriatic Sea (SAd) consists of a main overturning cell located between the Southern Adriatic Sea and the Northern Ionian Sea and driven by the SAd sink of dense water (Figure 1).

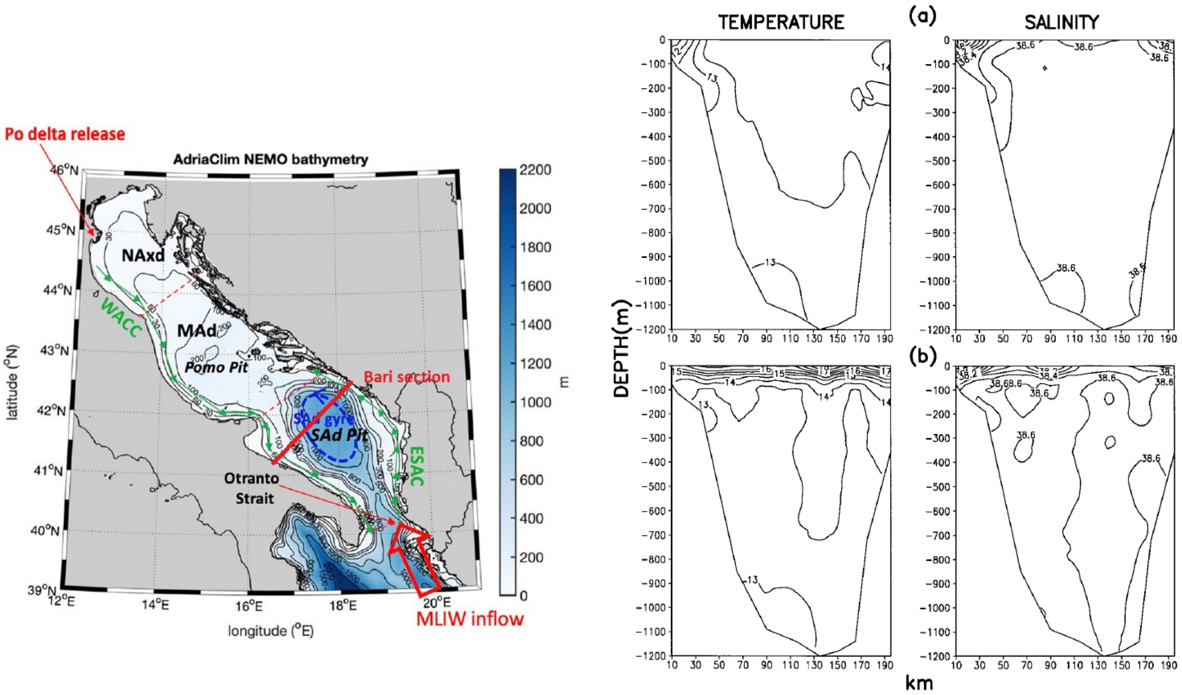


Figure 2: In the left panel, the Adriatic Sea basins; in the right the Bari vertical section

In particular, as observed in the Bari vertical section of Figure 1, representative of the central southern Adriatic sea, obtained from the analysis of a comprehensive historical hydrographic dataset of the last century (Artegiani et al., 1997), the characteristic winter conditions are defined by low temperature and low salinity coastal waters on the western shelf area of the Adriatic, with T <

13 °C and S < 38.6 psu in the deepest part of the section, confirming that the southern basin is an area of dense water formation. In Spring the dense water masses are still
evident at the bottom while the surface waters have decreased their salinity in the first 50 m, indicating that the river runoff waters affect also the southern Adriatic surface waters.

