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

### CASCADE

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

## D5.2.1

# Report on implemented restoration test and soft actions supporting endangered species

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## 1. Introduction

The main objective of **WP5 – Pilots for endangered species restoration and Integrated coastal/marine management system** was to demonstrate the usefulness of monitoring (observing and modelling) and spatial planning techniques by implementing coastal integrated management, restoration of marine ecosystems and support for endangered species and related ecosystems. D5.2.1 reports the outcomes of the **Activity 5.2 “Restoration actions supporting endangered species”**, which focused on implemented restoration tests and soft actions supporting endangered species and threatened ecosystems in CASCADE study areas. Marine habitat restoration is an emerging field to actively reverse the degradation and loss of natural ecosystems (Abelson et al., 2020). It often focuses on enhancing the abundance or distribution of habitat-forming organisms such as salt marshes, kelps, oyster reefs, seagrasses. Here the term *restoration* has been used in its broadest possible meaning (Airoldi et al., 2022), encompassing multiple forms of interventions to (1) facilitate and speed up the partial or full recovery of habitat-forming organisms and of the services they support, (2) reallocate, reinforce or redefine functional and healthy ecosystems, even if they may differ from what it known to have existed prior to human interference, or (3) preventative actions to protect and mitigate rather than compensate for damage to marine systems from predicted future changes. In the report are included the following case studies:

- Development and test of restoration actions for the native cordgrass *Sporobolus maritimus* in saltmarshes of the coastal lagoons of the Emilia Romagna region (**P2**) as well as other lagoons along the Italian side of the North Adriatic sea;
- Enlargement proposal of MPA Torre Guaceto and related issues. The extension of MPA area would increase the area with limitation to human activities thanks to newly proposed NTZ (No take zone) accordingly to the DPSIR model as one of the Responses to the driving forces (**P3**);
- Analysis of the threats and conservation and restoration actions for the Neretva River Delta ecosystems (**P4**);
- Analysis of possible measures to counteract and prevent the degradation of Nin Lagoon, including the Miljašić Jaruga (**P6**);
- Analysis of threats and identification of local restoration actions on coastal wetlands on Molise coast (**P7**);
- Development and test of restoration actions for identified threats to and losses of the endangered canopy-forming seaweed *Fucus virsoides* along the Istrian coast (**P8**);
- Installation of *P. nobilis* larval collectors, in order to restore population of the species, at Cetina Estuary site (**P9**). During 2019 *Pinna nobilis* has been listed as Critically Endangered on the IUCN Red List of Threatened Species due to drastic population

reduction caused by a MME (Mass Mortality Event).

- Analysis of threats and identification of local restoration actions on coastal dunes on Torre del Cerrano Marine Protected area (**P10**).

## 2. Description of the case studies: endangered species and issues addressed by the restoration tests/actions.

### 2.1. P2 *Sporobolus maritimus* vegetation in the North Adriatic sea: distribution and issues addressed by the restoration tests.

Saltmarshes are valuable coastal ecosystem that supports important ecosystem services, such as fishery resources (van Proosdij et al., 2010), nutrient regulation (Koch et al., 2009), coastal protection (Fairchild et al., 2021) and carbon sequestration (Ouyang and Lee, 2014). Unfortunately, salt marshes are in steady historical decline due to direct human activities (e.g., coastal reclamation and pollution) and global change related events (e.g., heatwaves, sea level rise; Gedan et al., 2009). The north-western Adriatic Sea is one of the few regions in Italy still hosting extensive and valuable remaining tracts of intact intertidal cordgrass meadows. The loss of saltmarsh area has been driven in large part by coastal development and land conversion (Airoldi and Beck, 2007), although extreme heatwaves like the particularly severe 2003 event in the Mediterranean have been linked to a shift in dominance from the perennial native grass *Sporobolus maritimus* to mudflats colonised by the annual succulent *Salicornia veneta* (Strain et al. 2017). While direct conversion is now restricted, saltmarshes are faced with other continuing human impacts, including excessive nutrient input from intensive farming, industry and dense urban centres in the watershed and RSLR driven both by global increasing sea level and localised land subsidence (Wong et al., 2015). Pockets of extensive coastal saltmarshes remain, but growth dynamics and basic ecological processes are poorly known, and these habitats are further threatened by the spread of the non-indigenous species *Sporobolus anglicus* (An et al., 2020; Shin et al., 2022) with significant negative consequences for the delivery of crucial ecosystems services (An et al., 2007; Kriwoken and Hedge, 2000; Wang et al., 2006).

*S. anglicus* is the fertile hybrid deriving from the union between *S. alterniflorus* (native of North America; An et al., 2007), and *S. maritimus* (native of Europe; (Neves et al., 2010), which originated in southern Britain at the end of 19th century after the accidental introduction of *S. alterniflorus* in that area (Gray et al., 1991). Since then, *S. anglicus* has been actively or accidentally spread all over the world, from Europe (Proença et al., 2019) to China (Zhi et al., 2007), Australia (Cutajar et al., 2012) and South Korea (Shin et al., 2022) for coastal protection purposes due to its strong capacity to stabilise the sediment (Shin et al., 2022; Wang et al., 2006). *S. anglicus* is a highly productive plant, capable to strongly modify the hydrodynamic conditions (Bouma et al., 2013, 2010, 2009), as well as to change the sediment biogeochemistry (Thompson, 1991). These modifications can negatively impact on soil native invertebrates (Hedge and Kriwoken, 2000), birds (Goss-Custard and Moser, 1988), and native plants (Hacker and Dethier, 2006). Therefore, to effectively manage marsh ecosystems under current global changes and implement successful restoration projects, it is necessary to understand the distribution of native and non-indigenous species (Gao et al.,

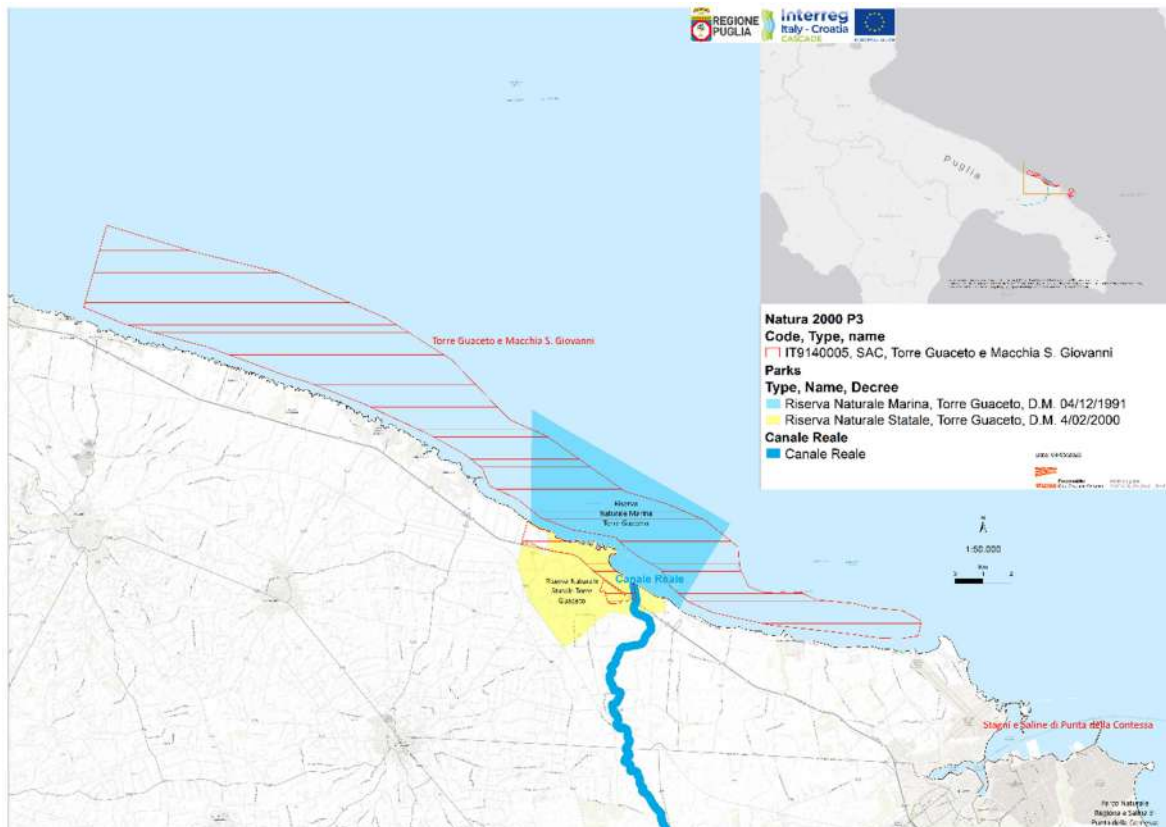
2016; Wan et al., 2009) as well as their ecological niches.

In southern Europe, the native *S. maritimus* can still form large populations (Castillo et al., 2005; Curado et al., 2014), but also here this species is slowly being replaced by *S. anglicus* (e.g., Wong et al., 2018). This ongoing replacement is evident along the north-west (NW) coast of the Adriatic Sea, where both species are present in the same areas (Wong et al., 2018). Lagoons and wetlands situated along the Adriatic Sea play a crucial role in sustaining fisheries and aquaculture, preserving cultural heritage, and safeguarding biodiversity (see D'Alpaos and D'Alpaos, 2021). It is therefore imperative to understand which conditions favour the spread of the non-indigenous species over the native species, and what are the consequences for the functioning of the ecosystem. This information will provide crucial knowledge on how to prevent the total replacement of the native *S. maritimus* by the non-indigenous *S. anglicus*, and for designing effective restoration plans which allow the recovery of the native species.

## **2.2. P3 Enlargement proposal of Torre Guaceto MPA and *maintenance of habitat suitability of the "Saline di Punta della Contessa" transitional water ecosystems for the flamingo bird***

The Pilot area 3 insists on the MPA of Torre Guaceto, on the other hand the MPA overlaps the wider Nature 2000 SAC IT9140005, Torre Guaceto e Macchia S. Giovanni, visible in Figure 1.





**Figure 1.** MPA of Torre Guaceto in comparison to the overlapped Natura 2000 SAC IT9140005 Torre Guaceto e Macchia S. Giovanni.

The Management Consortium of Torre Guaceto on the basis of presence of various habitat of conservation interest proposed an expansion of MPA to all the existing marine SAC to the North-West portion and to even wider portion in the South-East portion external to the existing SAC. The proposed enlargement would reach a marine area of about 9,070 ha, close to four time the existing MPA.

As a matter of fact the 2000 SAC (Special Area of Conservation) IT9140005 is 7978 ha of which 7,659 marine and 319 terrestrial. Considering only the marine area the SAC is about three times bigger the MPA and therefore host a wider proportion of habitat and species.

The proposed enlarged area includes habitat of conservation interest according Habitat Directive such as 1120\* Posidonia beds and 1170 Reefs. The habitat 1120\* Posidonia beds is priority which means “natural habitat types in danger of disappearance”.

On the other hand it was also recorded the presence of alien species *Caulerpa racemosa*, an alien species according to EASIN - European Alien Species Information Network currently present in all the Mediterranean.

The extension of MPA area would increase the area with limitation to human activities thanks to newly proposed NTZ (No take zone).

Conditions for the maintenance of habitat suitability of the "Saline di Punta della Contessa" transitional water ecosystems for the flamingo bird (*Phenicopterus roseus*) have been experimentally addressed.

"Saline di Punta della Contessa" is a Regional Protected Area sited in the coastal area of Brindisi (Apulia Region, Italy) that presents birdlife of particular interest and value. The study area is affected by different anthropogenic disturbances main linked to the agriculture and to the presence of industrial and manufacturing establishes (Pinna et al. 2007; Mastrolia et al. 2017; Tomaselli et al. 2021). Such perturbances threaten the persistence of the species that live or stay in the area. Describe inputs characterising ponds and the relationships between organisms is important to identify the main energy and contaminant pathways as well as, the compartment to focus on and take management action with priority (Calizza et al. 2015; Sporta Caputi et al. 2020a, b).

Reconstructing food webs means tracing all the main flow pathways of nutrients and contaminants from the basal resource to the top consumers, including avifauna of important ecological and social interest (Signa et al. 2019; Shao et al. 2020).

Stable isotope analysis of carbon ( $\delta^{13}C$ ) and nitrogen ( $\delta^{15}N$ ) is a valuable tool for identify both the organic and inorganic matter sources and the relationships between organisms (i.e., food webs). This method presents several advantages since: (i) the isotopic ratio of C and N elements in consumer tissues reflects that of the assimilated food sources in a predictable way, (ii)  $\delta^{13}C$  vary considerably among primary producers, taking on very different values between terrestrial and aquatic vegetation allowing to disentangle the contribution of various basal sources to food webs, (iii)  $\delta^{15}N$  provide information on the position of organisms in the food web and (iii) on the nature and source of nitrogen inputs (organic or inorganic and natural or anthropogenic) assimilated by primary producers allowing to track anthropogenic N pollution in water bodies and across trophic levels in food webs (Careddu et al. 2015; Fiorentino et al. 2017; Sporta Caputi et al. 2020b; Calizza et al. 2020 and literature therein).

In wetlands and, particularly, in transitional waters, waterbird base their diet wholly or at least in part in the living ponds (Fasola et al. 1989; Ji et al. 2021; Tokatli and Islam 2022). Stable isotope analysis of guano collected in the Artificial Pond surrounding area, showed gammarids living as the most important prey within this pond (95.00%  $\pm$ 0.42%) followed by *Ulva lactuca* (5.00%  $\pm$ 1.97%) in the very short-term diet of flamingo birds at "Saline Punta della Contessa". The food web analysis allowed to identify and quantify the main pathways of energy and matter along food chains. The future application of the analysis of contaminants in the basal resource compartments and in the fauna linked to the study

ponds associated with the analysis of the food web will allow to identify and quantify the presence, accumulation and transfer of pollutants along the food chains providing crucial information for the conservation and management of the ecosystem of the Saline di Punta della Contessa.

The "Saline di Punta della Contessa" Regional Natural Park represents an important coastal wetland characterised by the presence of different habitats of the EU directive 92/43 / EEC that present and support, several primary producers and animal species with important value, including birdlife temporarily or permanently stationed in the area. However, the anthropogenic pressures in the surrounding areas affect the ecosystems directly and indirectly threaten the persistence of communities (Petrosillo et al. 2010; Tomaselli et al. 2021). The study results suggest primary producers and sediment as priority compartment on which focus for the management of the ecosystem and the safeguarding of the aquatic biodiversity of the area since they, highly consumed directly and indirectly also by birds, are a source of accumulation, both in the short, medium and long term, of anthropogenic inputs and possible pollutants.

The study suggests the design of an intervention to maintain and improve the ecological condition and habitat suitability for the flamingo birds of the "Saline Punta della Contessa" transitional water ecosystems, through the management of the freshwater and marine inputs. The proposed management would contribute to conserve and improve the ecological status of the "Saline Punta della Contessa", as 1150 priority ecosystem type in the Natura 2000 directive, and the occurrence and abundance of the *Phenicopterus roseus* population, as species included in the Annex 1 of the Bird Directive and requiring special conservation measure even though the *Phenicopterus roseus* populations are showing trends of recovery.

### **2.3. P4 Natural values of the Neretva River delta**

The following description of Neretva River delta area is necessary to understand the functioning and the state of the pilot site.

With the aim of developing restoration activities for the establishment of a favourable state of biodiversity features in the pilot area, the first step was to define the biodiversity features that are described in the following text. The survival of the site's key species and habitats depends on environmental factors that reflect human impact.

In the Neretva River Delta, there are protected areas of nature according to the Croatian Nature Protection Act:

- ornithological special reserves: Prud, Pod Gredom, Orepak, Modro oko and Lake Desne
- ornithological-ichthyological special reserve Neretva River mouth
- significant landscape Predolac-Šibenica.

The Ramsar Convention on Wetlands (1971) designated the Neretva River Delta as an internationally important wetland. It has the most extensive complex of wetlands in the Croatian littoral, with well-developed water-fringe vegetation (the largest reedbeds in the country, sedge communities, and rushes), as well as floating and submerged vegetation around Neretva and its tributaries.

Also, it is a Natura 2000 site: HR100031 Delta Neretva (SPA), HR5000031 (SCI).

The Neretva Delta is the most valuable wetland on the eastern Adriatic coast and one of only a few wetlands remaining in the Mediterranean region of Europe. The mouth of the river Neretva is characterized by wide lagoons, sandflats, and saltmarshes. Though a large area of the wetland habitat has been transformed into agricultural lands, due to the branching network of channels, these areas are still important habitats for aquatic birds and a very important ichthyological area. Reclaimed land is covered by an agricultural landscape with many irrigation channels. The Neretva Delta has many lagoons, shallow sandy bays, low sandy shores, sand flats, salt beaches, etc. The delta, lagoons, and brackish waters are exceptionally important habitats because they create room for the intensive growth of fry, which later spend their life cycle in the sea or fresh water. Furthermore, these areas are important for the migration of anadromous and catadromous fish species. Almost 200 regularly occurring bird species use the Neretva Delta for breeding, migration, and wintering.

With a large number of endemic species and great diversity, the mouth of the Neretva River is one of the most interesting areas of Croatia.

The delta is surrounded by karst hills rich with underground water that supplies numerous springs, streams, and lakes. More than 80 registered caves and other underground habitats in these karst surroundings are home to rich fauna, including many threatened and endemic taxa.

The Ramsar site is the SPA Neretva Delta, which has at least 313 registered bird species. Altogether, there are around 193 regularly occurring species, out of which around 89 are breeding birds (Delta Neretve, 2022). The area is an important stopover point for birds migrating from Middle and Northeast Europe to Africa, as it is located on the Central European (Black Sea/Mediterranean) Flyway. Wintering birds, which are accompanied by residents during the winter, account for roughly one-third of all registered species.

The Neretva Delta is part of the wider transboundary wetland with the Hutovo Blato Ramsar site in Bosnia and Herzegovina. The same birds use both sites during migration, wintering, and even breeding. Some species breed in Hutovo Blato and feed in the Neretva Delta, like *Phalacrocorax pygmeus* and *Plegadis fascinellus*.

The Neretva Delta is home to more than 1% of the *Phalacrocorax pygmaeus* population in SE Europe and Turkey.

During the breeding season, *Plegadis fascinellus*, which breeds in Hutovo Blato in B&H (Ili, HOD, pers. comm.), regularly feeds in the Croatian part of the Neretva Delta, depending on

the phase of the breeding.

More than 10,000 waterbirds regularly winter in the Neretva Delta (Ili, HOD, pers. comm.), including several thousand ducks, up to 3,000 coots (*Fulica atra*), up to 2,000 individuals of *Larus ridibundus*, up to 2,000 individuals of *Larus michahellis*, and about 400 individuals of *Phalacrocorax carbo*, among others. The most common are *Anas platyrhynchos* and *Fulica atra*, but their numbers differ significantly from year to year, depending on weather conditions. During very cold winters, large numbers of geese stay in the estuary, mostly *Anser albifrons* and *Anser anser*. If one adds the wintering waterbirds of Hutovo Blato, which have bigger numbers because of large open-water habitats, the whole Lower Neretva area (the transboundary Ramsar site) probably reaches the criterion of 20,000 waterbirds.

As the Neretva Delta is situated on the Central European (Black Sea/Mediterranean) Flyway, this area is also important for the migration of *Grus grus*. During February and March, flocks of cranes are flying over the delta, and up to several hundred birds per day have been registered (Ili, HOD, pers. comm.). Although monitoring started only in 2011, data indicate that probably around 3,000 cranes migrate over the Neretva Delta (the threshold for the "bottleneck" of European importance according to the BirdLife criteria). The river mouth, with its shoals, sandbanks, and saltmarshes, is of greatest importance for the migration of waders, representing one of the two most important coastal sites for waders, along with the SW Dalmatia and Pag. Bird monitoring at the river mouth during the last several years indicates that the Neretva Delta probably satisfies the 1% level for the Central and SE populations of *Platalea leucorodia*. The river mouth represents one of only two breeding sites of *Charadrius alexandrinus* in Croatia and one of only two coastal breeding sites of *Himantopus himantopus*, the other being the SPA SW Dalmatia and Pag.

Reedbeds in the Neretva Delta are especially important for breeding *Botaurus stellaris* (50% of the national population), *Porzana pusilla* (83% of the national population), *Porzana parva* (25% of the national population), and *Porzana porzana* (17% of the national population). It also holds 12.5% of the Croatian population of *Ixobrychus minutus* as well as 17.5% of *Circus aeruginosus*.

