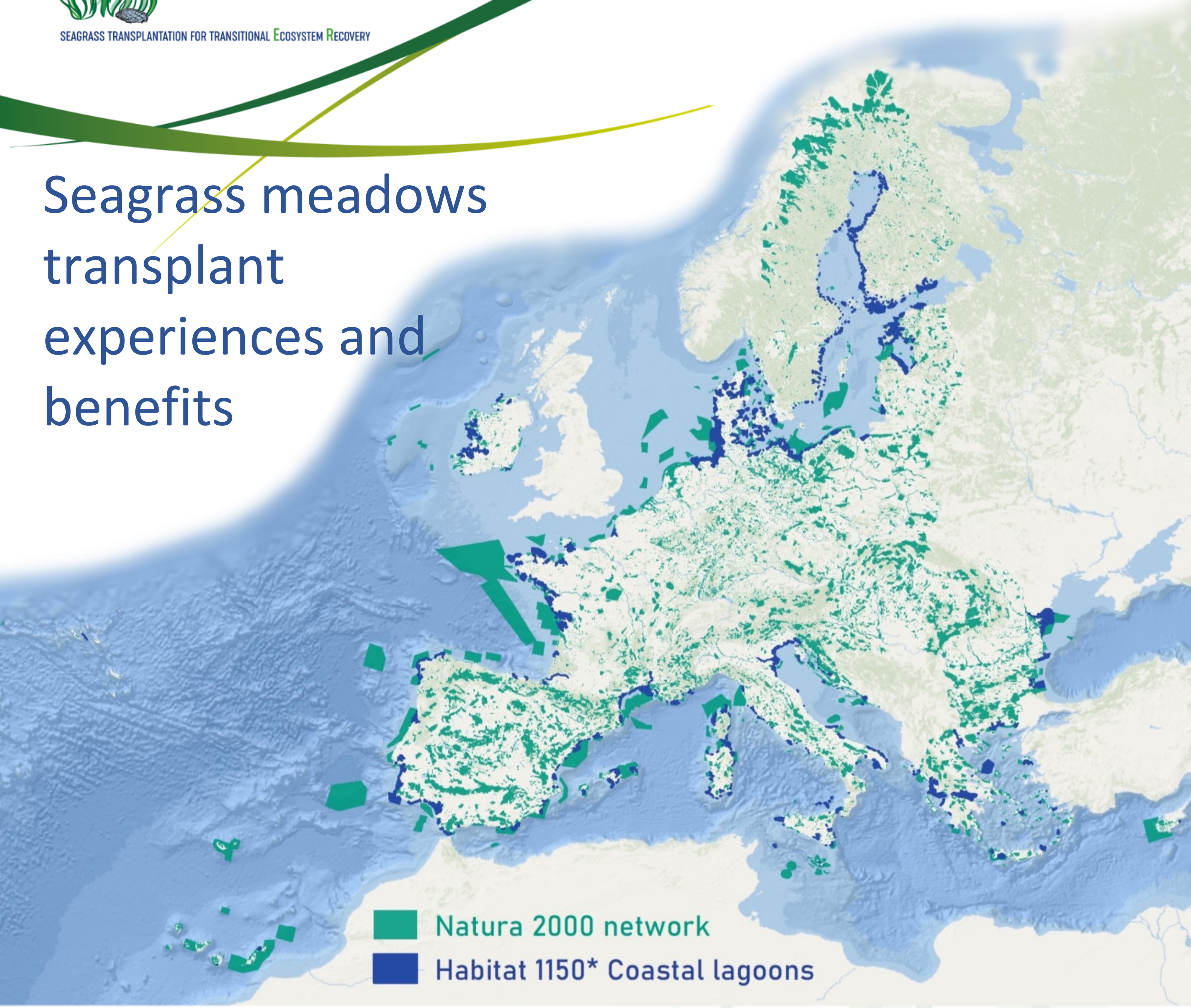
LIFE TRANSFER

SEAGRASS TRANSPLANTATION FOR TRANSITIONAL ECOSYSTEM RECOVERY

Handbook for Natura 2000 managers and technicians

Seagrass meadows transplant experiences and benefits



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Seagrasses

What seagrasses are and why we care

Seagrasses are marine flowering plants, which live permanently submerged, inhabiting shallow waters across various global regions, spanning from the tropics to the Arctic zones. This vast coverage makes them one of the most widespread coastal habitats on Earth. Seagrasses thrive as large submerged meadows, creating intricate, highly productive, and biologically diverse environments. With over 70 distinct species worldwide (Short et al. 2011), they live in 159 countries distributed over six continents, encompassing an area exceeding 300 000 km^2 (United Nations Environment, 2020).

Seagrasses deliver an array of valuable ecosystem services and nature-based solutions that profoundly enhance the well-being of both Earth's ecosystems and human populations residing in coastal regions also serving as significant actors in climate change mitigation and adaptation strategies. Despite occupying merely 0.1% of the ocean floor, these species function as remarkably efficient carbon reservoirs, storing as much as 18% of the global oceanic carbon (Bedulli et al, 2020; United Nations, 2022). Seagrasses can help to buffer and mitigate the effects of ocean acidification, thus contributing to the resilience of more delicate ecosystems and species, including crustose coralline algae and coral reefs. Furthermore, they assume a primary role as the first line of defence along coastlines, reducing wave energy and safeguarding local communities from floods and storms threats. Regrettably, seagrasses are among the coastal ecosystems with the lowest levels of protection (UNEP-WCMC et Short 2018; UNEP et IUCN 2019), frequently facing cumulative pressures arising from coastal urbanization, nutrient runoff, and the effects of climate change. A mere 26% of documented seagrass meadows lie within the boundaries of marine protected areas, in contrast to the 40% coverage for coral reefs and 43% for mangroves. The majority of seagrass habitats lack comprehensive management plans or safeguards against the