5.2. The Mediterranean forecasting system

In order to provide the seasonal and annual variations of the main hydrological and physico-chemical features of the Southern Adriatic Sea, the Mediterranean Forecasting System was used to assess the distribution of sea surface currents, sea surface temperature and salinity for the years 2020-2023.
The Mediterranean Forecasting System, also called MedFS, is run operationally at CMCC in the framework of the Copernicus Marine Service (Figure 2).
It is composed of a 2-way coupled ocean-wave modeling system based on NEMO and WaveWatch3 at a resolution of around 4 km and includes ocean measurements from satellite altimeters (Sea Level Anomaly).
In situ temperature and salinity vertical profiles are assimilated though the OceanVar (3DVAR) assimilation model.
The model is forced with high resolution ECMWF (European Centre for Medium-Range Weather Forecasts) atmospheric fields and is nested in the Atlantic Ocean through the Copernicus global ocean analyses and forecasting system.
The variables produced are: Temperature, Salinity, Currents, Sea Level and Mixed Layer Depth.

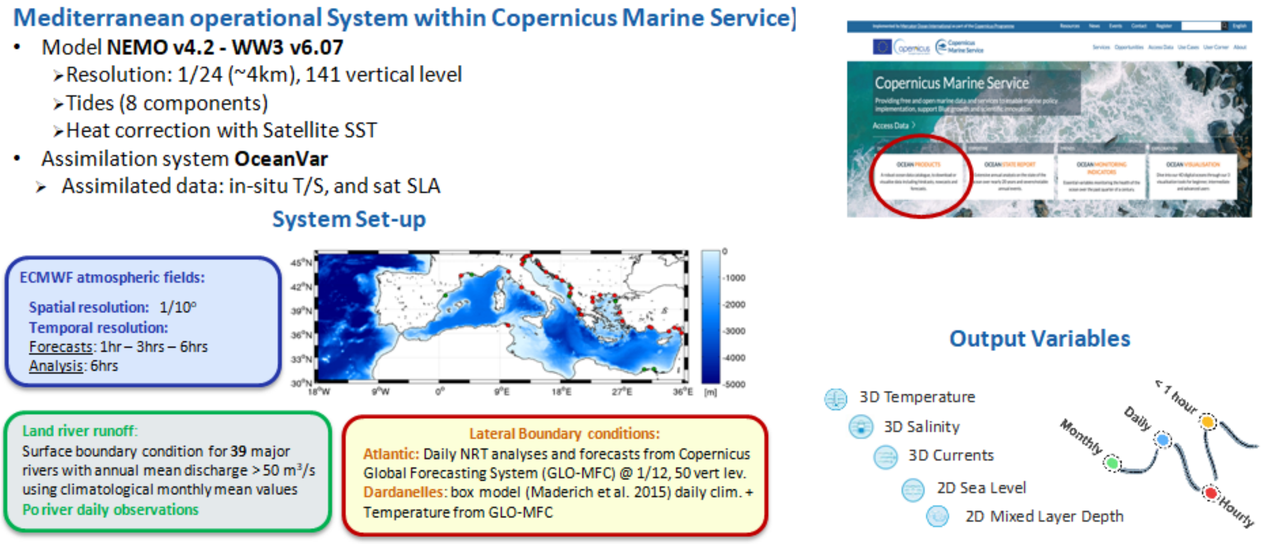


Figure 2: Scheme of the Mediterranean Forecasting System (MedFS)

5.2.1. Mean sea surface currents

The general circulation in the Southern Adriatic Sea (SAd) consists of a main overturning cell, the Southern Adriatic Gyre, located between the Southern Adriatic Sea and the Northern Ionian Sea and driven by the SAd sink of dense water (Figure 3).
The Southern Adriatic Gyre, a well-known cyclonic circulation feature, is present throughout the year, though its intensity and position vary by season. Additionally, the figure highlights the Western South Adriatic Current (WSAC), a southward flow along the Apulian coast, and the Eastern South Adriatic Current (ESAC), a northward flow along the Balkan coast.