The breeding of *Ardea purpurea* in the Neretva Delta was confirmed for the first time in 2013 when a colony of 25–30 p. was found in reedbeds (Bariša Ilić, HOD, pers. comm.). The Neretva Delta is the only breeding site for *Aythya nyroca* in the Mediterranean region of Croatia. The breeding of *Acrocephalus melanopogon* was registered for the first time in the Neretva Delta in 2011. Along with the SPA Cetina River, this is the only breeding site in the Mediterranean region of Croatia for this species. The reedbeds of the Neretva Delta represent the only breeding site in the Mediterranean region of Croatia for *Panurus biarmicus* and for *Acrocephalus schoenobenus*. They are also important for migrating and wintering birds, especially for wintering populations of *Acrocephalus melanopogon*, *Porzana parva*, *Porzana porzana*, *Porzana pusilla*, and *Rallus aquaticus*.

This area contains a high diversity of water habitats, including the delta, lagoons, brackish

waters, a network of channels, springs, streams with rheophilic characteristics, and lakes that are inhabited by almost 20 fish species endemic to the Adriatic basin and is one of two important sites for the endemic species *Squalius svallize*. Freshwater habitats with rheophilic characteristics are important for *Salmothymus (Salmo obtusirostris)*. Freshwater habitats with rheophilic characteristics and oligotrophic lakes, such as Lake Modro Oko, are important for *Salmo marmoratus*, which accounts for up to 60% of the total Croatian population. The brackish habitats of the site are important for *Pomatoschistus canestrinii* and *Knipowitschia panizzae*. It's one of two sites important for reproducing *Petromyzon marinus*, one of two sites important for *Lampetra zanandraei*, an endemic lamprey, as well as one of three sites important for *Knipowitschia croatica*. Other than that its the only important site for the endemic species *Chondrostoma knerii* is in Croatia, where 100% of the population lives. *Alosa fallax's* only significant site for reproduction. The only important site for the endemic species *Alburnus neretvae* (syn. *Alburnus albidus*) with a 100% Croatian population *Cobitis narentana* (syn. *C. taenia*) is the only endemic species with a 100% Croatian population.

Baćina Lakes are important for *Cobitis illyrica* (syn. *C. taenia*), and *Delminichthys adspersus* inhabits them as well. Delta Neretva is an important site for herpetofauna species *Elaphe quatuorlineata*, *Zamenis situla*, *Emys orbicularis*, *Mauremys rivulata* and *Testudo hermannii*. It is also the southernmost site of distribution for *Lutra lutra*. The site is important for 8310 caves that are not open to the public; the area is important for *Congeria kusceri*, the only living underground bivalve in the world; the species is found in 7 localities in the Delta Neretva site: two colonies (Jama u Predolcu hosting more than 72 000 individuals and Pukotina u tunelu polje Jezero - Peračko blato); one locality where only individual live specimens were found.

This is one of two sites important for the conservation of *Coenagrion ornatum* in the Mediterranean Biogeographical Region. Because of the large population (ca. 40% of the national population), the site is of great importance for the conservation of *Lindenia tetraphylla* in Croatia.

The largest *Miniopterus schreibersii* hibernation colony in the Mediterranean biogeographical region of Croatia is in Delta Neretve. as well as one of the 34 underground sites with 10,000 or more bats recorded in Europe. Internationally significant underground site for *Rhinolophus ferrumequinum* (nursery, migration), *Myotis emarginatus* (hibernation), and the southernmost known *Myotis capaccinii* nursery. The nursery and migration of *Rhinolophus hipposideros* rely on this location. *Rhinolophus euryale* also has a summer roost.

Important site for Mediterranean salt meadows (*Juncetalia maritimi*), as well as Mediterranean and thermo-Atlantic halophile scrubs (*Sarcocornetea fruticosi*) and *Salicornia* and other annuals colonizing mud and sand; these two habitat types occur together on the site, with *Salicornia* represented in a much smaller area. The area is considered to support a significant presence of coastal lagoons. It is an important site for the 3130 habitat type, with

some plant communities found nowhere else in Croatia. important site for habitat types 62A0 and 9320. Croatia has one of the best areas in the country for 92D0 southern riparian galleries and thickets (*Nerio-Tamaricetea* and *Securinegion tinctoriae*). It is one of the most representative sites for estuaries and an important site for mudflats and sandflats not covered by seawater at low tide.

According to the IUCN Red List of Threatened Species (2012), the Neretva Delta and its surrounding area support a significant number of globally threatened species in categories CR, EN, VU, and NT\*. They include: 1 VU and 2 NT mammals; 5 NT birds; 2 NT reptiles; 1 VU amphibian; 2 CR, 1 EN, and 5 VU fishes; and 1 VU mollusk. The Neretva Delta is also an important site for a number of species of European concern that are protected by the Birds Directive and the Habitats Directive, as well as by the Convention on the Conservation of European Wildlife and Natural Habitats. European Red List species include: 3 NT vascular plants; 1 VU and 4 NT mammals; 2 NT reptiles; 1 VU amphibian; 2 CR, 1 EN, and 5 VU fishes; 1 VU dragonfly; and 1 VU mollusk. Regarding regularly occurring ornithofauna, the Neretva Delta supports 80 birds under the BirdLife categories of Species of European Concern (SPEC 2 and SPEC 3—8 vulnerable, 34 declining, 5 rare, 32 depleted, and 1 localized) and 46 birds protected as Annex I species of the Birds Directive. Neretva Delta is important for 60 Croatian Red List species (8 CR, 19 EN, 10 VU, and 23 NT).

#### **2.4. P6 Endangered and rare species in Nin Lagoon, including the area of the Miljašić Jaruga**

Based on earlier research in the area of the Nin Lagoon and the narrower target area around the mouth of the Miljašić Jaruga, certain measures were implemented with the aim of creating conditions for the restoration of endangered species and supporting adaptation to various natural and anthropogenic effects in this area. The Nin lagoon, including the area of the Miljašić jaruga (Fig. P6.1), is characterized by numerous sandy beaches in the coastal zone, but also in the mediolittoral and infralittoral zones. The entire area is under the influence of erosion processes, triggered by the action of strong winds in the winter months and the inflow of water from the mainland. The target area in this project, along with other areas selected for monitoring as part of the CASCADE project, is the mouth of the Miljašić jaruga. Currently, this area serves for the drainage of a part of Ravni Kotar with a total area of over 100 km<sup>2</sup>, and through the hydraulic tunnel "Bokanjac" the drainage of the Bokanjački Blat area is connected to the watercourse Miljašić jaruga. With this, the water regime is occasionally torrential in nature and is formed occasionally during heavy rainfall, when large amounts of water appear that pose a danger to the surrounding lands and buildings due to possible flooding and erosion, with a significant impact on marine habitats and organisms that live on this microlocality. As part of this project task, certain actions and tests of adaptation possibilities were carried out on the currently established marine organisms at this location, with an emphasis on those rare and endangered species. Sea

gastropods stand out here, with certain rare taxa that are rarely present in other locations on the Adriatic. Also, special attention was paid to the possibilities of restoration of the *Pinna nobilis*, with several living specimens in the wider area of the Nin Lagoon, which represents an exceptional rarity in the Adriatic and the entire Mediterranean in general, considering the occurrence of mass mortality of this species since 2017 in the Mediterranean and in 2019 on the eastern coast of the Adriatic.

*Endangered and rare species and issues addressed by the restoration actions.*

#### Marine gastropods

Monitoring of the area around the mouth of the Miljašić riječica was started in the 1990s, and it turned out to be a very interesting malacological site. In the described area, 369 species of molluscs were recorded (without the class of cephalopods where 4 species are present). Of the listed 369 species of molluscs, as many as 272 belong to the class of gastropods (Gastropoda), 90 bivalves (Bivalvia), 5 species of polychaetes (Polyplacophara) and 2 species of arthropods (Scaphopoda). Of the recorded approx. 1200 species in the Adriatic Sea, almost a quarter of mollusk species live in this microlocation, which is an exceptional case of biodiversity on a small area near the Miljašić jaruga. The most valuable part in terms of the biodiversity found is the large stones and rocks on the right (north) side of the pier and the area next to it, where 2/3 of the recorded species of this microlocation are based.

In the last 5 years, it is evident that the number of individuals has decreased by about 50%, which could be attributed to global climate changes or increased anthropogenic pressure. The temperature of the sea has changed significantly, so in the summer there is a marked warming of the sea water, which in that microlocation reaches up to 28°C. This is very disastrous for the spawning of certain species and they are unable to survive such new conditions. Spawning of most molluscs takes place during winter and spring when the sea temperature is the lowest.

Of all the snails found, the first find for the Adriatic Sea should be mentioned the species *Doto koenneckeri*, then a potentially new species that has not been found anywhere else in the Adriatic or the Mediterranean, namely *Eatonina* sp. The special feature of the Miljašić jaruga microlocality is the large number of constantly present species of sea gastropods, such as *Alvania rudis*, *Turbonilla* sp. and *Ondina modiola* (Figs. P6.2-4). Analyzes using molecular methods are currently underway to confirm that these are the species mentioned. From all of the above, it is clear that this is a very specific microlocation that is an endemic habitat of potentially new and rare species of molluscs, a breeding ground for certain species, and this place should be under constant monitoring and/or scientific research in the future.

#### Noble pen shell *Pinna nobilis*

The noble pen shell *Pinna nobilis* is an endemic bivalve species that inhabits the



Mediterranean Sea (Fig. P6.5). Periska is also the largest bivalve mollusk in the Mediterranean Sea, and it is also among the largest bivalve molluscs in the world. It has a long life span (the highest recorded age was 27 years) and can grow up to 120 cm in length. It lives at depths from 0.5 to 60 m, most often on sedimentary bottoms overgrown with *Posidonia oceanica* meadows. Throughout history, the periska populations have been greatly reduced due to various anthropogenic and environmental influences. For this reason, the periska is listed as an endangered and protected species in Annex IV of Directive 92/43/EEC on the protection of natural habitats and wild plant and animal species (OJ L 206, 22.07.1992) and in Annex II of the Protocol on Specially Protected Areas and Biological diversity in the Mediterranean Convention on the protection of the Mediterranean Sea from pollution (Barcelona Convention). It is also listed as a strictly protected species in Annex I of the Ordinance on Strictly Protected Species (Official Gazette 144/2013). Until now, various types of pollution, invasive species, climate change and, as the most significant - the destruction of *P. oceanica* meadows - have been considered the greatest threats to the already endangered population of periskeleton.

In the early autumn of 2016, on the western coast of the Mediterranean, along the Spanish coast and around the Balearic Islands, a mass death of the bivalve *P. nobilis* was observed, affecting specimens of all sizes. Underwater visual censuses conducted at various locations along the Spanish Mediterranean coast revealed alarmingly high mortality rates, reaching up to 100% in some areas. The current situation (July 2017) is such that the deaths have spread to almost the entire Spanish part of the Mediterranean Sea, and deaths have also been recorded on the coasts of Sicily. A similar situation affected the eastern coast of the Adriatic, where the first symptoms and deaths were recorded from 2019, which affected all populations of this species in the Adriatic by 2022.

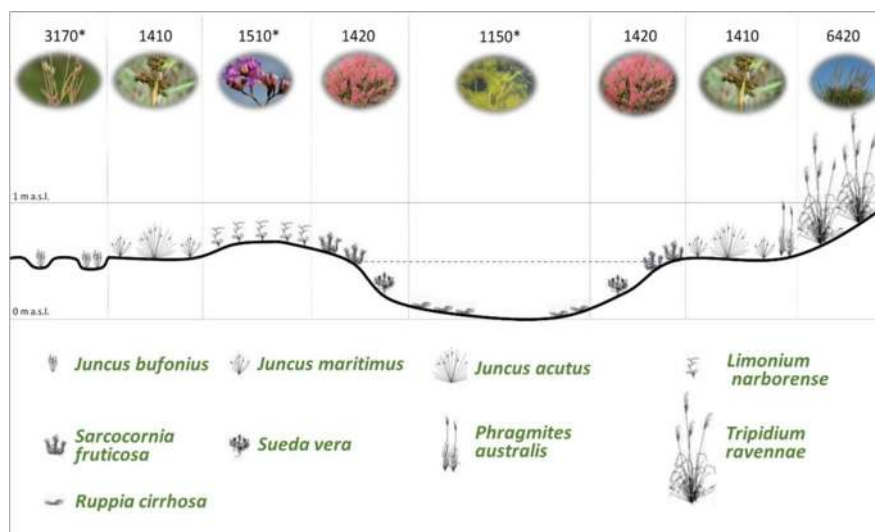
Experiences with similar phenomena in oysters indicate the impossibility of eradicating diseases or treating sick individuals in the open sea. Therefore, during the current epizootic that affected *P. nobilis*, only preventive measures can be applied. Also, it is necessary to establish monitoring of the condition of populations that are not affected in order to detect the further spread of the disease. As soon as possible, the possibility of the existence of resistant individuals should be investigated, which can be crucial for the development of a program to restore the population of resistant strains obtained through selective breeding. It is also of great importance to find potentially surviving juveniles in the affected area.

## **2.5. P7 restoration actions on Biferno mouth wetlands**

Salt marshes in the Biferno mouth are composed by a mosaic of habitats of Conservation Concern (HD 92/43/CEE; <http://vnr.unipg.it/habitat/index.jsp>) whose spatial variability (e.g. zonation) is shaped by the interplay of several environmental factors as: water table level, local micro morphology, substrate salinity and seashore distance. Vegetation zonation in the

Biferno mouth brackish mosaic is schematically reported in Figure 2 and briefly described in Table 1.

Biferno mouth wetlands also host several species of fauna, such as the migratory and sedentary birds (e.g. *Ixobrychus minutus*, *Gallinula chloropus*, *Phalacrocorax carbo*, *Ciconia nigra*, *Himantopus himantopus* and *Botaurus stellaris*), reptiles (i.e. *Hemys orbicularis*, *Testudo hermanni*), amphibians (e.g. *Epidalea viridis*) and bats.



**Figure 2.** Schematic profile depicting the typical brackish vegetation zonation in the Biferno River mouth (Pilot 7) and the respective EU habitat types along with their codes. Asterisks indicate EU priority habitats. A description of the habitats is reported in table 1.

Habitat name	Short name	Description	Dominant species
Coastal lagoons (EU habitat 1150*)	Coastal lagoons	Aquatic vegetation growing on shallow brackish waters with strong temporal variations in salinity and water depth, responding to differences in water table inputs, rainfalls and temperatures.	<i>Ruppia cirrhosa</i>
Mediterranean salt meadows ( <i>Juncetalia maritima</i> - EU habitat 1410)	Salt meadows	Subalophilic meadows of backdunal humid depressions with medium-high sandy substrates flooded by brackish water for medium-long period.	<i>Juncus acutus</i> , <i>J. maritimus</i>
Mediterranean and thermo-Atlantic halophilous scrubs ( <i>Sarcocornietea fruticosa</i> - EU habitat 1420)	Halophilous scrubs	Pauci-specific communities consisting of perennial halophytes, mainly chamaephytes and succulent nanophanerophytes, growing on periodically flooded areas	<i>Sarcocornia fruticosa</i>
Mediterranean salt steppes ( <i>Limonietales</i> - EU habitat 1510*)	Salt steppes	Halophilic perennial herbaceous species of the back side of the halophilous scrubs, on small dumps with salty soils (clayey, clayey-slimy or sandy), temporarily humid, but not submerged.	<i>Limonium narborens</i>
Mediterranean temporary ponds (EU habitat 3170*)	Temporary ponds vegetation	Amphibious vegetation given by small therophytic and geophytic species with late-winter/spring phenology, growing in small temporary ponds.	<i>Isolepis cernua</i> , <i>Juncus bufonius</i>

Sub-pannonic grasslands (EU habitat 6420)	steppic	Steppic grasslands	Reed vegetation growing on sandy-clay soils in contact with dune grasslands.	<i>Tripidium ravennae</i>
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**Table 1:** EU habitat names (EEC, 1992) along with their short name, brief description and the dominant species present in the Biferno mouth brackish mosaic. For the schematic description of brackish vegetation zonation see figure 2.

In 2016, the area was part of an environmental restoration program, funded by LIFE10 NAT/IT/00262 project which aimed at recovering the wetland ecosystem. The water flow pattern was re-established by opening the artificial wetland drainages and recovering the local hydrological regime (Prisco et al. 2017). Restoration included the demolition of artifacts, the reclamation of hazardous materials and the reconstruction of banks. Still, a boardwalk and a set of picket fences were put in place to protect salt marshes area from human trampling.

## 2.6. P8 First restoration experiment for endemic *Fucus virsoides* on the western Istrian coast

*Fucus virsoides* J. Agardh (Fucales, Phaeophyceae) is a species endemic to the Mediterranean, only found in its coldest part, the Adriatic Sea (Ribera et al., 1992). It is one of 10 species of its genus, as well as the only one inhabiting the Mediterranean basin (Guiry and Guiry, 2022). This species is limited to the intertidal zone of the Adriatic sea and is a functional equivalent to the much larger *Fucus* species that form substantial macroalgal forests in the intertidal zone of the oceanic coasts, as well as the Baltic and North seas (Guiry and Guiry, 2022). While currently distributed mostly on the rocky eastern coast, from Venice, Italy, to Albania, the species has historically been found in Ancona on the western coast until the 1960s, when it went extinct (Battelli, 2016; Kashta, 1996; Linardić, 1949; Mačić, 2006; Munda, 1972; Orlando-Bonaca et al., 2013; Rindi et al., 2020; Zavodnik, 1967). Although considered locally ubiquitous in the past, especially in the northern Adriatic, nowadays it can be regarded as functionally extinct due to a small number of isolated remnant settlements. *F. virsoides* settlements in their original size were able to support a noticeable biodiversity and biomass of smaller algae and animal species, although this was explored mostly during the mid-20th century, but this was also touched upon later during the 2010s, albeit on a small scale due to already severely degraded settlements (Čelig, 2010; Gljušić, 2016; Kučinar, 2014; Linardić, 1949; Munda, 1973; Zavodnik, 1967).

The species was considered to be very abundant and common on the Istrian coastline (northern Adriatic Sea), especially on the western coast, confirmed by floristic studies and herbarium collections from the 19th and early 20th century (Battelli and Alberti, 2003; Linardić, 1949; Munda, 2000; Schiffner, 1916; Vatova, 1928). Large abundances were also measured during the late 1960s and early 1970s in the vicinity of Rovinj, with up to 5 kg/m<sup>2</sup>

(218 g/400 cm<sup>2</sup>) measured in Rovinj at the time (Munda, 1973, 1972). Later, wet weight assessments were smaller, at 2.6–3.5 kg/m<sup>2</sup> (107–140 g/400 cm<sup>2</sup>) in 1999 and 0.5–2.5 kg/m<sup>2</sup> (20–100 g/400 cm<sup>2</sup>) in 2014, respectively (Kučinar, 2014; Zavodnik et al., 2002). Surprisingly, despite the perceived ordinariness, save for the settlements around Rovinj, almost no additional settlements were surveyed and mapped until the start of 21<sup>st</sup> century. This has, unfortunately, left a major gap in the distributional data for this species, which can cause serious obstacles in conservation legislation and management.

The distribution and abundance of *F. virsoides* on the Istrian coast has shrunk massively during the last 30 years, with some signs of regression mentioned in the literature as far back as the late 1960s and 1970s (Munda, 1980a, 1973, 1972; Zavodnik, 1967). This may imply the abundance of the species may have been even larger prior to the 1960s and that the regression may have started earlier. However, the older literature data are floristic in nature (herbarium collections and author accounts), and they do not contain quantifiable information, only presence/absence and geographic location (Battelli and Alberti, 2003; Linardić, 1949; Munda, 2000; Schiffner, 1916). Photographic evidence of the species' distribution is also very difficult to locate, with only a few usable examples. The implied reason for the regression of all fucalean algae since the 1960s was the ever-increasing pollution and eutrophication of coastal waters (Munda, 1993, 1980b, 1980a). Fucalean biomass in general was presumed to be recovering in some localities during the late 1990s and early 2000s, with *F. virsoides* included (Hanel, 2002; Iveša and Djakovac, 2015; Zavodnik et al., 2002). However, while some Fucalean species in the northern Adriatic were recovering well into the 2010s, *F. virsoides* regressed again, for example completely disappearing from the Slovenian coast (Battelli, 2016; Rindi et al., 2019).

More recent surveys from 2010–2016 have focused on specific parts of the coastline where the distribution was mapped, coverage was assessed, and biomass was measured, where possible (Čelig, 2010; Gljušić, 2016; Kučinar, 2014). These data were collected in order to be as comparable as possible with the data from Munda (1973, 1972). However, during 2016 surveys, destructive sampling (sample collection) for biomass assessment was abandoned due to low settlement density.

Most recently, *F. virsoides* distribution was fully mapped along the Istrian coast during 2021, showing a further regression of the species with several settlements lost and others degraded (Gljušić et al., 2022). Most of the surviving population on the Istrian coast is located in the vicinity of Poreč, Funtana, and Vrsar (Fig. P8.1).