impacts originating from human activities. A comprehensive analysis of 215 studies by Waycott et al., (2009) has uncovered a swift decline in seagrass habitats, experiencing an average annual reduction of 110 km^2 since 1980. Since the initial mapping of seagrass areas in 1879, about 30% of the previously known expanse has eroded, marking a considerable escalation in meadows coverage loss. Furthermore, no less than 22 out of the world's 72 seagrass species fall into the categories of Near Threatened, Vulnerable, and Endangered species on the International Union for Conservation of Nature Red List of Threatened Species. Noteworthy factors contributing to seagrass loss trend include: urban, industrial, and agricultural runoff, coastal urbanization, dredging, unregulated fishing and boating practices, and the influence of climate change and extreme climatic events (such as heat waves or raging storms). The repercussions of this decline in seagrass ecosystems extend to coastal biodiversity, inducing shifts in food chains and the depletion of harvestable resources (Sfriso et al., 2021). Despite the widespread global seagrass loss trend, regional improvements have been recorded at the regional level as numerous active projects for different species have so far been implemented. Preserving and restoring seagrass meadows offer the potential to address 26 targets and indicators associated with 10 Sustainable Development Goals (SDGs). These underwater ecosystems underpin a strategic significance for marine life, while concurrently delivering diverse advantages to terrestrial populations. Incorporating seagrass ecosystems within Nationally Determined Contributions is particularly significant due to their carbon storage and sequestration capacity, aiding nations in fulfilling their objectives outlined in the Paris Agreement and the United Nations Framework Convention on Climate Change (UNFCCC).

Figure 1: *Zostera marina* rhizomes ready for transplant.

The significance of seagrass ecosystems in climate change mitigation is emphasized by the potential emissions from worldwide seagrass degradation, that are estimated to approach 0.65 $GtCO_2$ annually (Hoegh-Guldberg et al. 2018). This emission level is approximately comparable to the annual emissions generated by the entire global shipping industry.