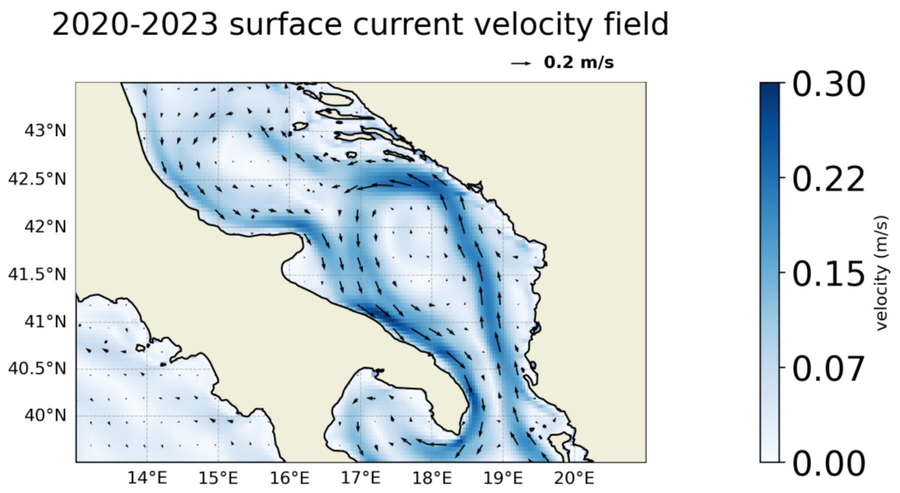


Figure 3: Mean sea surface currents in the period 2020-2023

These key circulation features exhibit distinct seasonal characteristics.
The plots reported in Figure 4 show the seasonal variations of sea surface currents (m/s) at the Southern Adriatic Sea for the years 2020-2023:

- in spring, the strength of the gyre is weak, and the WSAC is more confined along the Apulian coasts. The ESAC is still present but appears less intense than in winter;
- during the summer the WSAC intensifies, while the ESAC continue in weaking;
- in autumn, the currents intensify. The Southern Adriatic Gyre becomes more prominent;
- during winter, the Southern Adriatic Gyre exhibits asymmetry with reduced WSAC and stronger ESAC flow.