*F. virsoides* distribution is not well mapped in Croatia, and no recent data on its distribution exist along the neighbouring countries' coastlines. Even though the distribution in Istria has been mostly mapped, for the rest of Croatia, the distribution of *F. virsoides* is still unknown, with only hearsay reports available, but no published data are available (Miočić-Stošić and Pleslić, 2022).

Since the remaining known settlements in Istria are small, isolated, and in severe regression,

it is prudent to develop a method for enhancing or planting new settlements of *F. virsoides*. Here, the first ex situ experimental restoration of *F. virsoides* in Croatia is presented. It was tested whether *F. virsoides* can be grown ex situ in laboratory conditions and planted into a location previously hosting the species, reintroducing the species on a small scale in order to assess survivability. Furthermore, it was explored whether there are any differences in growth regarding the upper and lower positioning of planting plots in the intertidal zone.

### **2.7. P9 Pinna nobilis Mass Mortality Event**

In 2019 *P. nobilis* has been listed as Critically Endangered on the IUCN Red List of Threatened Species due to the drastic population size reduction caused by the still ongoing MME and the fact that the causing pathogen is still present in the environment.

*P. nobilis* is also listed in Annex IV of Habitat Directive

In order to follow eventual recovery of impacted populations at Cetina Estuary site, *P. nobilis* larval collectors were installed. The availability of juveniles obtained from larval collectors will allow population of *P. nobilis* restoration.

### **2.8. P10 Recover of coastal dune ecosystems by soft conservation actions. Proposal for Torre del Cerrano Marina Protected area.**

Coastal dune landscapes are dynamic mosaics hosting high values of biodiversity which assures essential benefits to society. Coastal dunes provide unique habitat assemblages due to a strong environmental sea-inland gradient, which supports a highly specialized flora and fauna sharing relatively few species with other terrestrial ecosystems. The unique dune plant diversity is not only valuable itself (so-called ES “Existence value of biodiversity”), but it also underpins other Ecosystem services, both directly (e.g. Protection from wind and aerosol, Erosion regulation, and Recreation and Tourism) and indirectly (e.g. Climate regulation).

There is an increasing agreement about that coastal dunes are not just a place for recreational activities and that it is necessary to integrate spatial planning, stakeholders’ interests and the conservation of ecosystems processes for human well-being through a multidisciplinary approach. Still, conflicts frequently arise between the socio-economic interests in the beaches’ exploitation for tourism and the conservation measures to protect these vulnerable ecosystems.

For instance, human trampling associated with recreational activities is the most common disturbance on sand dune habitats, especially during the summer period. Trampling often means an extremely negative effect, causing substrate erosion, decreasing of plant community’s diversity and wildlife dismissal from no longer suitable habitats. To face trampling effects, regulatory and management tools are undertaken to balance conservation with tourism needs. Several studies have focused on the negative impact of activities related

to beach tourism on coastal dunes vegetation, coupled with the implementation of management plans and actions. Dune restoration usually foresees strategies to limit coastal erosion and trampling through fencing, building elevated boardwalks, vehicular access restrictions, revegetation of degraded areas and environmental education programs. Previous studies found indicators of rehabilitation of coastal habitats, in terms of dune vegetation's density increase, coastal erosion reduction and revegetation with native plants leading to dune accretion. Access control fencing, boardwalks and vegetation replanting are among the most effective methods largely used in many countries to promote successful dune restoration.

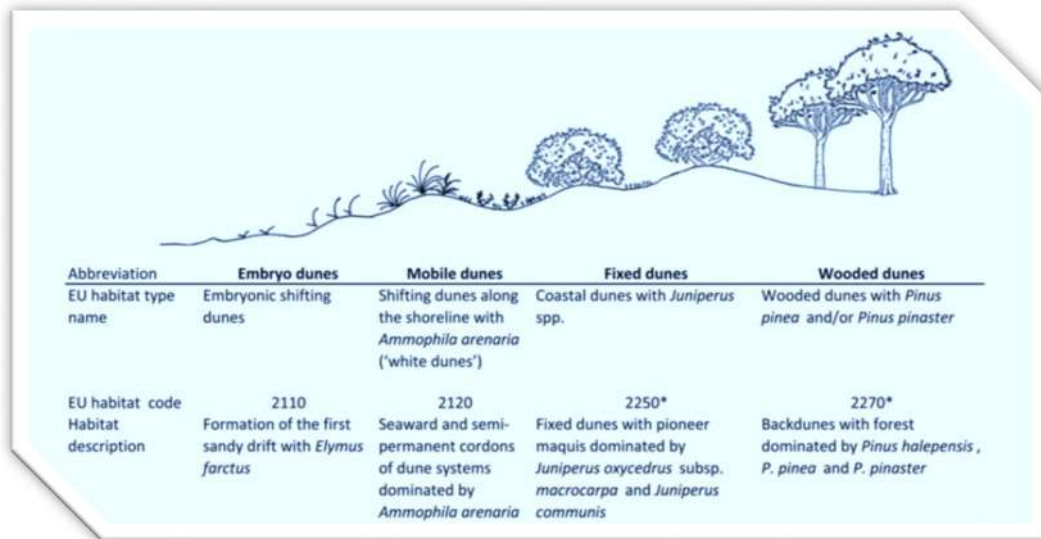
Coastal dune zonation in central Italy is characterized by the following habitats schematically reported in figure.

**Embryonic shifting dunes** (EC 2110). Formations of the coast representing the first stages of dune construction, constituted by ripples or raised sand surfaces of the upper beach or by a seaward fringe of small embryonic dunes at the foot of the tall dunes. This habitat consists of pioneer communities, dominated by *Elymus farctus*, which represent the first stages of plant colonization and contain some therophytes belonging to annual vegetation of the drift line.

**Shifting dunes along the shoreline with *Ammophila arenaria*** ('white dunes') (EC 2120): Taller mobile dunes forming the seaward cordons of dune systems, colonized by a perennial herb community dominated by the rhizomatous tussock grass *Ammophila arenaria* ssp. *australis*. This habitat is generally in sequential contact with the embryonic shifting dunes on the seaward side and with the fixed dunes with *Cisto-Lavanduletalia* dune sclerophyllous scrubs (habitat type 2260) and *Juniperus oxycedrus* ssp. *macrocarpa* maquis on the landward side.

**Coastal dunes with *Juniperus* spp.** (2250\*): This habitat type comprises juniper scrub on coastal stable sand dunes in a variety of situations. *Junipers* are coniferous plants, shrubs or small trees, native to Mediterranean coasts, growing into a pyramid shape, with branches growing from its base. In Northern Adriatic coast *Juniperus communis* is more common whereas in central and southern Adriatic sectors *Juniperus oxycedrus* subsp. *macrocarpa* is dominant. The habitat has often sequential contact with *Cisto-Lavanduletalia* dune sclerophyllous scrubs (habitat type 2260) and with Wooded dunes with *Pinus pinea* and/or *Pinus pinaster* (habitat type 2270) on the landward side.

**Wooded dunes with *Pinus pinea* and/or *Pinus pinaster*** (2270\*). Coastal dunes colonised by Mediterranean and Atlantic thermophilous pines (*Pinus halepensis*, *P. pinea*, *P. pinaster*), often corresponding to substitution facies of artificial origin or to climax formations of evergreen oak (*Quercus ilex*). On the Italian Adriatic coast, these are mostly plantations and are rarely natural formations, even if they host maquis and evergreen oak in the undergrowth. They generally occupy the inland stable sector of dune systems. Many stations are currently threatened by marine erosion.



**Figure 3.** A schematic profile (Drius et al., 2016) describing the typical Mediterranean coastal dune vegetation zonation and the relative EU habitat types (EEC, 1992) along with their codes in the central Adriatic Coast

The current analysis depicts the results of conservation actions carried out in the context of the project LIFE NAT/IT/000262 MAESTRALE (<http://www.lifemaestrale.eu>). Specifically, relative to the construction of wooden boardwalks perpendicular to the shoreline implemented for limiting human trampling on the whole dune system, from the first habitats near to sea-line towards those inland. In order to assure trampling reduction, also access bars were set up able to prevent vehicular entrance from the main road. Environmental education activities were also designed so as to raise public awareness regarding coastal habitat's conservation.

### 3. Report on the outcomes of restoration tests and actions

#### 3.1. P2 *Sporobolus maritimus* vegetation in the North Adriatic sea

To tackle the extensive regression of *S. maritimus* along the Italian coastline of the North Adriatic sea, a series of studies were carried out. First an extensive survey of the lagoons and wetlands of the northern Adriatic was undertaken to map the distribution of *S. anglicus* and *S. maritimus*. Results from this survey demonstrated the absence of *S. maritimus* southern of the Venice lagoon, while showed variable co-occurrence in Venice lagoon and other northern lagoons (session 3.1.1). Second, an extensive sampling of the saltmarsh restoration work carried out in Venice lagoon over the past 30 years (about 11 km<sup>2</sup> of artificial salt marshes were constructed/reconstructed reusing dredged sediments to maintain functional navigation channels) carried out aiming at: i) testing if the approach used in the Venice lagoon is effective at restoring the native *S. maritimus* in the region; and ii) unravel if *S. maritimus* and *S. anglicus* occupy the same niche, i.e., they grow at the same marsh height and under similar environmental conditions (e.g. sediment type). Results from this study indicated that current restoration actions are not particularly effective at restoring *S. maritimus*. It was hypothesised this might be due to the fact that restored marshes tend to have a greater proportion of sandy sediments, which might limit the growth of *S. maritimus* but not that of *S. anglicus*. For this reason, third, it was performed a manipulative experiment where plants of both species were transplanted in either mud or sandy sediments. Fourth, to understand what factors currently limit the growth of *S. maritimus* south of Venice Lagoon and if the restoration of *S. maritimus* would be feasible in those areas, clumps of *S. maritimus* were transplanted in three CASCADE locations: Vallona (Rosolina), Bellocchio and Baiona.

##### 3.1.1. Description of the restoration actions and tests

###### *Spatial mapping of Sporobolus maritimus and Sporobolus anglicus*

Spatial mapping of *Sporobolus* species were carried out along the coast of NW Adriatic Sea (Mediterranean Sea). The area considered for mapping the species distribution ranges approximately 400 km from Ravenna (Lido di Spina) in Italy to the Seča in Slovenia (Fig. P2.1). For the mapping and species distribution, an extensive field investigation were carried out in the major lagoon systems (e.g., Rosolina Mare, Venice lagoon, Marano and Grado lagoon) along the coast of NW Adriatic Sea. Field investigations have been conducted between 2014 (Aug to Oct) and 2021 (May to Oct). For species identification reference plant samples were collected from the field and brought to the laboratory to identify until species level with morphometric (especially leaf angle) and pollen characteristics (Wong et al., 2018). QGIS version 3.22.3 was then used to plot the species distribution in the map.

*Understanding the effectiveness of artificial islands for S. maritimus restoration and to what extent S. maritimus and S. anglicus have overlapping niches.*



The Venice lagoon (north-eastern coast of Italy) is the largest in the Mediterranean Sea, with an area of about 550 km<sup>2</sup> and an average depth of about 1.1 m. It is among the most important lagoon system in Europe due to its high productivity, aquaculture, and history (Barausse et al., 2015). Three major inlets, Lido, Malamocco, and Chioggia, connect the Lagoon to the Adriatic Sea. The lagoon is a microtidal system (average range ~ 0.6 m up to 1 m during spring tides), and it is characterized by strong physico-chemical and morphological gradients (Cecconi, 2005; Molinaroli et al., 2007; Solidoro et al., 2004). Human populations have inhabited the Venice Lagoon since centuries, largely re-shaping these territories, including the diversion of two major rivers (Brenta and Piave) in the 16th and 17th centuries to avoid sediment infilling of lagoon channels (Solidoro et al., 2010). The consequent long-term sediment starvation and salinity modifications, coupled with land reclamation, urban sprawl, land subsidence, and sea level rise caused strong degradation of salt marshes, with a loss of 72% in surface area, from 170 km<sup>2</sup> to only 37 km<sup>2</sup> (Barausse et al., 2015; D'Alpaos and Defina, 2007; Rizzetto and Tosi, 2012). To counter-act this loss and to recover salt marsh areas, during 1985-2009 more than 80 tidal islands were created using local sediment (dredged for shipping canals maintenance), ultimately restoring >11 km<sup>2</sup> of salt marshes (Cecconi, 2005). Sediments varied in grain size, from silt to sand, and were contained using rows of post made of logs (~30 cm in diameter) and/or 2-3 rows of gabions filled with rocks in combination with geotextiles on the inner side (Cecconi, 2005; Scarton et al., 2013). In the following years, at some islands small to large parts of the artificial structures were removed to allow for a more natural evolution of the tidal flat and the formation of tidal channels (Scarton et al., 2013).

These particular settings were ideal for covering a wide range of environmental factors (e.g., from muddy to sandy sediment types) as well as for unravelling the effectiveness of restoration techniques in providing a suitable habitat for the native *S. maritimus*. In this study, three types of salt marshes were considered: Natural (N), without visible artificial containing structures either due to lack of recent human interventions or to a more natural evolution following restoration; Restored type 1 sites (R1), with structures (either log poles, gabions, or both) but which also had low shore tracts where artificial structures were removed; and Restored type 2 sites (R2), with artificial structures still intact and abrupt margins at the low shore. Three sites with comparable hydrological setting were identified for each category over an area ~ 3 x 10 km wide.

Sampling was carried out between May 2021 and July 2021. At each site, it was sampled the low shore, the area closest to the edge of the marsh where bare ground and pioneer species are present. Monospecific patches (about 0,5-2 m diameter) of either the native *S. maritimus* or the non-native *S. anglicus*, embedded within large areas of unvegetated, bare sediment, were searched and sampled. At each site, it were randomly sampled four quadrats (0.5 × 0.5 m) for each vegetation type, *S. maritimus* and *S. anglicus*. In each quadrat, surface elevation (marsh height) was measured using real time kinetics (RTK) GNSS and referred to the Italian Ordinance Datum (Genoa, 1942). It was then visually recorded total vegetation

cover (%); canopy height was measured based on “drop disc” methods following (Stewart et al., 2001), using a Styrofoam disc (weighing: ~80 g, diameter: 30 cm) and a calibrated stick used as ruler. Afterwards, all the aboveground biomass (AGB) were clipped at the base, and two sediment cores (20 cm in depth and 9 cm diameter), which were stored separately in ziplock plastic bags, were collected. Cores were used for subsequent analyses of belowground biomass (BGB). An extra sediment core was collected from each quadrat to analyse the sediment composition.

The samples for the analysis of AGB and BGB were carefully washed on a 0.5 mm sieve under tap water to remove all sand and silt. Samples were oven-dried at 70°C until constant weight and they were weighed to the nearest 0.001 g using a precision scale (Alldred et al., 2017). Sediment cores were air dried in the laboratory and passed through a 500 µm sieve to discard gravels, plant materials and mollusc shell. Sediment electrical conductivity (EC) and pH were measured in a 1:5 (w/v) soil-distilled water ratio extract (Smith and Doran, 2015), using a multiparametric probe (Hanna HI9829). Bulk density (BD; g cm<sup>-3</sup>) was obtained by following formula (Thorne et al., 2014). Further, a sub-sample of known weight (Wi) was ignited in a muffle furnace for 5 hours at 550°C. The combusted sediment was weighted (Wf), and the organic matter content (OM %) was calculated as the difference between the initial weight and final weight after combustion ( $W_i - W_f / W_i$ ). Clay (%) was obtained by sieving (<63 µm) and further weighting the combusted sediment following Deneff et al. (2001).

#### *Unravelling the role of sediment grain size in mediating the presence/absence of *Sporobolus* species in restored artificial marshes.*

Considering the absence of *S. maritimus*, but not of *S. anglicus*, in restored sandy sites (see results section 3.1.2), in summer 2022, a manipulative experiment was conducted to answer the following research question: is the absence of *S. maritimus* on restored marshes determined by different soil types between natural and restored marshes?

A salt marsh in the Central Venice Lagoon (Fig. P2.2) was chosen where both species of *S. maritimus* and *S. anglicus* were naturally present. The experiment consisted in transplanting clumps of both the native and the invasive *Sporobolus* in plots with two different sediment treatments, muddy sediment from the natural marsh and sandy sediment from an artificial marsh. In June 2022 it was started to prepare the substrate for the subsequent transplantation: twenty plots of 50x50 cm length along a transect of 60 m, at the same marsh height where patches of both *Sporobolus* species naturally occurred, were set. Ten plots were filled with silty sediment from the same natural marsh, the other ten were filled with sandy sediment taken from a nearby restored marsh. After filling the plots, a layer of jute fiber was put on top of them to facilitate the stabilisation of the sediment for two weeks (Fig. P2.3). During the second phase the jute fiber was removed and proceeded with the transplantation (Fig. P2.3). Clumps of *S. maritimus* and *S. anglicus* were taken from nearby

patches, washed from the sediment with water from a marsh's creek, and stems were counted in order to have thirty of them for each transplant clump. Afterwards, five clumps for each species were planted in the plots either with sandy or muddy sediment. Additionally, ten plots of untouched stems of both species nearby the treatment were set as references plots to investigate the ability of un-manipulated plant to survive at the location throughout the experiment. Also, five clumps of thirty stems each were taken to the lab and measured for plant traits and initial biomass. Sediment samples were taken to characterize sediment parameters such as organic matter, grain size, salinity, Ph and Bulk Density. At the end of the experiment (September 2022) all transplanted clumps for both species were collected and plants traits, alive, and dead biomass were measured to assess the survival rate of the clumps.

#### *Transplantation experiment of Sporobolus maritimus in P2 areas southern of Venice Lagoon.*

In parallel to the above experiment, in July 2022, it was set up another transplantation experiment consisting of additional transplantations of *S. maritimus* southern the Venice Lagoon. With this experiment it was investigated if *S. maritimus* can grow further south than the Venice lagoon or if climatic (e.g. temperature) conditions prevent that. Three lagoons where the native species is not observed anymore (Fig. P2.4; Wong et al., 2018) were selected, but where *S. anglicus* grows indicating that a suitable habitat for *S. maritimus* may be present. The three sites are respectively Vallona Lagoon (45° 0'56.93"N, 12°22'14.28"E), Bellocchio Lagoon (44°37'54.23"N, 12°15'44.61"E) and Baiona Lagoon (44°31'28.83"N, 12°15'54.14"E). Vallona Lagoon (close to Rosolina) is in the current-day Po Delta while Bellocchio Lagoon and Baiona Lagoon are located respectively 50 km and 66 km south of Vallona Lagoon in the fossil Po Delta which is no longer part of the active delta (Consorzio di Bonifica Delta del Po, 2013). These lagoons are small and sheltered by natural or man-made barriers but are freely influenced by tides. The low intertidal salt marsh assemblage is similar at all three lagoons, predominantly comprising of mono-specific tussocks of *Sporobolus anglicus* interspersed with *Salicornia veneta* and bare soil (Wong et al., 2015).

In each of the three sites, clumps of 30 individuals each of *S. maritimus* were planted (Fig. P2.5); the donor population was a natural salt marsh located in the central Venice Lagoon. In this case, since the work did not involve the use of different sediment treatments, roots of the clumps were washed by sediment to avoid to over-stressed the plants. On each site temperature sensors (HOBO data loggers) were put in the soil to monitor temperature variations for 3 months (from July to September). During summer and autumn, the transplants survival, measures soil properties (salinity, pH, OM content, granulometry) and downloaded temperature data from the sensors (Fig. P2.6) were checked. No destructive sampling has been conducted yet because the experiment is still in progress.

### 3.1.2. Outcomes of the restoration actions and tests

#### *Distribution of *S. maritimus* and *S. anglicus* in the Nord-Est Adriatic.*

Overall, our sampling along the NW Adriatic Sea revealed that the non-indigenous *S. anglicus* was the common species found along this stretch of coast (Fig. P2.1). In particular, in Bellocchio, Vallona, and Baiona only the non-indigenous *S. anglicus* or *S. townsendii* were observed. Relatively greater abundance of *S. maritimus* was found in the Venice lagoon compared to other studied lagoons. Surprisingly, no occurrence of the *S. maritimus* was reported in the sites sampled in the Marano lagoon. In the Grado lagoon, it could observe only few patches of the native *S. maritimus* (Fig. P2.1).

#### *Habitat distribution of *S. maritimus* and *S. anglicus* in the Venice Lagoon.*

In the study focusing in the Venice lagoon, the main striking result was the lack of *S. maritimus* in both restoration types and the absence of *S. anglicus* from one type of restoration. Interestingly, the height of the low shore did not vary among restored marshes (Fig. P2.7), indicating that restored marshes are subjected to the same flooding regime as natural ones. Also, both type of restored marshes showed similar levels to natural sites with respect to clay fraction, organic matter, pH, and salinity (Fig. P2.8). In contrast, R1 marshes had higher bulk density (i.e. were sandier) than natural sites, but not so with respect to R2 marshes (Fig. P2.8). Natural and R2 sites had comparable BD. Thus, the lack of *S. maritimus* but the presence of *S. anglicus* in R1 marshes, suggested that sediment grain size may differently influence mediate establishment and/or growth capacity of the two species.