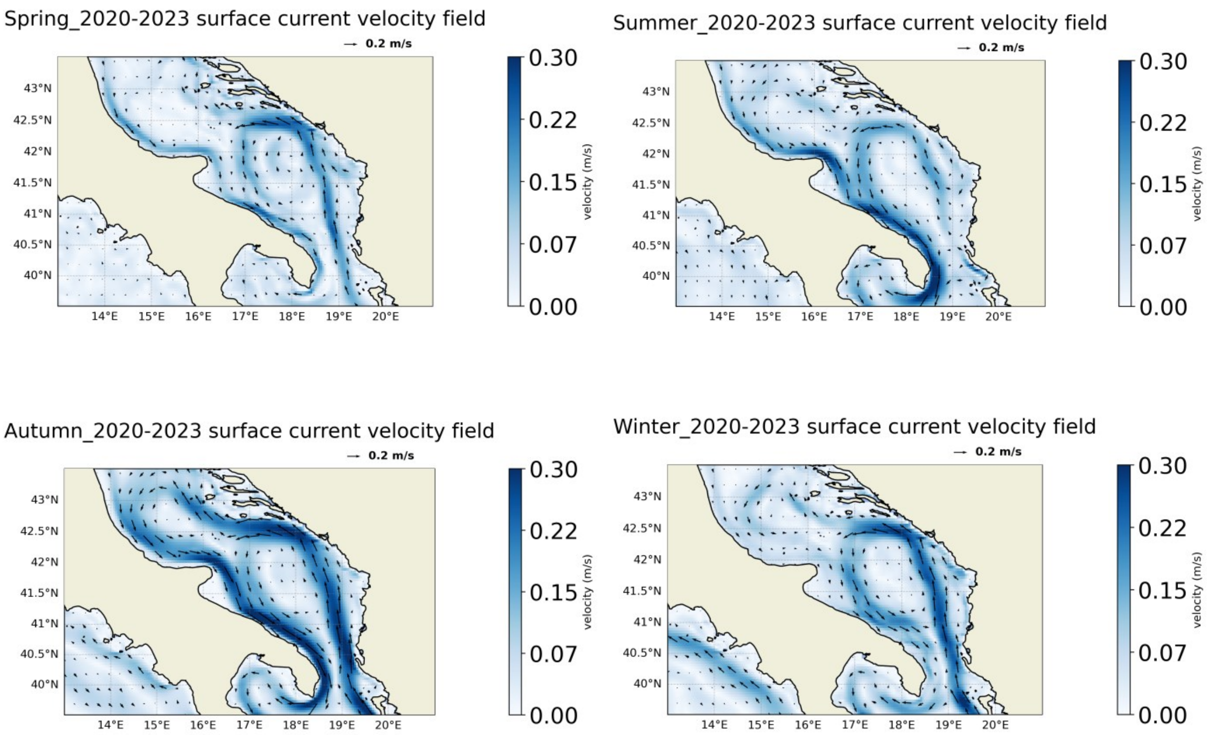


Figure 4: Mean sea surface currents in the period 2020-2023

Figure 5 illustrates seasonal variations of sea surface currents (m/s) at the Southern Adriatic Sea for the year 2018-2019 from very high-resolution coastal model system. As observed in the previous analysis, the figure highlights the Western South Adriatic Current (WSAC), a southward flow along the Apulian coast, and the Eastern South Adriatic Current (ESAC), a northward flow along the Balkan coast. These key circulation features exhibit distinct seasonal characteristics:

- Winter (DJF): During winter, the Southern Adriatic Gyre exhibits asymmetry with reduced WSAC and stronger ESAC flow.
- Spring (MAM): In spring, the strength of the gyre slightly decrease, and the WSAC is more confined along the Apulian coasts. The current system shows a more relaxed structure as the gyre shifts slightly southeastward. The ESAC maintains its presence but appears less intense than in winter.
- Summer (JJA): During the summer the WSAC intensifies, while the ESAC continue in weaking.
- Autumn (SON): In autumn, the currents begin to intensify again. The Southern Adriatic Gyre becomes more prominent, resembling its winter structure, and the WSAC strengthens along the Apulian coast. The ESAC shows increased flow as well, signaling the transition the most energetic circulation regime.

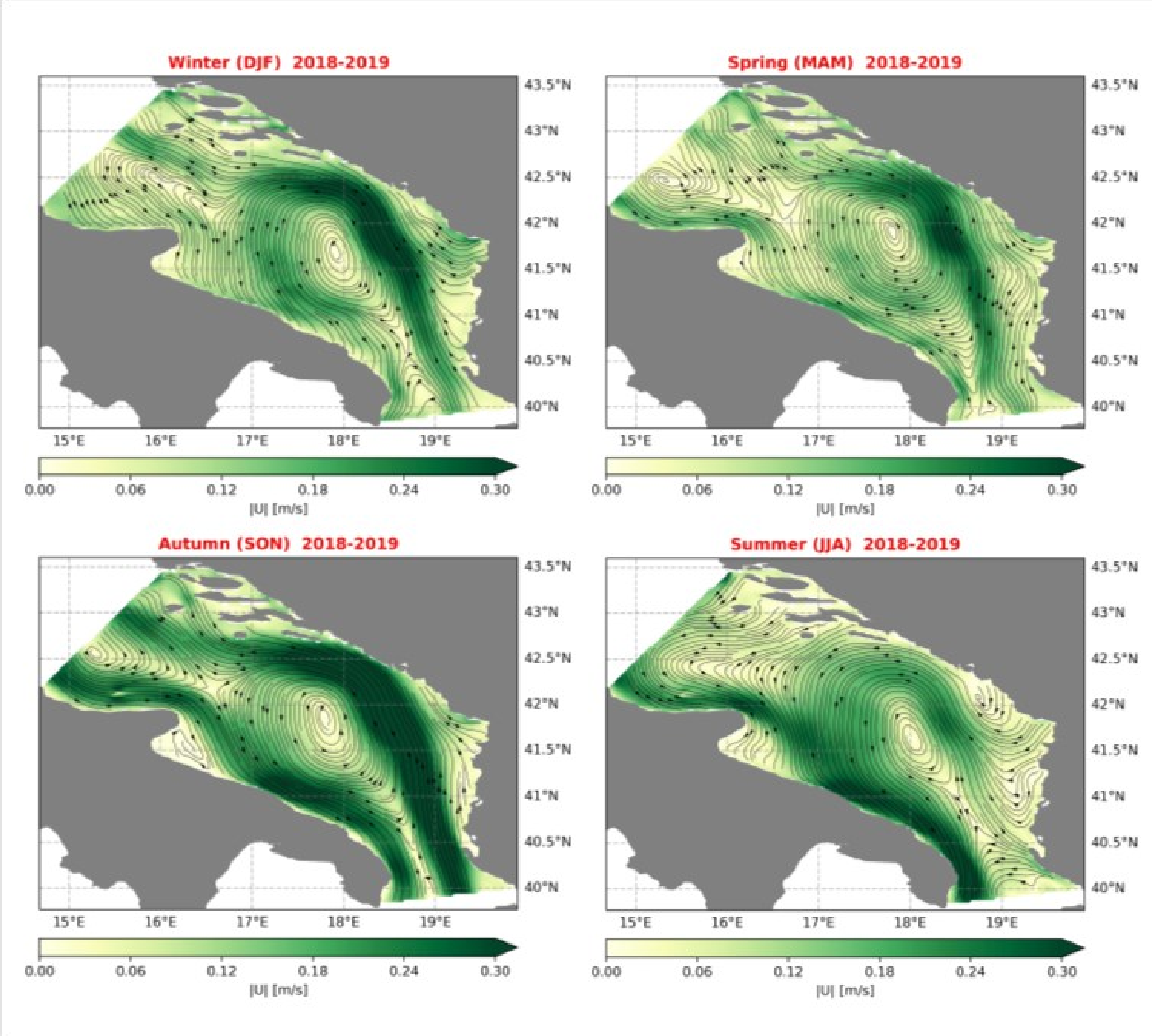


Figure 5: Seasonal-average basin-scale surface circulation in the Southern Adriatic Sea from very high-resolution coastal model system

5.2.2. Mean Sea Surface Temperature

To analyse the seasonal mean and the annual mean of surface temperature in the Southern Adriatic Sea the Mediterranean Forecasting System (MedFS) was run for the period 2020-2023.
As observed in figure 6, mean sea surface temperature values during the period 2020-2023 vary between 19 and 20°C.

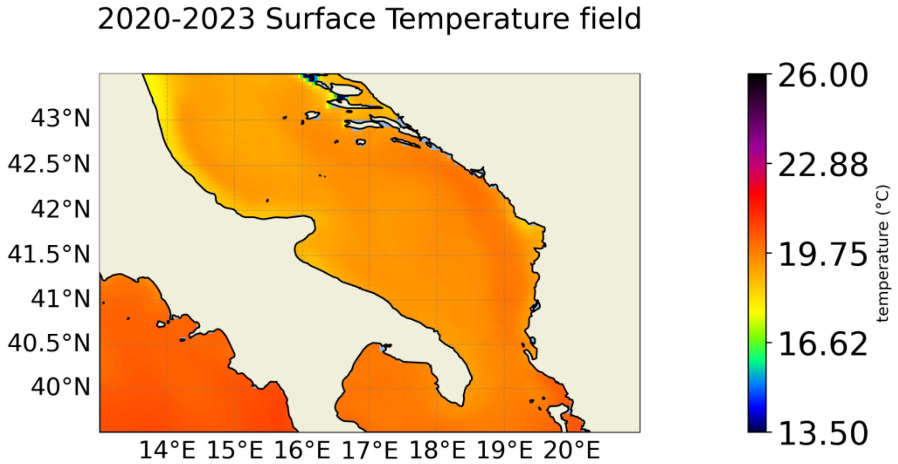


Figure 6: Mean Sea Surface Temperature 2020-2023

Sea surface temperature is dominated by a strong seasonal variability and influenced by surface atmospheric variability and circulation. It varies from around 13 °C minimum during winter and more than 26 °C during summer period (Figure 7).