Regarding vegetation characteristics, it could compare only *S. anglicus* variables between natural and R1 sites. *S. anglicus* showed similarity between R1 and natural marshes in vegetation cover, canopy height, aboveground biomass, and belowground biomass (Fig. P2.9). This result suggests that despite the higher bulk density in R1 marshes, this factor does not influence the growth of plants.

#### *Testing sediment grain size as a possible limiting factor of *S. maritimus* restoration actions*

*Sporobolus maritimus* was unable to survive the transplantation in either sediment type; only two stems survived out of all the ten plots. Surprisingly, these two stems belonged to one sandy sediment treatment. Untouched *S. maritimus* plots showed a healthy status, indicating that uncontrolled environmental factors did not influence the study. High mortality of *S. maritimus* prevented statistical analysis. In contrast, *Sporobolus anglicus* survived the transplantation into both sediment types and no significant differences were found between alive and dead biomass between mud and sand treatments (Fig. P2.10). Overall, our results suggest that there was not a sediment effect but an effect on manipulation. Thus, the factor affecting the survival of the transplants was the manipulation and mechanical stress of the transplantation rather than the sediment type.

Interestingly, however, the manipulation affected the survival of *S. maritimus* only and not that of *S. anglicus*. Our findings arise two notable points: a) *S. maritimus* might be more vulnerable to mechanical stresses (be it natural or induced by humans) and this might affect the success rate of its survival. On the contrary, *S. anglicus* is more resistant to manipulations and might better rely on the spreading of propagules through current or through human interventions which make it more successful on colonizing different types of salt marshes and to spread in wider areas; b) restoration of diverse communities presents a challenge when not all species within a target assemblage have the same capacity for recruitment and establishment in restored areas. Species with high recruitment rates or high levels of stress tolerance can dominate restored sites and create monospecific or low diversity assemblages. These results should be considered when planning conservation actions that aim to facilitate the growing and the spreading of the native species instead of the invasive one. These assumptions need to be validated with further experiments.

#### *Survival of transplants of S. maritimus at P2 study areas southern of Venice Lagoon.*

According to preliminary observations, the transplants had survived the summer season and suffered low levels of mortality. This suggests that the species could be potentially restored at P2 sites south of Venice Lagoon. Sampling is in progress to monitor whether the transplanted specimens survived the winter and whether they restart the vegetative growth this spring/summer.

### **3.2. P3 Enlargement proposal of Torre Guaceto MPA**

#### **3.2.1. Description of the restoration actions and tests**

The proposal of extension originated from the habitat spatial distribution outside the current MPA of Torre Guaceto. As a matter of fact, the habitats of conservation interest are present in the current MPA but they extend also outside the MPA. The habitats cover all the area of the existing SAC IT9140005 Torre Guaceto e Macchia S. Giovanni and in the South-East portion they extend outside the SAC IT9140005 therefore the area of MPA extension proposal is bigger than the marine SAC portion.

The habitats of conservation interest are two habitats listed in Annex I of Habitats Directive (92/43/EEC) of Open sea and tidal areas, specifically code 1120\* Posidonia beds and code 1170 Reefs. The asterisk \* close to the code according the Habitats Directive indicates priority habitat types.

Habitats are not evenly distributed but nested with mosaics of habitats classified according EUNIS which does not necessarily entitle a protection status and in some cases alien species such as *Caulerpa racemosa*. Anyhow on the whole the majority of habitat, 63% are considered necessary for the conservation and only the remaining 37% are classified as not

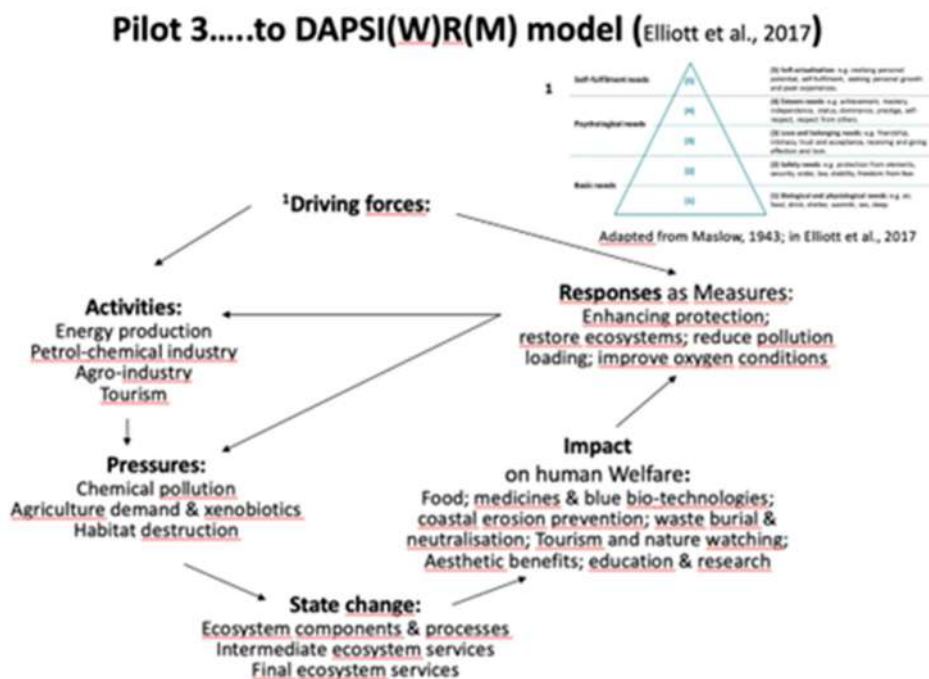
of high interest.

The presence of habitat 1120\* Posidonia beds classified as priority entitle the possibility to submit proposal for restoration actions with an higher co-financing rate inside the LIFE programme, up to 75% opposite to 60% if priority habitat or species are not present.

### 3.2.2. Outcomes of the restoration actions and tests

The DPSIR model (Drivers, Pressures, State, Impact, and Response) elaborate for the Pilot 3 management area was resumed in deliverable “D5.1.1 Report and datasets on assessment of hazards, impacts and vulnerability”. In the case of P3 the DPSIR model was presented as the usual standard but the University of Salento elaborate also the DAPSI(W)R(M) model (Elliott et al., 2017), “better highlighting the human need drivers requiring activities causing pressures on the ecological status changes which determine impacts on human welfare in the management area. DAPSI(W)R(M) model has also the advantage to clearly disentangle pressures which could be managed within the management area of interests and those pressures which are generated outside the area and, therefore, cannot be managed locally.”

The DAPSI(W)R(M) model, presented “Enhancing protection” as one of the Responses to the driving forces, envisaging therefore the need of proposal of the extension of MPA of Torre Guaceto.



**Figure 4.** the DAPSI(W)R(M) model (Elliott et al., 2017) elaborated by University of Salento.

Besides the “Enhancing protection” the second response of the DAPSI(W)R(M) model was “restore ecosystems”, which is highlighted by the proposal extension:

- 1) due to the presence of alien species *Caulerpa racemosa* in mosaic to habitat of conservation interest;
- 2) due to the presence of damages induced by anchoring as well trawling.

Last but not least ecosystem restoration is second pillar of EU Biodiversity Strategy for 2030 and Restoring marine ecosystems is represented by Target 15 – “The negative impacts on sensitive species and habitats, including on the seabed through fishing and extraction activities, are substantially reduced to achieve good environmental status.”

### **3.3. P4 Neretva River Delta**

#### **3.3.1. Description of the restoration actions and tests**

*Description of the detected impacts and pressures.*

The detection of impact was followed by a detailed elaboration of the natural values of the Neretva River Delta. In order to address the main points for assessing different applicable restoration actions, it is a necessity to take into account all the activities in the area and its geographical position.

With the beginning of the first land reclamation and changes in the course of the Neretva River, especially contemporary interventions in the last 50 years, man began to significantly change the natural characteristics of the delta and thus dictate the economic orientation and the location and form of settlement. Until then, the local community lived on fisheries, hunting, and agriculture, which depended on traditional methods of land reclamation.

Intensive reclamations in the second half of the 20th century that drained wetlands created land adequate for commercial agriculture but also resulted in closing migration paths and destroying habitat for fish species (*Anguilla anguilla*), which formed the basis of traditional fisheries. Today, the local economy relies on intensive agriculture, mostly of citrus fruits, which is followed by increased use of pesticides and artificial fertilizers that are easily washed through the soil into the watercourses of the delta and then into the transitional waters and the sea.

Hunting and fishing are recreational activities without significant economic value. But still,

they are performed illegally and therefore have a devastating effect on the Neretva River Delta's protected bird and fish populations.

The cargo seaport in Ploče is an important part of the local economy in the area, ranking second in Croatia in terms of transshipment and serving as a multi-purpose port for the transshipment of almost all commodities represented in international maritime transport. An integral part of the port of Ploče is the Metković port, which is located 20 km upstream on the river Neretva. This is significant because the Neretva Delta's specific geographic location created the precondition for the formation of important traffic intersections of major roads, rail, and maritime transport; however, this advantageous traffic position has drawbacks, such as pollution of the delta's transition waters and sea, the introduction of invasive marine species, habitat degradation, and so on.

There is also a recorded intensification of tourist activity due to the significantly different offer of the rest of the Croatian coast. Tourism is great for the development of the local community but has the side effect of contributing to pollution and the destruction of fragile coastal habitats because of the concentration of visitors and vehicles.

The final identified impact is cross-border pressure, which manifests itself in water pollution (both in plastic and organic and chemical substances) as well as changes in water regime caused by upstream hydropower plants.

### **3.3.2. Outcomes of the restoration actions and tests**

*The collected views of local communities.*

The first local stakeholder workshop was based on the work of participants divided into three groups, while each group brought together participants with different professional backgrounds. Through teamwork and discussion, every group formed a set of five different measures that are necessary for preventing further degradation of the Neretva Pilot Area.

Through this discussion, all of the previously mentioned patterns were observed in correlation with their impact on the biodiversity values, and this process resulted in the formulation of the set of measures necessary for preventing further degradation of the Neretva pilot area. The next workshop emphasized the environmental aspects in order to develop a long-term strategy that could be applicable to processes that have the potential to impact and degrade the Neretva Pilot Area.

This participatory process made it clear that the stakeholders became deeply interested and more involved in the process of planning the measures. All of this clearly indicated an increase in their knowledge and awareness about the problem of the pilot area. From this, it was concluded that work on increasing stakeholder awareness has a long-term positive effect on the willingness of the local community to react in political processes, lobbying decision-makers to introduce necessary changes for the benefit of the ecosystem and the



local community itself.

This pilot area is of great importance for biodiversity locally, regionally, and globally, but equally, the pressures on the stability and state of biodiversity are very different and complex. The listed pressures act cumulatively on the pilot area's biodiversity state, so the only restoration option is a joint improvement of various impacts in order to achieve area recovery. It is necessary to highlight that often these impacts derive from the different sectoral authorities, and joint action is difficult. So, as previously mentioned, the main soft reconstruction action planned is an awareness-raising campaign for the Pilot area's stakeholders with the goal of empowering the local community to take part in the proposal, adoption, and modification of policies in local, regional, and cross-border issues. in a way that improves biodiversity and quality of life. Since it is the only way influence all the different sectors at once.

### **3.4. P6 Protection of endangered and rare species in Nin Lagoon, including the Miljašić Jaruga**

#### **3.4.1. Description of the restoration actions and tests**

##### *Protection and the support of the marine gastropods*

With regard to the planning of the construction interventions in this area, which include the demolition of the currently coastal wall on both sides of the Miljašić jaruga and the construction of new piers in a variant where both piers are constructed as a combination of a vertical wall on the inside and an embankment type on the outside. Part of the construction is made of larger stone blocks, which will be used for the dissipation of wave energy, and part of the construction is made with flat surfaces for access to piers, bathing and mooring of recreational vessels. The conceptual solution for the reconstruction of the mouth of the Miljašić jaruga envisages works that include the removal of stone lining from the eastern and western wings, the removal of concrete parts of the eastern wing, excavation of the bottom (deepening of the bed), the construction of the eastern and western wings and the connection to the pedestrian infrastructure.

The conceptual solution envisages the arrangement of pedestrian areas with stone, and the use of part of the existing stone on the location. In order to ensure a uniform flow in the area of the mouth, the bottom of the bed will be deepened to an elevation of -2.9 m. a.s.l. Therefore, it is planned to excavate 4,800 m<sup>3</sup> (silt, sand, gravel) that needs to be disposed of at an underwater location or at a landfill on land. With regard to the planned interventions, it is to be expected that increased sedimentation will occur in this micro-locality during and after the execution of the works and a possible significant impact on the locally present organisms. This especially applies to species that do not have fast mobility on the substrate and the ability to adapt to new habitats with significantly different ecological conditions, such as currently present snails.

Given the possible significant impacts of the above-mentioned planned operations on the population of sea snails, as part of this report, considerations were made on the introduction of 2 methods of supporting the adaptation of these species to new conditions. The measures themselves stem from two basic effects that these construction interventions will potentially have on rare and endangered species of snails:

*1. During the interventions themselves, when a significant impact is expected due to sedimentation caused by the desilting of silt deposits in the narrower area of the Miljašić Jaruga:*

Possible adjustment measures that have been considered are that the existing stones are not taken out of the sea during the demolition of the current stone and concrete wall, but that before the start of construction they are moved to a deeper location with the help of mechanization and divers. In this way, the marine organisms that are numerous inside the stones will not be so heavily influenced by the sedimentation processes during the construction of the new coast of the Miljašić jaruga. In that case, these individuals would have the possibility of staying at the existing installations in the sea, surviving in conditions that are identical or at least similar to those they had in the existing location. Survival based on the transfer of stones to a nearby zone where the impact of construction will not be visible would enable these organisms to eventually repopulate other areas, when the impact of construction is no longer visible. It is very likely that with a certain lapse of time, the same organisms or their descendants settled again in the area of the Miljašić jaruga, if elements with similar characteristics are used during new construction operations.

Such relocation operations are demanding from both the technical and the biological side, but they have proven to be relatively successful in practice, with examples of the transfer of protected species, such as the transfer of the noble *Pinna nobilis* from the port of Pula to the NP Brijuni. For successful technical feasibility, it is necessary to carefully select the location for the transfer of stone elements so that they do not have an impact on the maritime route and other habitats and species, which is why the transfer itself should be preceded by a detailed biological examination in order to precisely determine the microlocation for the transfer, taking into account the distance from the impact of the intervention and the compatibility of environmental conditions between these two locations. At the same time, it is necessary to ensure the agreement with the competent authorities for the safety of navigation at sea. It is recommended to carry out a regular annual biological survey of these localities in order to determine the processes of adaptation of certain species of snails to the newly created conditions.

*2. Characteristics, design and types of materials and elements that will be used in the construction of the new Miljašić ravine channel:*

During the construction, the proposed version was executed as a combination of a vertical wall on the inside and an embankment type on the outside. It is the exterior and its design and choice of materials that will play a decisive role in the repopulation of this habitat with

the species that previously lived there. The inner part of the channel is exposed to excessive salinity and temperature oscillations during the occurrence of high water, and therefore the biodiversity on that side is much lower and is mainly inhabited by organisms that only occasionally go there in search of food or shelter. In order to ensure a faster acceptance of fouling organisms and later other benthic species (such as bivalves and snails) on the outside, it suggests the design of beam elements that would have a neutral pH and a rough surface structure, as well as various cavities and depressions that have been shown as effective in similar situations.

### Protection and support of Noble pen shell *Pinna nobilis*

For the purpose of measures to support the critically endangered species *Pinna nobilis*, a detailed search of the remaining populations in the wider area of Zadar County was carried out in 2022. Of all the living individuals found in the summer of 2022, the largest number (a total of 7 individuals) was found inside or in the immediate vicinity of the Nin Lagoon. As part of the project, in cooperation with the Directorate for Nature Protection at the Ministry of Environmental Protection and Energy, a protective cage was installed on two surviving individuals located in a busy location (bathers and boats), one of which later died during the winter 2022. Considering the great biodiversity of bivalves and gastropods in the area of the Nin Lagoon, it proposes the establishment of permanent monitoring which should contribute to the recovery and protection of this species and which should consist of the following elements:

#### *1. Installation of a collector for the reception of larvae in the area of the Nin Lagoon.*

Considering that a certain number of surviving individuals in the area of the Nin Lagoon has been determined, it suggests that at the site of the Miljašić ravine, during construction, the installation of a signaling buoy of larger dimensions on which devices could be placed for the continuous monitoring of various biotic and abiotic parameters of the sea and the collector for the reception of fry with the aim of receiving pericarp fry (Fig. P6.6).

#### *2. Monitoring of a wider area for the purpose of finding and protecting adult individuals.*

The surviving individuals of the *P. nobilis* are potential individuals that show resistance to disease agents and are very important in repopulation. Therefore, it is necessary to locate as many living individuals as possible and ensure their protection (Fig. P6.7).

#### *3. Raising the awareness of the local population and tourists about the threat of this species.*

Considering that the main economic branch in this area is tourism, it is necessary to educate the population in order to raise awareness about the threat of this species. Also, visitors need to show the importance of preserving this and other rare species that live in this area. These measures have proven to be successful in protecting species and finding a greater number of surviving individuals.

### 3.5. P7 restoration experiment for brackish areas on the Biferno mouth (Pilot 7)

The analysis of vegetation dynamics on restored salt marshes in the Adriatic coast in Central Italy (Biferno brackish area, Pilot 7) revealed consistent changes on floristic composition and an improved conservation status. Specifically, the different plant guilds offer clear indications of ecosystem improvement. In restored areas, there was a significant increment of diagnostic species and a significant decrease of ruderal and alien plants, depicting an improvement of the environmental conditions after the restoration actions carried out in 2016 by the project LIFE + MAESTRALE (NAT/IT/000262).

As expected, in the Adriatic coast, the native plant diversity tends to recover. The observed recolonization can suggest the incipient establishment of a self-sustaining ecosystem status. A reduction in the number of species in the salt marsh mosaic was observed, probably linked to the successional process that led the salt marsh mosaic towards more natural conditions characterized by pauci-specific plant communities with average richness ranging from 4 to 13 species (Table 2)

	Mosaic		Salt meadows		Halophilous scrubs		Salt steppes	
	T1	T2	T1	T2	T1	T2	T1	T2
Total number of species	71	54	57	38	23	18	21	12
Number of diagnostic species	24	22	14	13	6	4	1	2
Number of ruderal species	21	10	15	9	2	0	5	0
Number of alien species	5	4	3	3	1	1	3	1

**Table 2.** Number of species over time (T1: 2010 and T2: 2020/21) for the entire mosaic and for each EU Habitat type. Salt meadows (EU habitat 1410); Halophilous scrubs (EU habitat 1420) and Salt steppes (EU habitat 1510\*).

The richness decline could respond to the interplay of different processes favoured by the restoration of wetlands and the construction of boardwalks (Prisco et al. 2017), as:

- 1) the expansion and gain in cover of the salt tolerant native species (*Artemisia caerulescens*, *Halimione portulacoides*, *Limonium narbonense* and *Sarcocornia fruticosa*) that have morphological and physiological adaptations to live on saline environments aided by the reconstruction of ponds and wetland,
- 2) the reduction and loss of ruderal species (e.g. *Arundo plinii*, *Melilotus albus* and *Vicia sativa*) partially due to the decrease of human trampling disturbance prevented by dedicated paths for tourists and visitors of the area (Prisco et al. 2017),
- 3) the low number of alien species is likely due to the competition with the native halophilic species that increased their cover over time. But the persistence of some exotic species could be due to the fact that they also grow in croplands and farms in the neighbouring fields and

4) the loss of plant species from other ecosystems of the close coastal dune mosaic, likely due to wetland restoration interventions.

Indeed 2021 species composition of each habitat type appeared closer to mature conditions (e.g. increase of diagnostic species) and the vegetation zonation along the brackish ecological gradient seemed less fragmented. Moreover, the different EU habitats of the brackish mosaic are currently quite distinguishable in the field and their species composition seemed closer to typical halophytic species assemblage.

The analysis of ecological groups and Ellenberg bio-indicator values over time (Pignatti 2005) revealed important changes in the entire brackish mosaic (from 2010 to 2020/21) and such changes varied across the different habitat types. In the whole brackish mosaic a significant increase in the cover of diagnostic species and a significant decrease in the cover and richness of ruderals were registered. As observed at mosaic level, significant gains of diagnostic species cover in the salts meadows and steppes and a significant decrease of their richness per plot in the halophilous scrubs were registered. As regards the ruderal species, a significant decrease in cover and richness in halophilous scrubs and salt steppes were observed. Concerning alien species, it was found a significant decrease in cover in salt steppes. Concerning the Ellenberg salinity value, the analysis showed a significant increase over time in brackish mosaic and in halophilous scrubs and salt steppes.