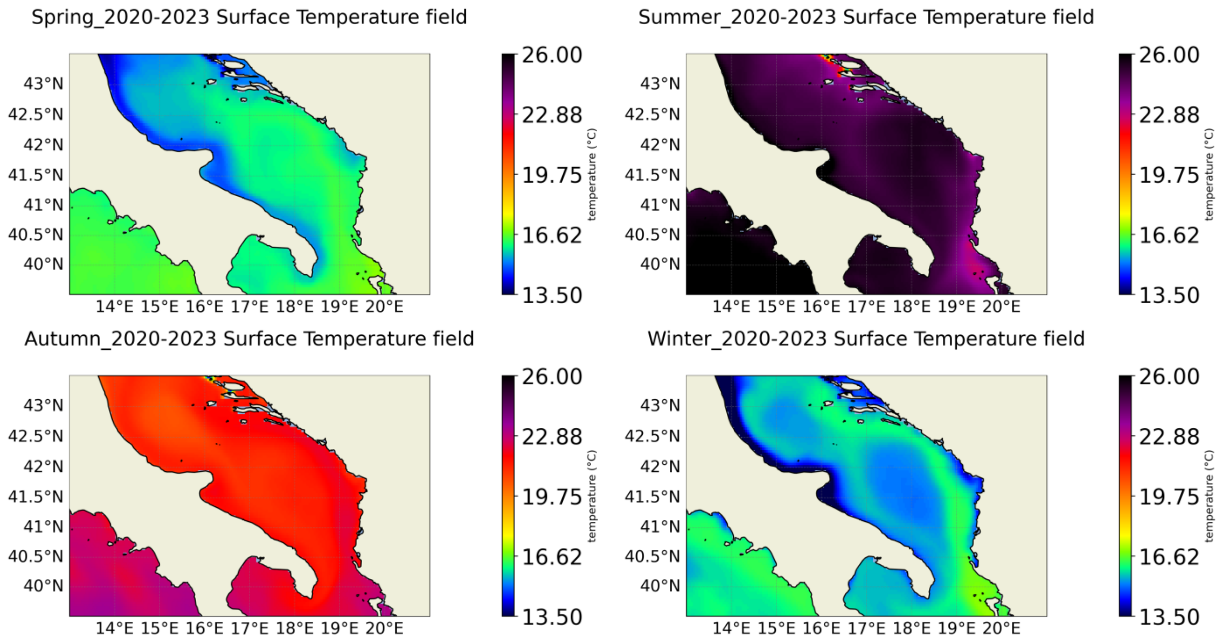


Figure 7: Seasonal Sea Surface Temperature variations in the period 2020-2023

5.2.3. Mean Sea Surface Salinity

Sea surface salinity values are strongly influenced by rivers, by surface atmospheric variability and circulation. During the period 2020-2023 salinity values vary between 38 and 39 psu (Figure 8).

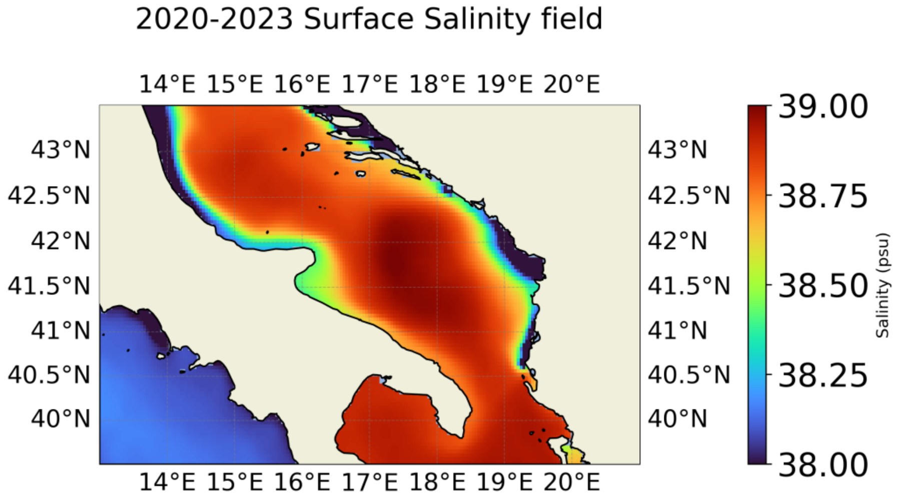
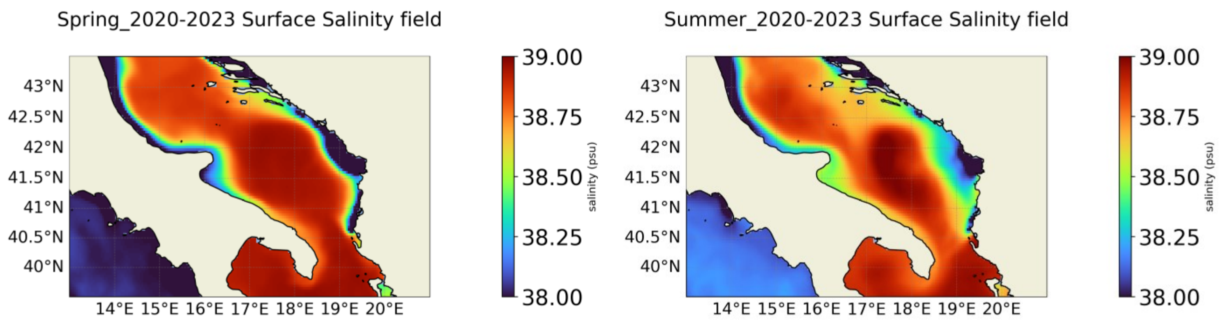


Figure 8: Seasonal Sea Surface Salinity 2020-2023

As observed in figure 9, lower values along the coastal areas are observed, strongly influenced by river inputs.



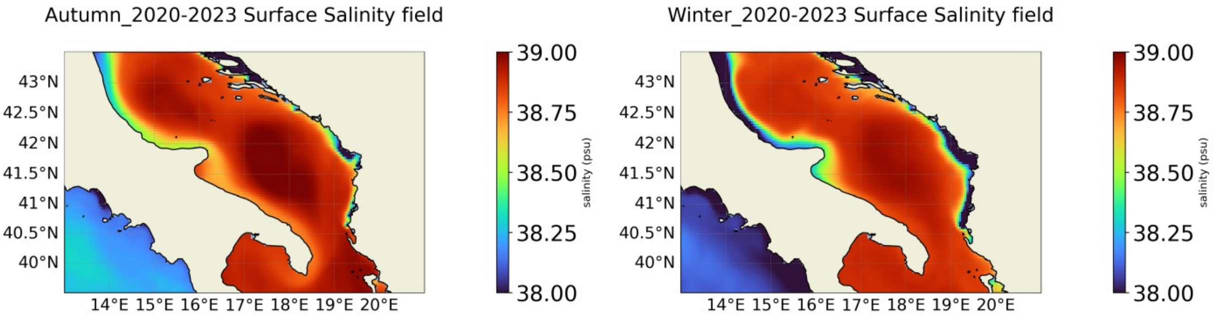


Figure 9: Seasonal Sea Surface Temperature variations in the period 2020-2023

5.3. References

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The Mediterranean Forecasting System: https://medfs.cmcc.it/backend/public/medfs/short-description.html