The species that, according to SIMPER analysis (similarity percentage) (Table 3), contributed 50% of floristic changes in the salt meadows habitat are diagnostics and thermophilous ( $T \geq 7$ ) with low-medium Ellenberg Salinity values. The cover of these species increased over time, except for *Juncus maritimus* that decreased. In halophilous scrubs and salt steppes habitats the temporal changes are given by the increase of some halophilous species (e.g. *Sarcocornia fruticosa* and *Limonium narborensis* with Ellenberg Salinity value of 8 - 9) and by the reduction of species with low Ellenberg Salinity value (e.g. *Juncus maritimus* and *Plantago crassifolia* with 6 and 1 indicator value).

Species	Ellenberg Values		Species Contribution (%)	Cumulative Contribution (%)	Mean Cover		
	S	T			T1	T2	
Salt meadows	<i>Plantago crassifolia</i>	1	8	13,24	13,24	11,3	30,5
	<i>Juncus maritimus</i>	6	7	10,78	24,03	22,1	13,5
	<i>Schoenus nigricans</i>	1	7	8,647	32,67	7,71	19,8
	<i>Artemisia caerulescens</i>	9	7	6,662	39,33	5,63	17,2
	<i>Juncus littoralis</i>	5	8	4,757	44,09	5,46	7,58
	<i>Juncus acutus</i>	5	8	4,755	48,85	1,5	11,1
	<i>Elymus acutus</i>	3	7	4,129	52,98	3,63	8,83
Halophilous scrubs	<i>Sarcocornia fruticosa</i>	8	9	18,85	18,85	34,5	58,3
	<i>Halimione portulacoides</i>	8	9	14,78	33,63	12	26,5
	<i>Juncus maritimus</i>	6	7	12,61	46,24	22,1	4,88
	<i>Artemisia caerulescens</i>	9	7	8,336	54,58	1,5	14,3
Salt steppes	<i>Limonium narborens</i>	8	7	23,94	23,94	4,8	67,5
	<i>Plantago crassifolia</i>	1	8	15,18	39,12	40,8	0
	<i>Halimione portulacoides</i>	8	9	12,39	51,51	0	32,5

**Table 3.** Plant species contribution to the temporal floristic changes and species mean cover (from 2010 to 2020) in the different EU Habitats assessed by the similarity percentage procedure. For each taxon, the Ellenberg ecological indicator values for salinity (S) and temperature (T) are also reported. Salt meadows (EU habitat 1410); halophilous scrubs (EU habitat 1420) and Salt steppes (EU habitat 1510\*).

### 3.6. P8 First restoration experiment for endemic *Fucus virsoides* on the western Istrian Coast

#### 3.6.1. Description of the restoration actions and tests

##### *Seeding*

The fertile receptacles were collected by hand from a surviving settlement in Funtana, near Poreč—Western Istrian coast on 23 April 2021 (Fig. P8.1). Receptacles were checked for fertility in situ by cutting a transverse section of the receptacle either by a razorblade or by a tip of a fingernail. Afterwards, the thick conceptacles with female gametes were clearly observable using a camera with a larger than 1:1 macro capability (Olympus Tough TG-6) (Fig. P8.7A–C). While both male and female gametes are produced in the same receptacle, the male gametes are only observable under high magnification of a microscope (Olympus SZX 12). A small aquarium filled with unfiltered natural seawater (5 L) was used to hold fragmented limestone tiles (i.e., stones) of a similar size (Fig. P8.8). A small net was filled with cca. 30 fertile receptacles from multiple individuals and placed in the aquarium on the water surface for the fertilization to happen in the water and the zygotes to settle on the stones (Fig. P8.8). Aeration and mixing were performed by an air pump.

### Early Growth

After germlings became visible (1 week), stones were moved to a bigger, 24 L closed aquarium system with controlled temperature and light (at 16–18 °C, LED GNC SilverMoon Marine, 148  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , 12 h light/dark period). Full water changes were also performed weekly. Stones were photographed in order for the height of the germlings to be measured using ImageJ (Rasband, 2023) (Rasband, n.d.). Unfortunately, this was not successful during the early growth period due to highly unreliable results. However, in September 2021, when germlings became more prominent and measurable, seeded stones were moved to outer open-flow stone basins with fluorescent tube lightning (Phillips Master TL-D 36 W/865, 6000 K, 95  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) in the Center for Marine Research courtyard in order to accelerate their growth. These basins (1300 L) have a constant flow of natural unfiltered seawater pumped from the nearby bay. From then until planting (two months), *F. virsoides* recruits were kept under those conditions, and 20 random specimens were used in order to assess the average size of thalli and the standard error. Light intensity and temperature were measured during the process by Hobo Pendant MX Temperature/Light Data Logger logged every 60 min in order to monitor the conditions.

### Planting

The recruited individuals were planted in Muća, Rovinj, which previously hosted *F. virsoides* settlements (Fig. P8.9). The previous settlement was marked with a blue epoxy in 2014 (Kučinar, 2014), and planting was performed by attaching the stones by the same epoxy (Sub Coat Xt Azzuro 2/1, Venezzi within the vicinity of these markings. Plots were covered by mesh cages (2 plots, 100  $\text{cm}^2$ , 4 stones per plot, covered with protective cage 12 × 12 × 10 cm in size, mesh opening of 1 cm) in order to protect the recruits from potential grazers (such as limpets and other gastropods, hermit crabs, *Sarpa salpa*, and urchins). Each of the two cages were placed on approximately the upper and lower edges of the mid-tidal zone (~3 m apart) in order to assess if positioning of the plots can significantly affect the growth and survivability. The cages were repaired when damaged and cleaned, as necessary, from fouling. Percent cover was monitored via photography, thallus height was measured manually with a ruler, and timing of fertility was monitored in situ. This was performed during the next 12 months. In each plot, 10 random recruits (and later juveniles and adults) were used in order to measure the average growth and standard error.

### Statistical Analysis

The thalli length of *F. virsoides* was analysed by two-way ANOVA using “Position” (2 levels: Upper and Lower) and “Time” (8 levels: November 2021, January 2022, February 2022, March 2022, May 2022, July 2022 August 2022, November 2022) as fixed factors. Prior to ANOVA, data were tested for homogeneity of variance using Cochran’s C-test. The significant

“Position × Time” ( $p < 0.003$ ) interaction was analysed by post hoc Student-Newman-Keuls (SNK) tests. Analysis of variance (ANOVA) was performed by GMAV-5 for Windows.

### 3.6.2. Outcomes of the restoration actions and tests

#### *Early Growth*

Settled germlings became visible just a few hours after the seeding process started. Eight stone fragments (cca. 200 cm<sup>2</sup> in total) were successfully seeded by germlings and retained recruits until the planting phase (Fig. P8.2). Other stones were not densely seeded enough, and recruits did not manage to reach a suitable size before succumbing to fouling. The recruits effectively showed no growth for the first four months (May to September) and were too small to accurately measure. After they were moved to the outdoor system in September 2021 at the average size of 0.228 cm ( $\pm 0.016$  SE), an unexpected increase in average size up to 0.437 cm ( $\pm 0.036$  SE) was measured two months later in November 2021, just before planting (Fig. P8.2D).

#### *Post-Planting Growth*

After planting *F. virsoides* in November 2021 in the designated positions, the growth accelerated dramatically (Fig. P8.3). On the lower position, algae reached 1.470 cm ( $\pm 0.159$  SE) after two months, 3.520 cm ( $\pm 0.193$  SE) after five months, 5.540 cm ( $\pm 0.135$  SE) after seven months, and finally 8.420 cm ( $\pm 0.283$  SE) in height with fertile receptacles nine months post planting (Fig. P8.4). On the upper position, algae reached 1.908 cm ( $\pm 0.105$  SE) after two months, 3.250 cm ( $\pm 0.282$  SE) after five months, 6.800 cm ( $\pm 0.902$  SE) after seven months, and finally 7.020 cm ( $\pm 0.305$  SE) nine months post planting (Fig. P8.4). In August 2022 (10 months post planting), the growth stagnated at 8 cm ( $\pm 0.301$  SE) on the lower position, but it increased to 7.700 cm ( $\pm 0.397$  SE) on the upper position. In November 2022 (1 year post planting), a reduction in the average size of thalli was measured at 7.06 cm ( $\pm 0.412$  SE) on the upper as well as a strong reduction 3.840 cm ( $\pm 0.753$  SE) on the lower position. The average size of thalli across all measurement times is presented in Fig. P8.4.

In late August 2022, the protective cage on the lower position was destroyed by unknown means and fully grown, fertile thalli were exposed to grazers, which consumed most of the algal fronds, leaving only holdfasts (Fig. P8.5). This destruction was the cause of such a strong decrease in the average height for the plots at the lower position in November 2022 (Fig. P8.4). Interestingly though, some of the surviving specimens from the destroyed cage were still found to be fertile.

#### *Statistical Analyses*



Two-way ANOVA and the SNK test for the “Position x Time” interaction showed that thallus length in May 2022 (seven months post planting) and November 2022 (one year post planting) was higher in the lower cage position than in the upper cage position. In July 2022 (after eight months), the thallus length was higher in the upper cage position, and no differences in thallus length between the upper and lower cage positions were found in all other times examined (Table 4). The difference between positions in November 2022 (one year post planting) can easily be explained by the destruction of the protective cage and overgrazing on the lower position in late August 2022 (after the August measurement).

Source	df	MS	F	p
Position	1	2.4337	0.38	0.5556
Time	7	160.2287	104.77	0.0000
Position x Time	7	6.3555	4.16	0.0003
Residual	144	1.5293		

Cochran’s test: 0.33,  $p < 0.05$

SNK test for the interaction Position x Time:

November 2021: Upper = Lower	May 2022: Upper > Lower
January 2022: Upper = Lower	July 2022: Upper < Lower
February 2022: Upper = Lower	August 2022: Upper = Lower
March 2022: Upper = Lower	November 2022: Upper > Lower

\* Factors: Position (fixed, 2 levels—Upper and Lower), Time (random, 8 levels—November 2021, January 2022, February 2022, March 2022, May 22, July 2022, August 2022, November 2022). Number of replicates per each combination of factor levels  $n = 10$ . Total number of replicates  $n = 160$ .

**Table 4.** Two-way ANOVA testing the effects of two positions of *Fucus virsoides* in the intertidal (upper and lower) on thallus growth.

### 3.7. P9 Cetina river mouth *P. nobilis* restoration

#### 3.7.1. Description of the restoration actions and tests

The Mass Mortality Event (MME) stroke Noble Pen shell (*Pinna nobilis*) population in the Croatian part of the Adriatic Sea from May 2019 onwards (Čižmek H. et al, 2020). Main cause of specie mass mortality is considered to be a combination of multiple pathogens with *Mycobacterium sp.* and *Haplosporidium pinnae* being most of the time involved (Carella F. et al, 2020). Once common specie to Natura 2000 site Cetina Estuary (P9) from 2019 become rare and very soon threatened by extinction (Kurtović Mrčelić J., 2019). In 2019 *P. nobilis* has been listed as Critically Endangered on the IUCN Red List of Threatened Species due to the drastic population size reduction caused by the still ongoing MME and the fact that the causing pathogen is still present in the environment. The natural recovery of impacted populations will solely depend on resistant individuals and recruitment. Recruitment can be

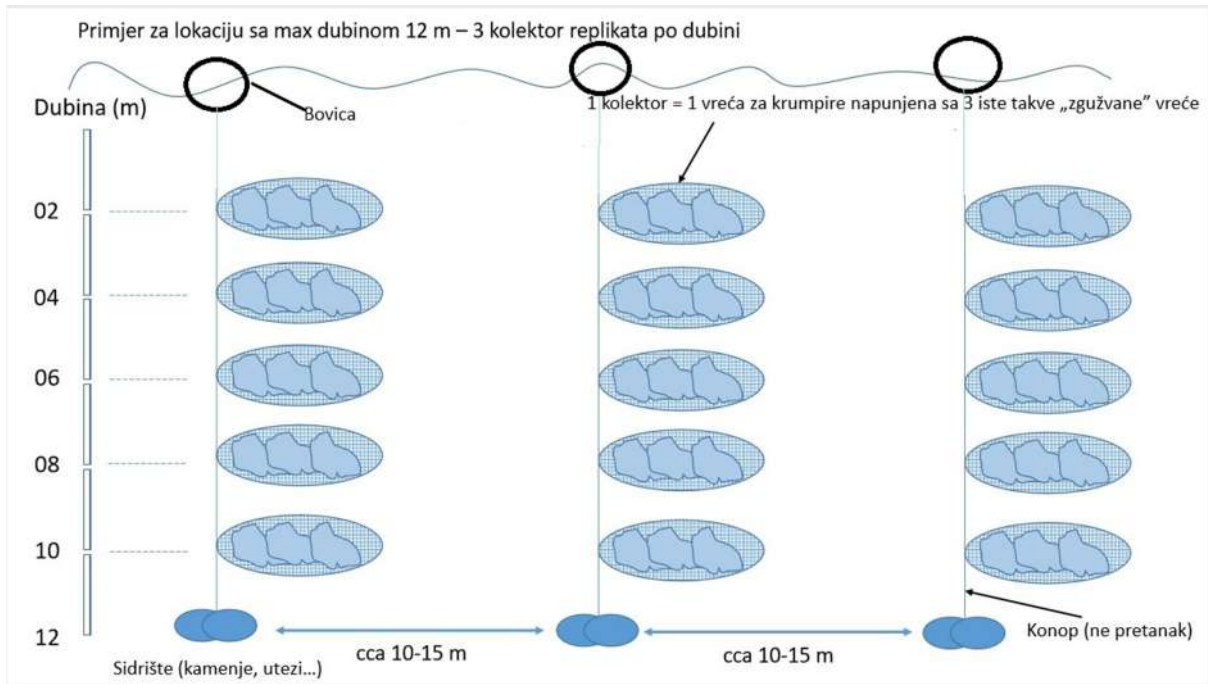
monitored using *P. nobilis* larval collectors (Kersting D. et al., 2019). Restoration actions could then be undertaken with juveniles obtained from larval collectors.

In order to follow eventual recovery of impacted populations at Cetina Estuary site, *P. nobilis* larval collectors were installed at 3 sites, namely Water polo playground, Shallow mark and Mala Luka Bay in 2020., 2021. and 2022. (Figure5).



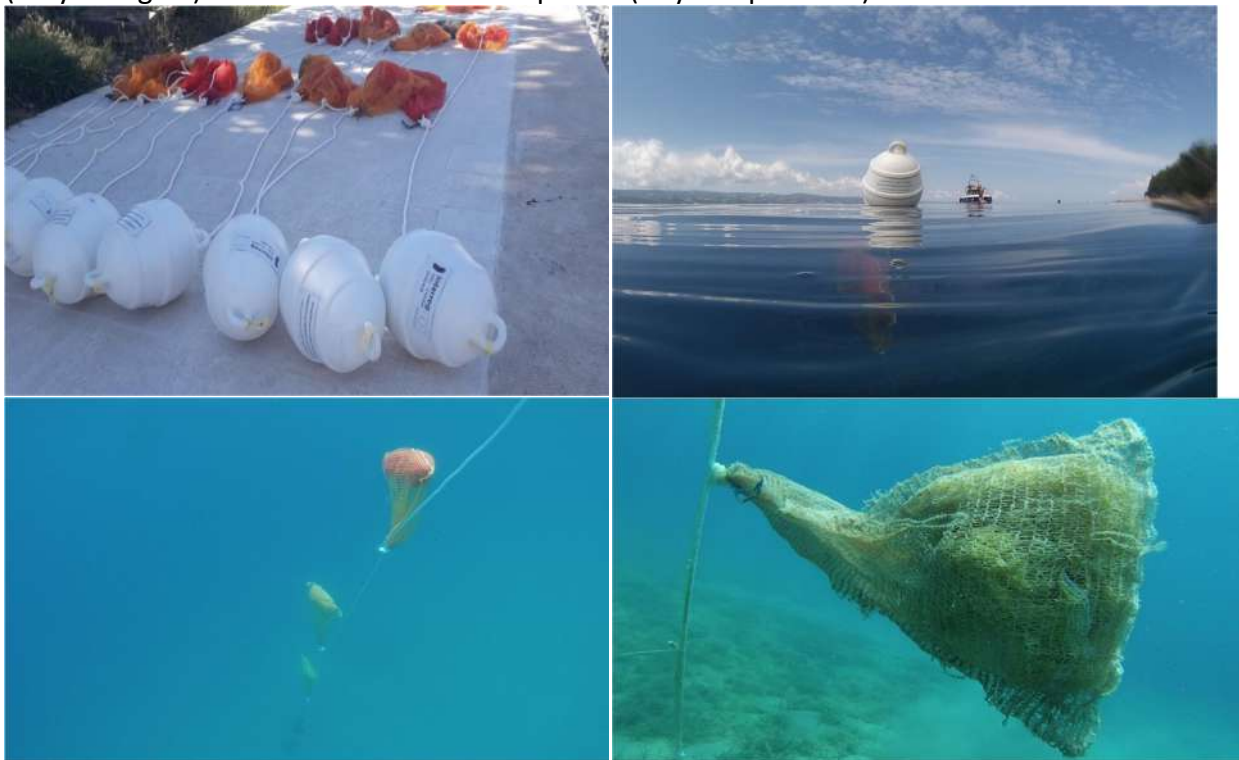
**Figure 5.** Sites for monitoring of Noble Pen shell (*Pinna nobilis*) recruitment: 1) Water polo playground (HTRS96 515605.220; 4811327.260, 4m depth), 2) Shallow mark (HTRS96 515345.760; 4810867.340, 6m depth) and 3) Mala Luka Bay (HTRS96 517358.460; 4808803.840, 12m depth). Map source: Bioportal, ©2019 Hrvatska agencija za okoliš i prirodu.

Water polo playground before MME was location with highest population density for the Cetina Estuary site, 57 individuals/m<sup>2</sup> (Kurtović Mrčelić J., 2019). Shallow mark was location with mid density population while Mala Luka Bay had few scattered individuals. Mala Luka is however characterized by sea currents from the south that transport water masses towards the first two locations. In 2020. permanent mooring lines were constructed for installation and removal of larval collectors each year. An anchoring screw is placed in the sea bottom holding all mooring line floating thanks to the floating buoy, with CASCADE visibility elements, placed at the sea surface. Each location has 3 replicas spaced apart minimum 10m. Each year *P. nobilis* larval collectors were constructed according to Kersting D. et al. (2019) *Short guidance for the construction, installation and removal of Pinna nobilis larval collectors* and placed at different depths with a mutual distance of 2 m (Figures 6 and 7). The dimensions of each collector are 50x80 cm. In such a way, while floating up and down, collectors collect marine organisms carried out by sea currents from the whole water column.



**Figure 6.** Schema of *Pinna nobilis* larval collectors with 3 replicas placed at one site

Larval collectors are placed in the water column by SCUBA diving at latest in June, and removed at earliest in October (Table 5) to cover the main reproduction period of *P. nobilis* (May – August) and the main settlement period (July – September).



**Figure 7.** Installed *P. nobilis* larval collectors

Each removed collector was processed by 1-2 persons in separate 70L container filled with fresh seawater, where all fouling organisms were manually separated. Organisms from Pinnidae family were identified first and if found removed in a separate smaller container and measured. Then all content of the collectors was filtered through a 30µm sieve and stored in 96% alcohol until further laboratory analysis (Figure 8).

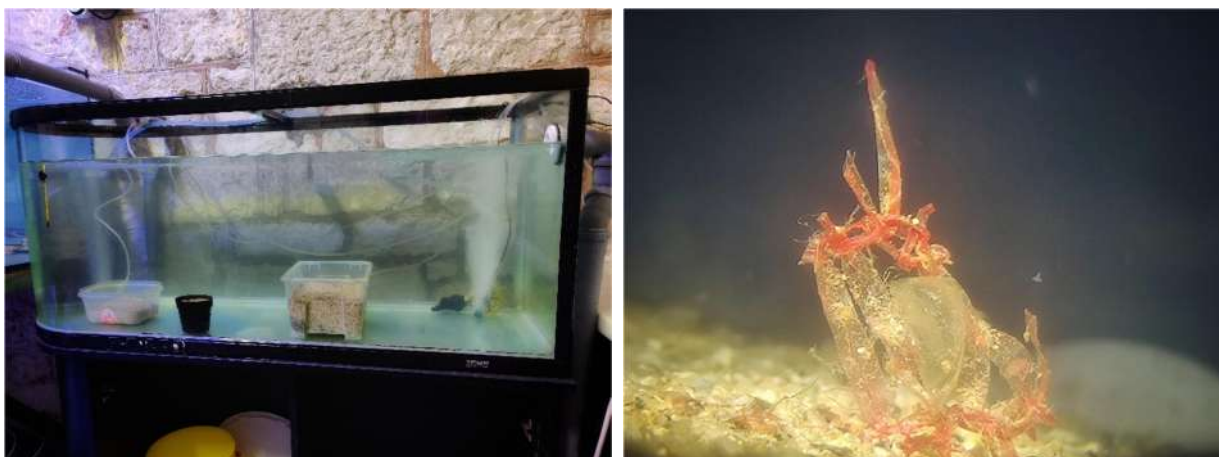


**Figure 8.** Processing of fouling community including individuals from Pinnidae family

### **3.7.2. Outcomes of the restoration actions and tests**

In 3 years period, only in year 2020 recruitment was recorded. One individual from Pinnidae family of maximum antero-posterior shell length (Ht) of 12 mm was found on the 2<sup>nd</sup> replica mooring line, in collector placed at 6m depth, at Mala Luka Bay site (Table 5). The found individual was transported to the Pula Aquarium in a dedicated portable aquarium for breeding in ex-situ conditions (Figure 9). Furthermore, the individual was identified as *Pinna nobilis* specie. In ex-situ conditions individual reached 32 mm (Ht) in half-year period (growth of 20 mm). However, individual died in June 04<sup>th</sup> 2021. Further tastings on the presence of pathogens show that the individual was positive for *Haplosporidium Pinnae* (Bel Dajković M.

A., June 2021).



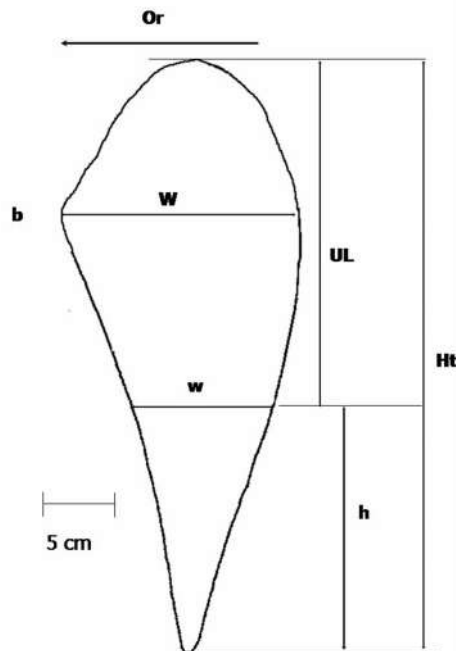
**Figure 9.** Breeding of *P. nobilis* in ex-situ conditions in Aquarium Pula

site	coordinates_HTRS96	depth_m	date installed	date recovered	No. p. rudis	N. p. nobilis	No. Pinctada
Water polo playground	515605.220; 4811327.260	4	12.06.2020	10.11.2020	0	0	0
Shallow mark	515345.760; 4810867.340	6	12.06.2020	28.11.2020	0	0	0
Mala luka bay	517358.460; 4808803.840	12	17.06.2020	12.11.2020	0	1	1
Water polo playground	515605.220; 4811327.260	4	25.05.2021	01.10.2021	0	0	0
Shallow mark	515345.760; 4810867.340	6	25.05.2021	10.11.2021	0	0	0
Mala luka bay	517358.460; 4808803.840	12	25.05.2021	11.11.2021	0	0	0
Water polo playground	515605.220; 4811327.260	4	28.04.2022	28.10.2022	0	0	0
Shallow mark	515345.760; 4810867.340	6	10.05.2022	28.10.2022	0	0	0
Mala luka bay	517358.460; 4808803.840	12	10.05.2022	3.11.2022	0	0	0

**Table 5.:** Results from monitoring of *P. nobilis* recruitment

In February 2022, thanks to citizens science approach developed through national project *Conservation of the noble pen shell in the Adriatic Sea* (haop.hr/hr/projekti; <https://www.facebook.com/CROpinnanobilis>), a live in-situ individual of Noble Pen shell was recorded at Cetina Estuary near local Aquapark (coordinates: 43.439110, 16.689698) at 2m depth inside *Cymodocea nodosa* meadow. After the start of the MME in the Croatian part of the Adriatic Sea in 2019, this is for now the only recorded living individual of the Noble Pen shell south of Zadar and has a symbolic name *New Hope*.

Dimensions of the individual shell were taken to estimate maximum antero-posterior shell length (Ht). According to Garcia-March et al. (2007) three measurements are basic to estimate Ht, i.e. maximum and minimum width (W and w) and unburied length (UL). Gape orientation (Or) is also important from an ecological point of view (Figure 10). Measurements were taken by a ruler (Figure 11b).



**Figure 10.** Measurements of interest to estimate maximum shell length (Ht) of *Pinna nobilis*. b, bend. h, length of the buried part. Or, orientation of the gape. UL, unburied length. W and w maximum and minimum width (García-March et al., 2007).

Several equations have been proposed up to date, based on simple or multiple regression, for the estimation of Maximum shell length (Ht) of *Pinna nobilis*, although probably each population will have intrinsic parameters in the regression between Ht and unburied shell dimensions.

Equation by de Gaulejac and Vicente (1990) for the Population of Diana lagoon (Corsica)

$$Ht = 2.186W + 1.6508$$

$$R = 0.98$$

Equation By García -March and Ferrer (1995) for the population of Moraira (Spain)

$$h = 1.79w + 0.5$$

$$R = 0.99$$

$$Ht = h + UL$$

Equation By García -March (2006) for the population of Moraira (Spain)

$$Ht = 1.29W^{1,24}$$

$$R = 0.98$$

Based on these equations maximum shell length (Ht) of New Hope was estimated at the time of its discovery which was between 22 and 25 cm (Table 6). Although age and growth

estimates of this species are not available for the Cetina Estuary population, according to data obtained for other Mediterranean populations living in similar environments it is possible to suppose that New Hope is 3 to 4 years old (García-March et al., 2020). New Hope is in that case originated from the recruitment of year 2018 or 2019. It is an individual that resisted to all causes that generated MME withing *P. nobilis* populations at Cetina Estuary in 2019.

New Hope already survived developmental phase when there is a high risk of predation. Individual settled at very shallow depth, of 2m only, where the impact of waves from the south and southeast quadrants is considerable during winter time. Fortunately, it settled in *Cymodocea nodosa* meadow that provides him a great anchoring position for its byssus threads. It also settled near Aquapark which is an opportunity and a threat to the individual at the same time. Opportunity because boats do not anchor neither catch fish at that location and threat because there is a high concentration of Aquapark visitors that can damage the individual. However individual is far enough from the Aquapark so visitors cannot jump on it. Another benefit is floating beach fens. Aquapark and beach fens are installed during the summer season only, beginning of June, and retrieved beginning of October. Because of all these circumstances decision was taken to construct a cage which is not completely closed and has a shape of a tent (Fig. 7a). Because of shallow depths (not possible to put a massive concrete block) and *Cymodocea nodosa* meadow presence, cage was anchored with an eco-mooring system, a screw screwed 0,5m deep into the sand at 2 opposite sides of the cage. In such a way there is a lower waves impact on the cage, lower overgrowth of the cage, individual is accessible for taking measurements and at the same time is protected from eventual mechanical damage. In addition, warning panels were put at each side of the cage to inform visitor that the species is strictly protected and should not be disturbed. Its condition is being monitored each month from May to November, and once during the winter period (Figure 11c). During a period of 15 months, the individual has grown at least 10 cm and his maximum shell length (Ht) is estimated to be between 34 cm and 38 cm (Table 6).



**Figure 11.** Living specimen of the Noble Pen shell New Hope: installation of the cage over the individual (a), taking of the measurements with a ruler (b), individual taken in photo during winter time.

Name	Date	w (cm)	W (cm)	UL (cm)	Ht1 (cm)	Ht2 (cm)	Ht3 (cm)
New Hope	24.02.2022.	5	10,6	12,6	24,1	22,1	24,8
New Hope	06.06.2023.	7,7	15,2	19,8	37,7	34,1	34,9
<b>Difference</b>	15 months	2,7	4,6	7,2	13,6	12,0	10,1

**Table 6.** Estimation of New Hope maximum shell length (Ht).

### 3.8. P10 outcomes of restoration tests and actions on costal dunes habitats on central Adriatic coast

Only 2 years after the boardwalks' installation across the dune systems in the central Adriatic coast, quite encouraging effects of the implemented conservation action were observed. Species richness increased in all macro-habitats, especially in the first vegetation communities near to the shoreline.

Notice that the effects of trampling, as well as changes in species cover or richness, strongly vary across dune zonation. In dune grasslands some focal species showed a cover increase, such as *Vulpia fasciculata* and *Silene colorata* as, the number of ruderal and exotic species also increased after the boardwalks' installation.



Dune grasslands, a mosaic of nitrophilous annual species with ephemeral spring blooming, resulted the most affected by the presence of fast-growing ruderal and exotic species

It was observed minor changes in the more stable fixed dunes macro-habitat dominated by woody vegetation, which was less impacted by the transit of the bathers. These habitats are characterised by a greater density of vegetation, sometimes spiny, so visitors usually avoid them and prefer to use well delineated paths through the pine forest to reach the sea.

The tests areas implemented by UNIMOL on central Adriatic coastal sand dunes pinpointed a positive effect of boardwalks in promoting fast recovery of dune vegetation, especially of beach and mobile dune, both in terms of species richness and cover. In fact, previous studies reported that in dune ecosystems plant communities improved after excluding trampling pressure avoiding bather's access through boardwalks or stabilising and reversing shoreline erosion.

#### 4. Recommendation for future restoration and conservation actions in the Pilot areas

The future restoration actions will be based on the changes in local and cross-border policies and their long-lasting effect on the state of the Pilot sites. The results of a multidisciplinary integrated analysis of existing data and the results of CASCADE experimental tests and existing studies, as well as the results of the research campaign carried out through the project, should include the impact of all investigated relevant factors on biodiversity as well as the local society, and mitigation methods should be defined. Of utmost importance is that this information will be usable in the future to meet the needs of nature conservation, spatial planning, flood protection systems, and addressing cross-border issues.

Here the main conclusions for the different case studies are summarized.

The studies carried out on the native *S. maritimus* cordgrass in coastal lagoons and other lagoons along the North Adriatic sea (Pilot 2) have shown that restoration of diverse communities presents a challenge when not all species within a target assemblage have the same capacity for recruitment and establishment in restored areas. Species with high recruitment rates or high levels of stress tolerance can dominate restored sites and create monospecific or low diversity assemblages. These results should be considered when planning conservation actions that aim to facilitate the growing and the spreading of the native species instead of the invasive one. Overall the restoration of *S. maritimus* cordgrass at P2 sites south of Venice lagoon seems feasible, but it is unclear how such restoration would respond to future climatic stressors. Further experiments are now in progress to understand how to future proof any restoration actions in the region.

The measures derived as a conclusion of a participatory process in the Neretva River delta (Pilot 4) include:

1. Encouraging the improvement of cross-border cooperation with Bosnia and Herzegovina, regarding waste management and Neretva River basin water status as well as joint scientific research and work when defining optimal chemical parameters related to good status of waters and autochthonous species.
2. Advocating the importance of including geological research of the rocks and sediments around surface and ground water bodies with the aim of defining the impact on quality regarding the origin of heavy metals in water.
3. Advocating changes in the legislative framework and encouraging the continuation of the Neretva area nature park declaration in joint action with Bosnia and Herzegovina with the aim of protecting the entire ecosystem of the Neretva area
4. Raising farmer's awareness of environmentally friendly agriculture and the importance of farmers' joint action in terms of environmental pollution and climate changes consequences.

5. Education and raising awareness of the local population about the importance of nature protection
6. Advocating the development of a joint authority's system for water and coastal pollution protection and notification system for deviations outside of optimal range in Croatia and Bosnia and Herzegovina.

The measures derived as a conclusion of the Nin Lagoon (Pilot 6) actions include:

- 1) The entire area of the Nin Lagoon is characterized by a high biodiversity index. The micro-site of the Miljašić jaruga, despite the fact that it was created by anthropogenic influence, i.e. by construction with the filling of large stones, is characterized by high biodiversity of mollusk species. Rare species of sea gastropods stand out in particular.
- 2) Underwater research has also indicated the once numerous presence of the endangered species of the noble pen shell *Pinna nobilis*, whose population is now significantly reduced due to the occurrence of mass mortality. Nevertheless, of all surviving individuals in the area of Zadar County and beyond, the largest number of individuals was found during 2022 within the Nin Lagoon and near the Miljašić ravine itself.
- 3) During the planned activities for the construction of future interventions at the mouth of the Miljašić ravine, the technical interventions described above are proposed, which should respect the biological characteristics of the currently present species, so as not to damage the biodiversity of this area.

The analysis and evaluation of floristic and ecological changes in Biferno brackish wetland (Pilot 7) after a restoration intervention evidenced variations on ecological features and species occurrence and abundance pattern across the different EU habitats conforming the brackish mosaic: salt meadows, halophilous scrubs and salt steppes (respectively 1410, 1420 and 1510\*). Such changes are most likely related to an intertwining of environmental changes (restoration actions, climate change and coastal erosion).

It was observed, after the restoration action, a general improvement of the naturalness of the Biferno mouth with a successional process that led the salt marsh mosaic towards typical paucispecific plant communities. Moreover, the observed expansion of hypersaline communities may be also related to other environmental drivers as climate change (e.g. rise of local temperatures, the decline of summer precipitation) and coastal erosion that affected this section of Adriatic coast. These environmental changes likely exposed Biferno wetland to an increase on water salt concentration and to a greater influence of salt aerosol which favored the expansion of halophilous diagnostic species and the rarefaction and loss of ruderal and alien plant taxa.

The excessive expansion of hypersaline coastal wetlands (e.g., succulent plant-dominated marshes) at the expense of other coastal wetland types requiring higher rainfall and freshwater inputs (e.g., graminoid-salt marshes), may be mitigated through further

restoration actions removing artificial drainages to increase the intake of freshwater in the brackish area.

The implemented work contributes to improve the current knowledge on the vegetation dynamics after a restoration intervention on coastal brackish areas and our observations results demonstrate that, before adequate hydraulic work and reduced human pressure, these fragile ecosystems, are able to recover in a few years.

It was collected valuable information for coastal salt marshes monitoring of conservation initiatives in the Mediterranean and it was asserted that the re-visitation approach, based on historical plots represents a cost-effective monitoring procedure that matches the need of periodical reporting requested by the European HD.

The studies carried out on the restoration of *F. Virsoides* in P8 have shown that while *F. virsoides* remains on the brink of regional extinction on the Istrian coast, ex situ cultivation and planting of this species using a simple method is possible, at least at the small-scale. This was primarily successful due to the unpredictably fast growth of the *F. virsoides* after the planting and the resilience of the grown thalli. Even though the growth of the planted *F. virsoides* showed some differences according to the positioning in the intertidal zone, this does not present an obstacle for the possible further restoration attempts on the Istrian coast. Further research pertaining to the interactions of this species within its restored environment will help inform best practises for scaling up restoration techniques with the goal of re-establishing new settlements in the future.

Concerning the implementation of boardwalks and other soft protection actions to protect coastal dune ecosystems at Torre del Cerrano (Pilot 10) as well as for the central Adriatic sandy coasts, the positive effect of such actions can be affirmed. Still, there is often a general lack of knowledge among residents and politicians about how vegetation can stabilise dunes and protect the beach by sea erosion, how plants can be damaged by trampling and, thus, why management initiatives, such as boardwalks, dune fence and planting events, are valuable. An important issue to determine concerns how tourism and human activities on the coasts can assist with dunes conservation rather than hindering it. Although limiting dune access and trampling is important to support conservation actions in Mediterranean coastal areas affected by mass tourism, this should be coupled with the promotion of a nature-aware tourism, where informed citizens are encouraged to monitor and conserve biodiversity as part of their own natural heritage. Environmental researchers have the big responsibility publicizing the importance of nature conservation through a focused dissemination in line with socioeconomic needs. An integrated environmental management that envisages citizens as the leader of their own future can be aspired to only through dialogue and knowledge transmission.

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## 6. List of figures

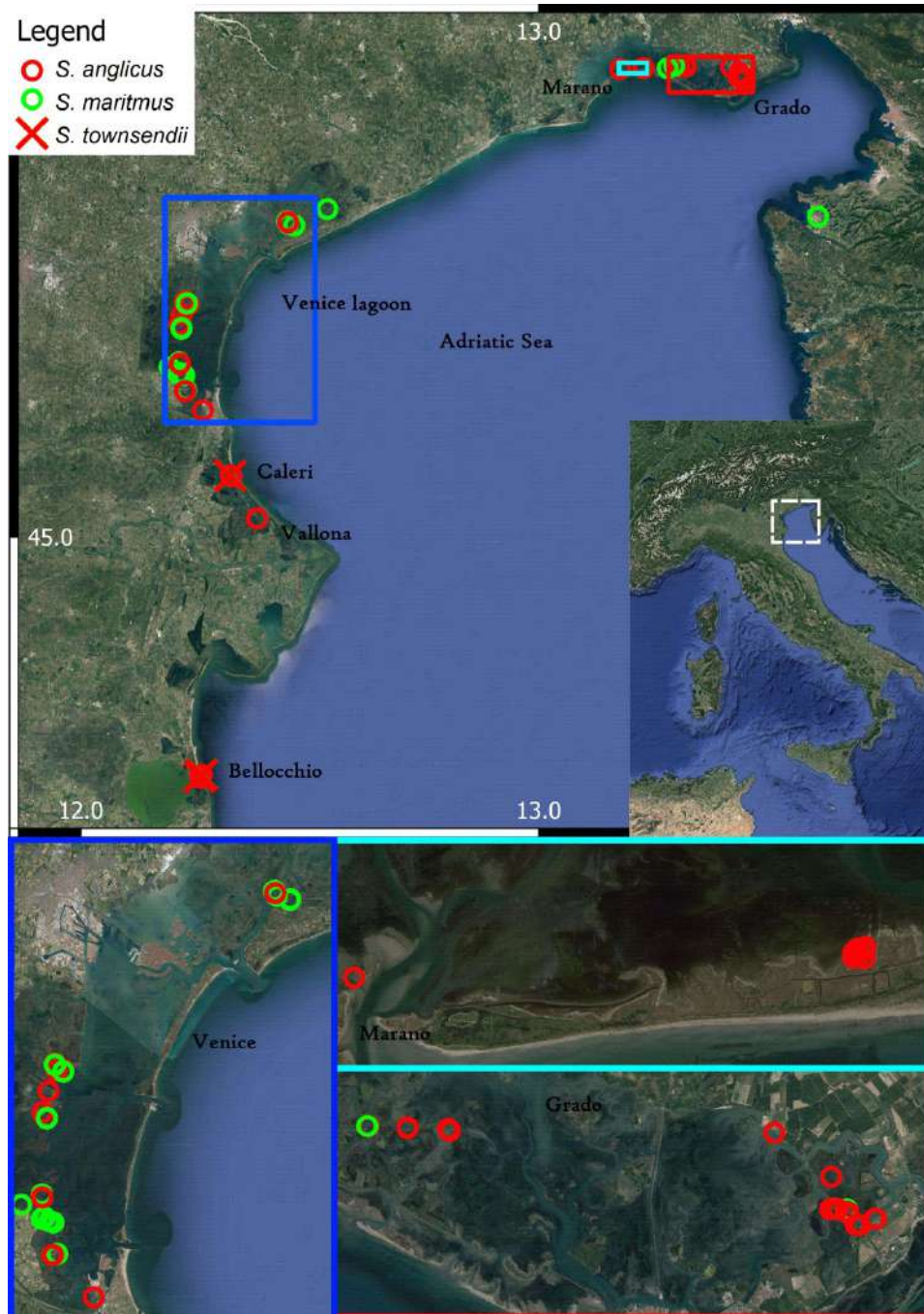


Fig. P2. 1. Map showing the spatial distribution range of *Sporobolus* species from Ravenna (Italy) to Seča (Slovenia).

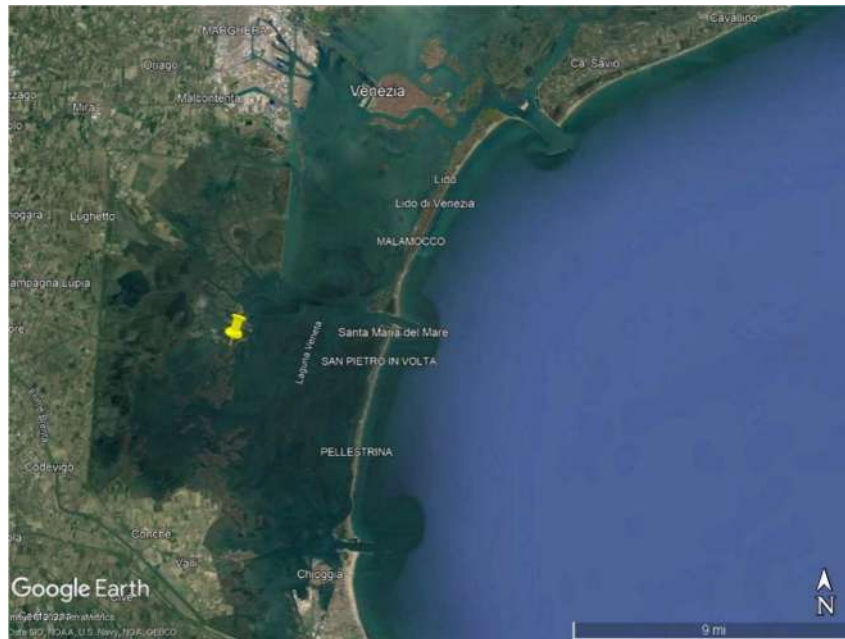


Fig. P2. 2. Map showing the location (yellow pin) in the Central Venice Lagoon where the transplant experiment with *S. anglicus* and *S. maritimus* was carried out.



Fig. P2. 3. (Top left) Plots covered with jute fiber for substrate preparation. (Top right) Plots with transplants of *S. anglicus* and *S. maritimus* on transplantation day in July 2022.

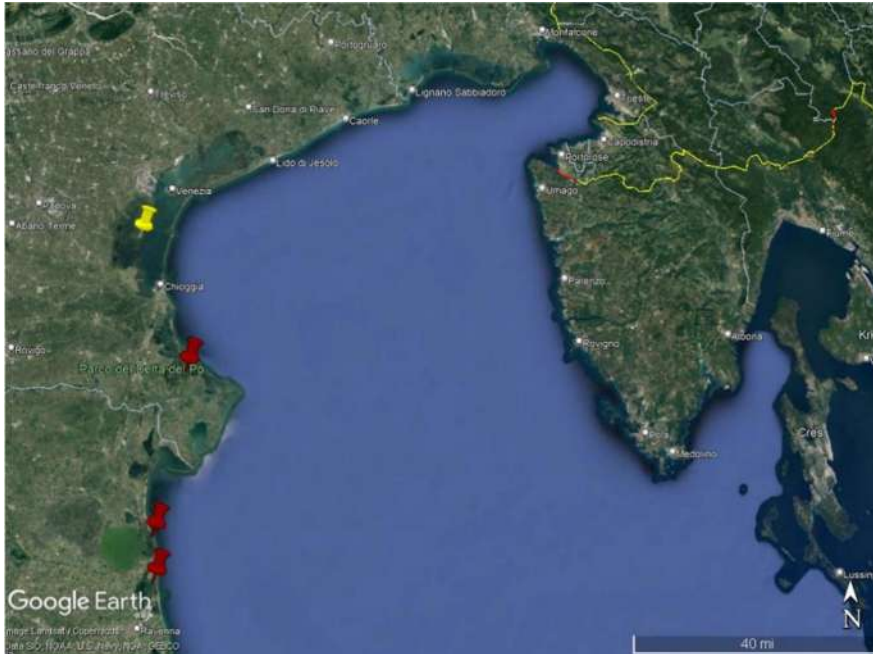


Fig. P2. 4. Map showing the position of the three sites involved in the *Sporobolus maritimus* transplantation: (yellow pin) transplant donor site in the Venice Lagoon, (red pins from north to south) Vallona (Rosolina), Bellocchio and Baiona Lagoon.



Fig. P2. 5. Transplants photos on the transplantation day on the 7th-8th July 2022: (Top left) control transplant at the donor

site, (top right) transplants at Vallona (Rosolina) site, (bottom left) transplants at Bellocchio site, (bottom right) transplants at Baiona site.

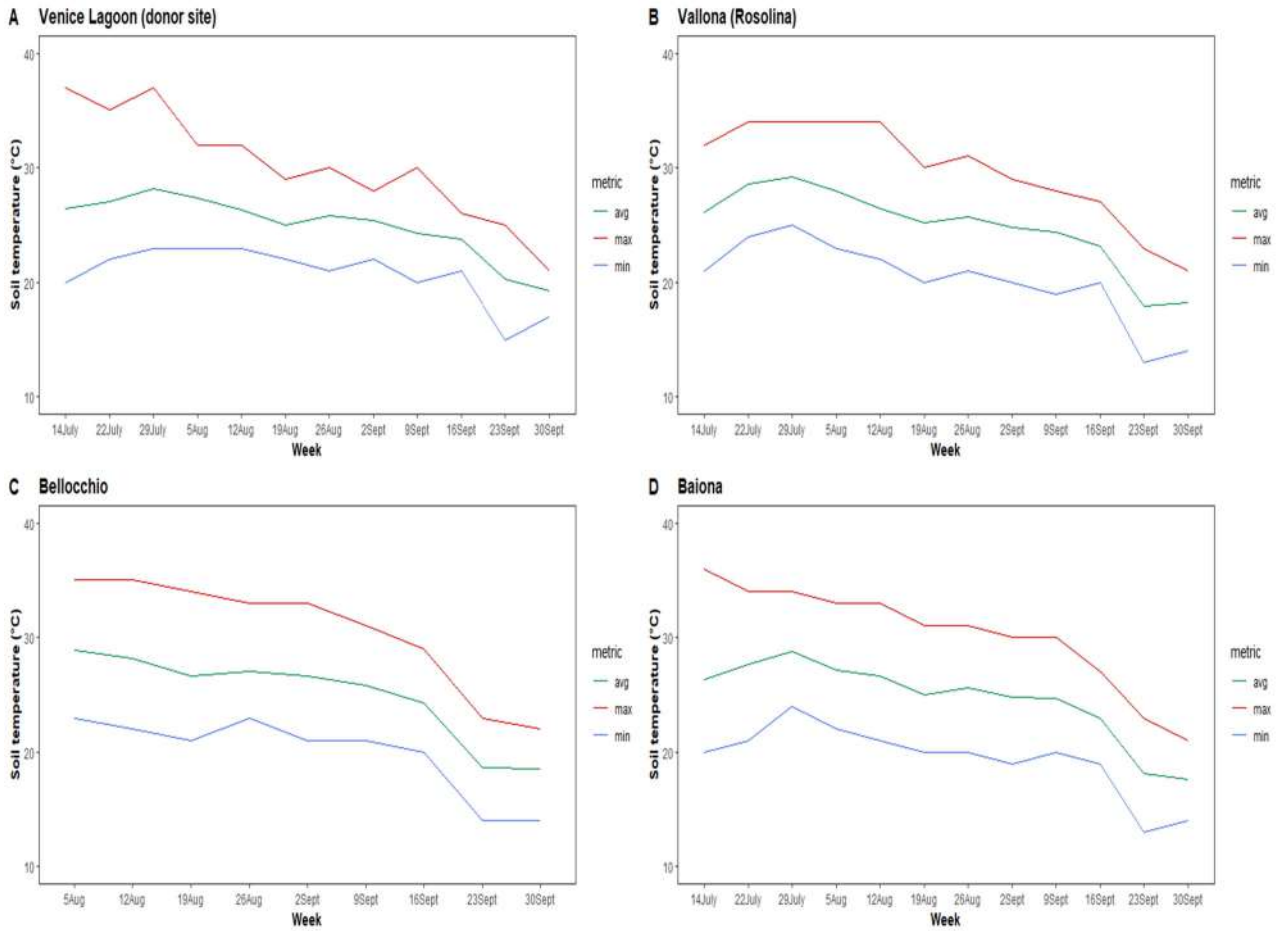


Fig. P2. 6. Graph showing average (green), maximum (red) and minimum (blue) temperature weekly data measured with Hobo data loggers at the four sites from July to September. (Bellocchio data of July are missing due to the malfunctioning of a Hobo sensor).



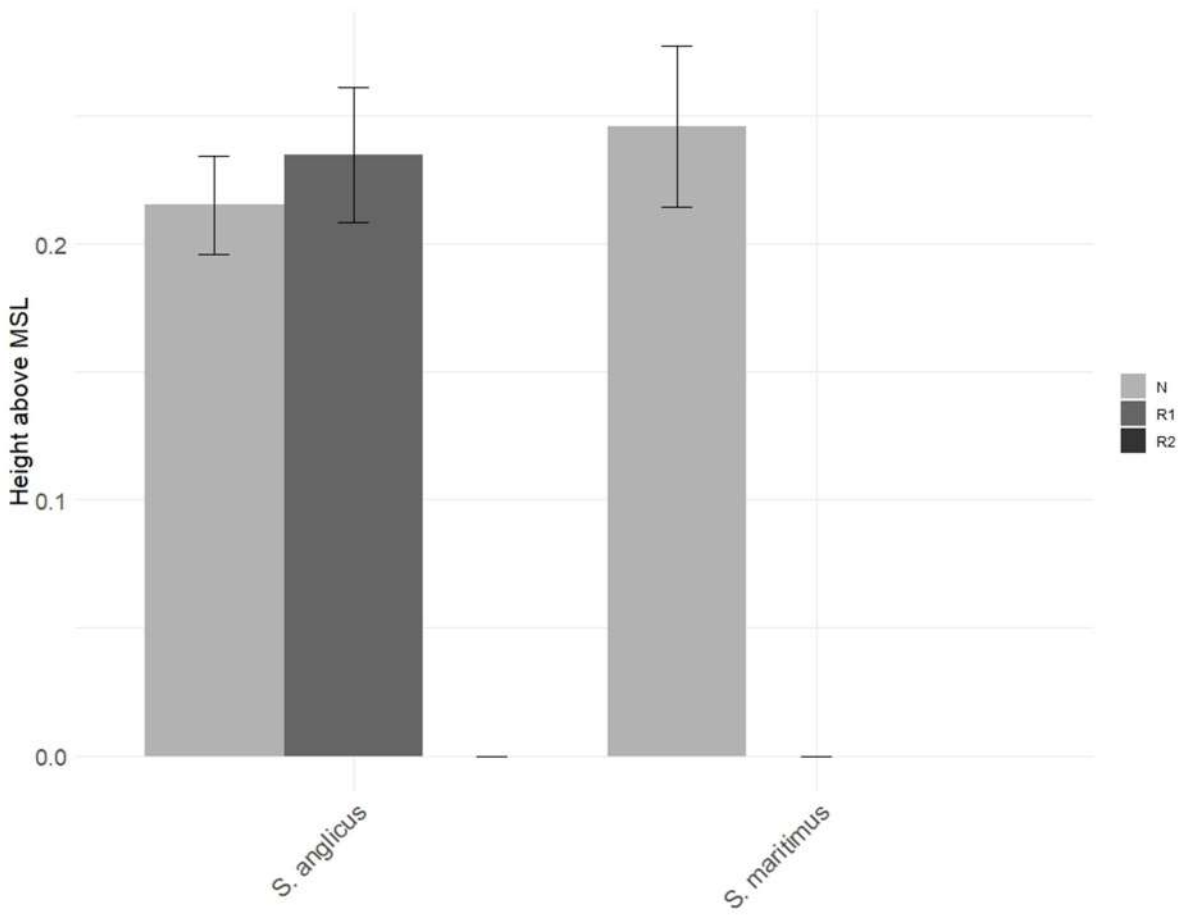


Fig. P2. 7. Barplots showing the soil height above MSL of the foreshore at natural (N), restored type 1 (R1) and restored type 2 (R2) sites. Bars represent Mean  $\pm$  SE.

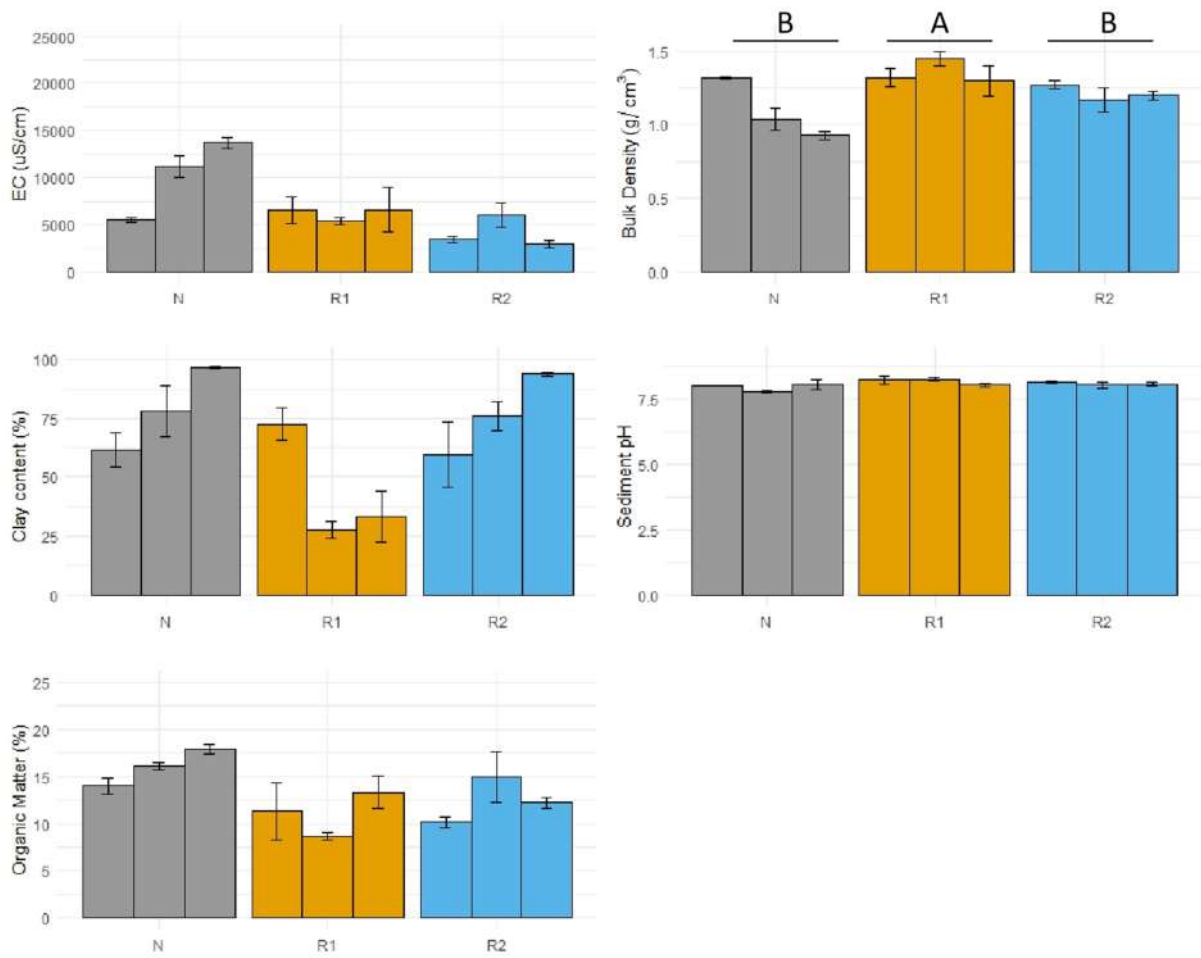


Fig. P2. 8. Bar plots showing EC, BD, Clay content, pH and OM values measured at natural (N), restored type 1 (R1) and restores type 2 (R2) sites. Bars represent Mean  $\pm$  SE. Letters denote statistically significant differences at  $\alpha = 0.05$ .

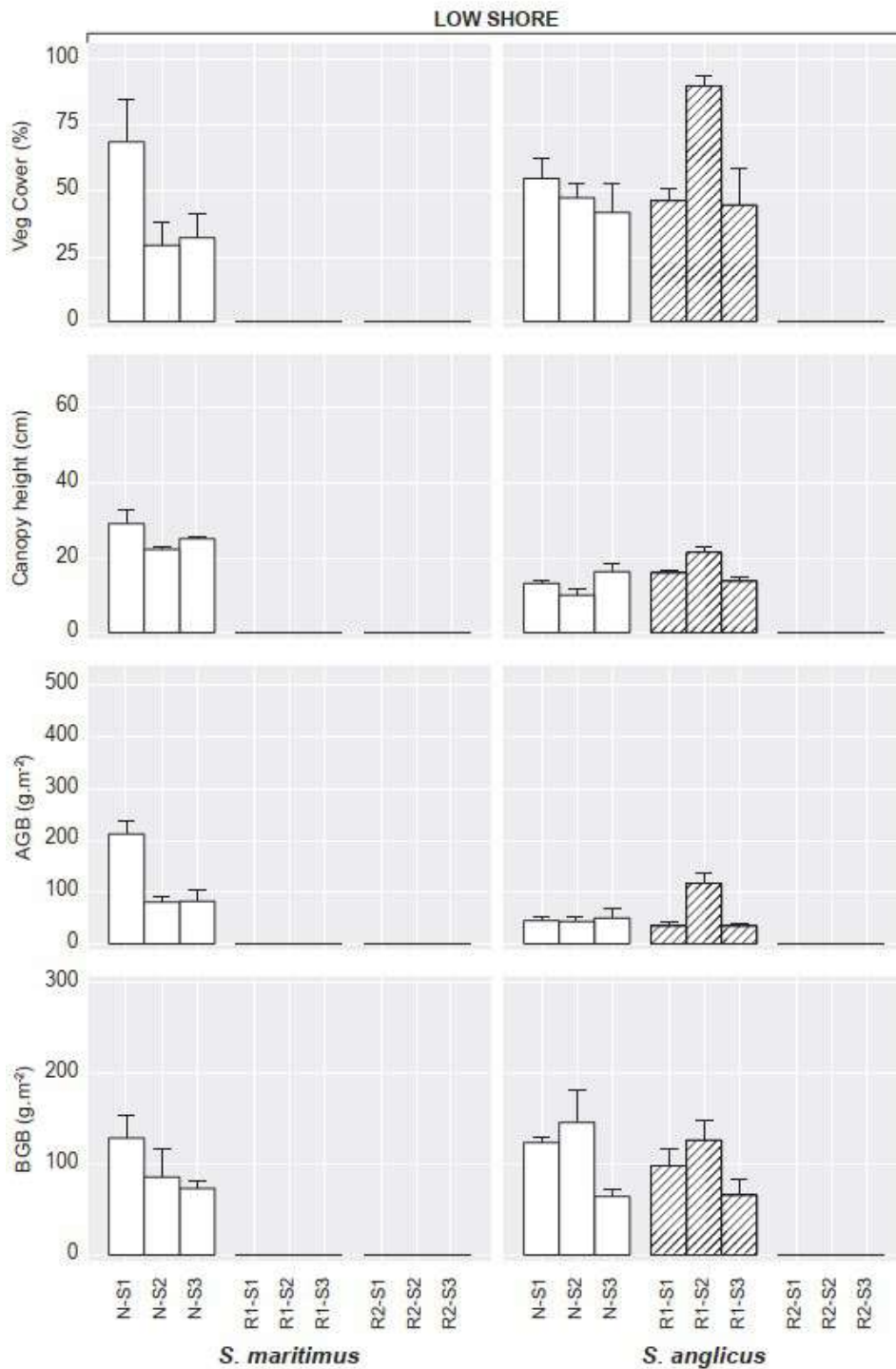


Fig. P2. 9. Bar plots showing vegetation characteristics (Veg. Cover, Canopy height, AGB and BGB) of *S. maritimus* (left) and *S. anglicus* (right) at natural (N), restored type 1 (R1) and restored type 2 (R2) sites. Bars represent Mean  $\pm$  SE.

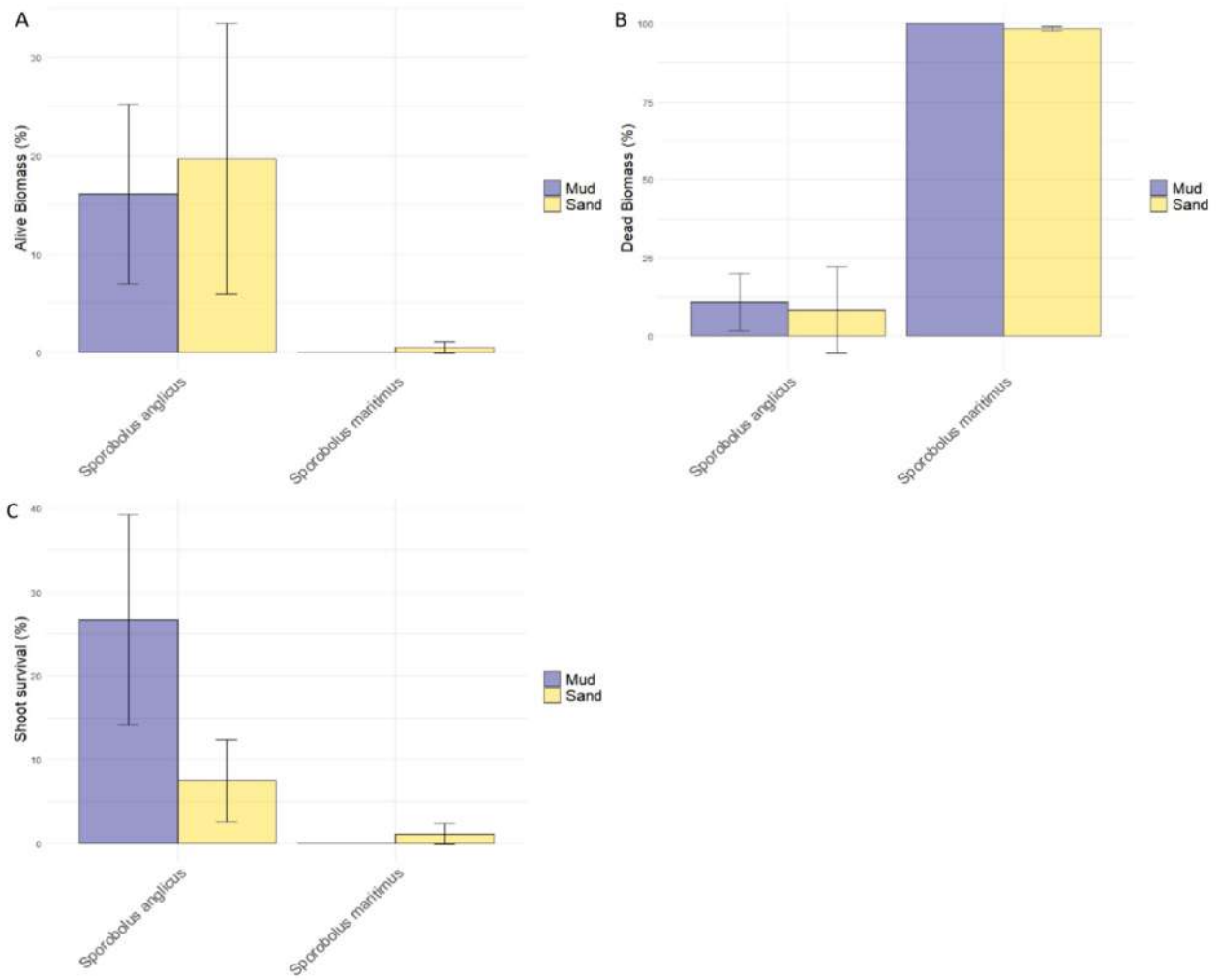


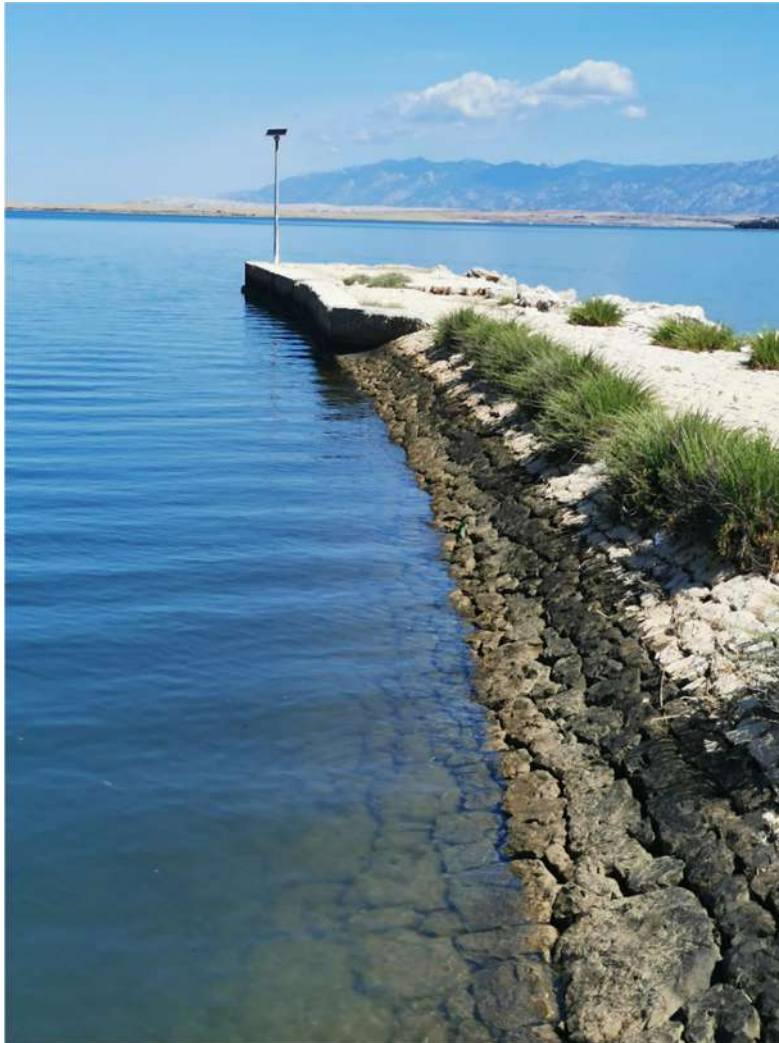
Fig. P2. 10. Percentage of alive biomass (A), dead biomass (B), and survived shoots (C) of *S. maritimus* and *S. anglicus* in mud and sand sediment type. Bars represent Mean  $\pm$  SE. In *S. maritimus*: mud N = 4, sand N = 6. In *S. anglicus*: mud N = 6, sand N = 4.



*Fig. P2. 11. Area in which an environmental requalification program for recovering wetland ecosystems was implemented on the central Adriatic coast.*



*Fig. P2. 12. wooden broad-walk installed on central Adriatic coast for protecting sand dunes from trampling and for aiding nature to re-establish the natural dune coastal zonation.*



*Fig. P6. 1. Site Miljašić jaruga during summer 2022.*



*Fig. P6. 2. Rare gastropod Turbonilla sp. found at the site.*



*Fig. P6. 3. Rare gastropod Ondina modiola found at the site.*





*Fig. P6. 4. Rare gastropod Alvania rudis found at the site.*



*Fig. P6. 5. Noble pen shell Pinna nobilis individual before the mass mortality event.*



*Fig. P6. 6. Collectors checked for the presence of larvae.*



*Fig. P6. 7. Individual of Pinna nobilis within the protective cage in Nin lagoon.*

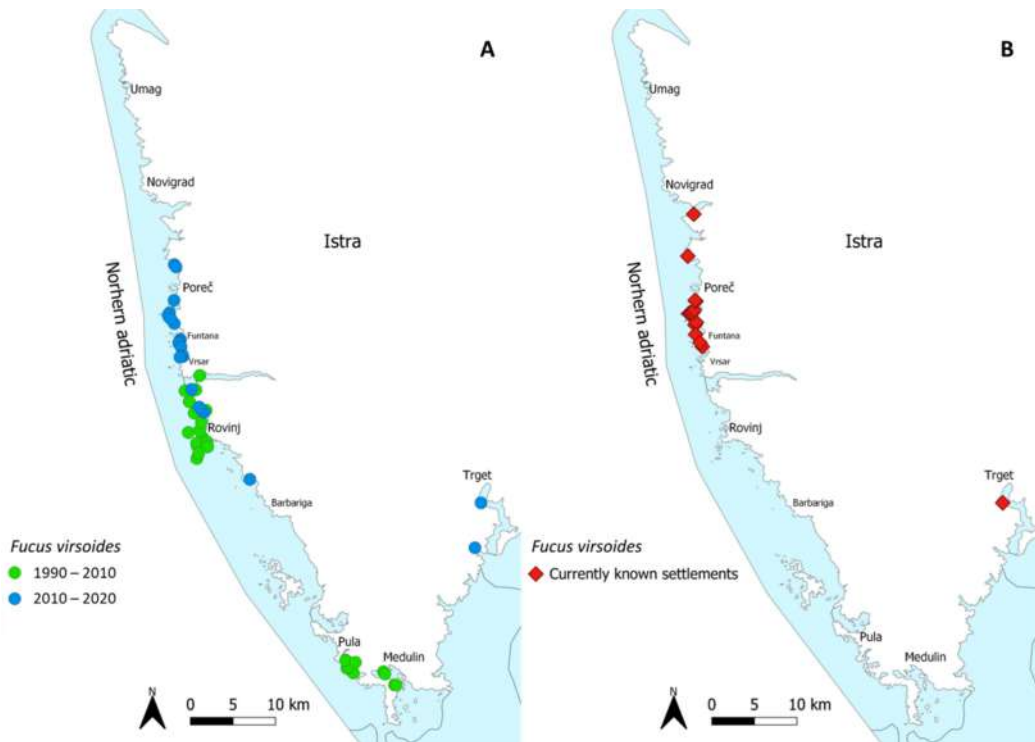


Fig. P8. 1. Historically known settlements of *Fucus virsoides* along the Istrian coast from 1990–2020 (A) and its current known settlements from mapping in 2021 (B).

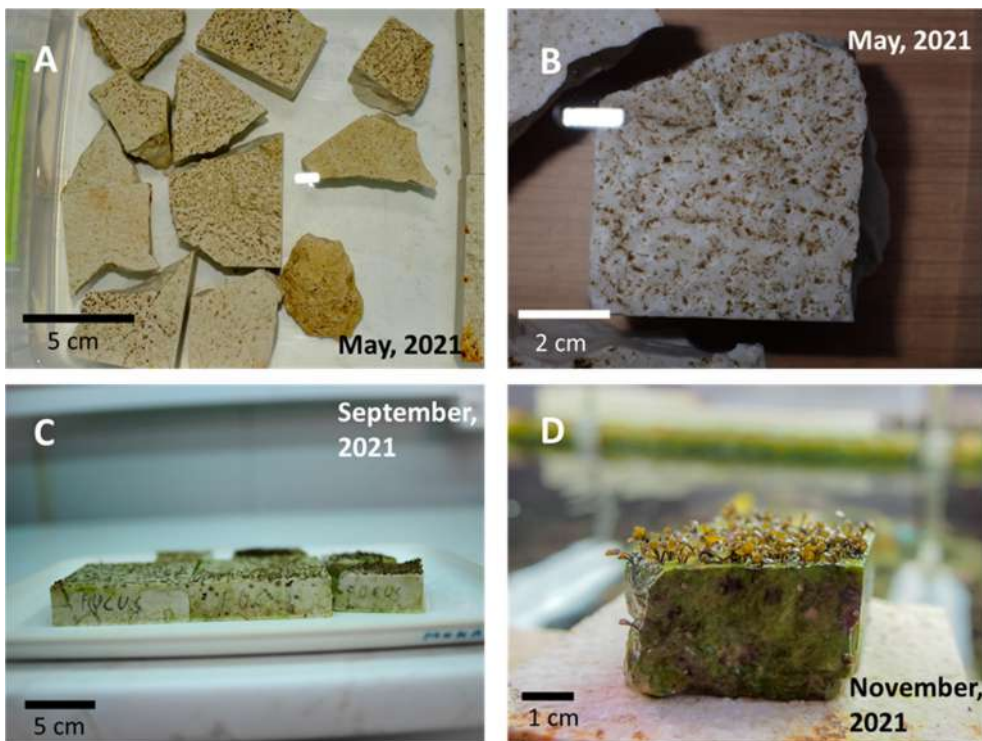


Fig. P8. 2. Early growth of *Fucus virsoides* recruits from May (A, B), September (C) and November 2021 (D).



Fig. P8. 3. Post planting growth of *Fucus virsoides* over a one-year period. Photographs were taken in November 2021 (A), January 2022 (B), May 2022 (C), July 2022 (D), August 2022 (E) and November 2022 (F).

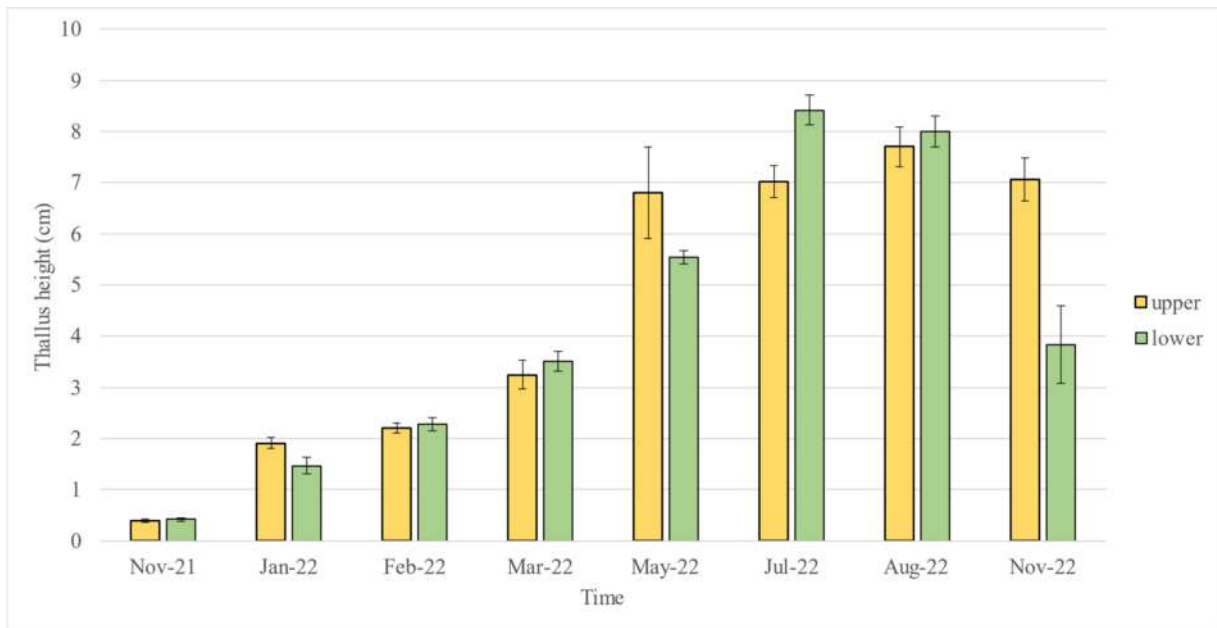


Fig. P8. 4. Growth of planted *Fucus virsoides* over a one-year period. Each measurement represents an average of 10 measured thalli. Data are presented as mean  $\pm$  SE. Colours represent upper (yellow) and lower (green) positions for plots.



Fig. P8. 5. Destroyed anti-grazer cage with consumed *Fucus virsoides*.



Fig. P8. 6. Fucalean species damaged during springtime low water-high air temperature event on southern (A) and western (B) Istrian coast.

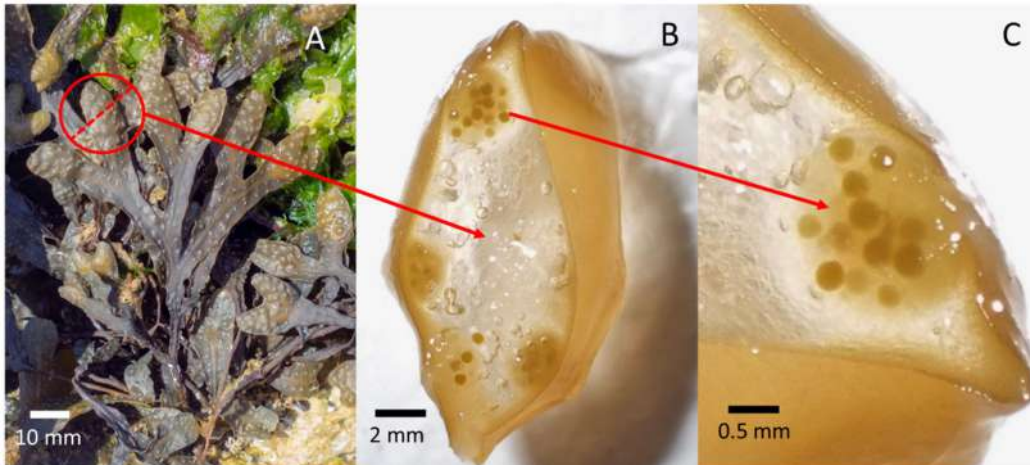


Fig. P8. 7. *Fucus virsoides* thallus with receptacle marked with a red circle (A), transverse section of a receptacle showing conceptacles (B), magnification showing conceptacle with mature gametangia (C).

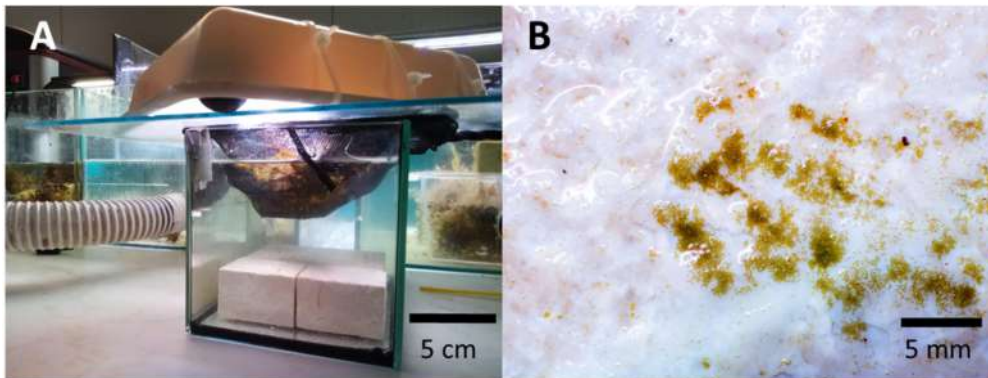


Fig. P8. 8. Seeding of *Fucus virsoides* onto limestone tiles (A) and early stage germlings (B).

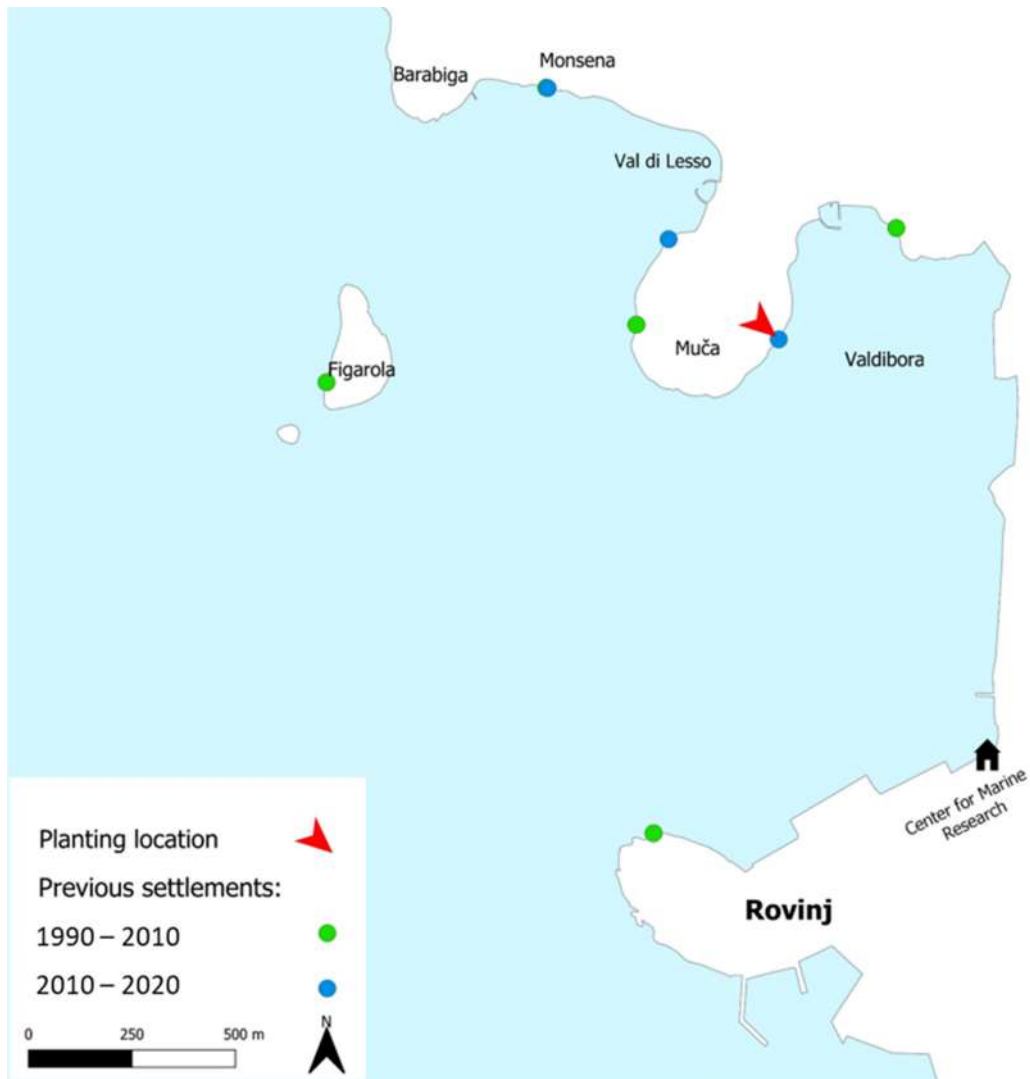


Fig. P8. 9. Past distribution of *Fucus virsoides* settlements in the Rovinj area. Area of planting is shown with a red mark.