Seagrasses play a crucial role in sustaining approximately 20 percent of the planet's major fisheries (Unsworth et al. 2018). In the Mediterranean region alone, these fisheries hold a collective value of no less than €200 million annually (Jackson et al. 2015). Moreover, the decline of seagrass habitats has been directly associated with swift reductions in fish populations (McArthur and Boland 2006). Increasing awareness about their significance for community welfare, whether in terms of ensuring food security through increased fish yields, enhancing water quality via seagrass filtration, safeguarding coastlines against erosion, storms, and floods, or their vital role in carbon sequestration and storage, will serve as a catalyst for global endeavours aimed at conserving, more effectively managing, and restoring these ecosystems.

In order to draw worldwide attention to the significance of these marine environments in May 2022, the United Nation General Assembly adopted the measure A/RES/76/265, designating the 1st of March as World Seagrass Day. This resolution emphasizes the pressing need to promote public awareness and foster initiatives that safeguard seagrass preservation and restoration, thereby supporting their well-being and restoration. The resolution therefore recognizes that restoring the health of seagrass meadows and consequently the services and functions of these ecosystems plays a crucial role in attaining the Sustainable Development Goals.

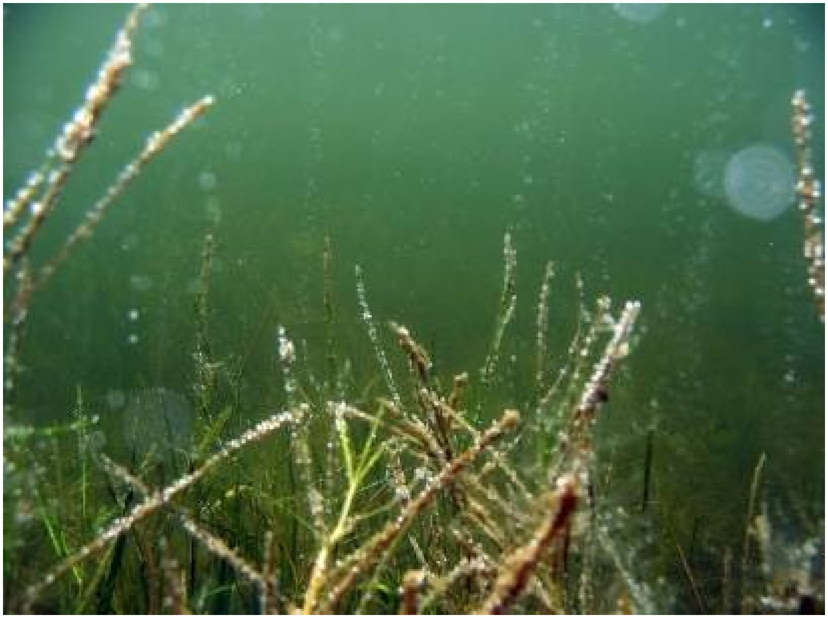

Figure 2: *Ruppia cirrhosa* releasing oxygen.

Species of seagrass targeted by LIFE TRANSFER

Aquatic plants comprise a group of monocots adapted to living completely submerged. They are plants that produce flowers known as Angiosperms, but in accordance with the most modern classification, they are to be attributed to the phylum of Magnoliophyta Cronquist, Takhtajan& W. Zimmermann, 1966. In the Italian transition environments there are 5 families: Cymodoceaceae N. Taylor in Womersley, 1984; Posidoniaceae Lotsy, 1984; Potamogetonaceae Dumortier, 1829; Zosteraceae Dumortier in Womersley, 1984 and Zannichelliaceae Dumortier in Womersley, 1984. Among the aforementioned families, 3 species can be identified as seagrasses in Italian transitional water systems: *Cymodocea nodosa, Zostera marina, Zostera noltei.* Added to these are two other species commonly found in brackish waters: *Ruppia cirrhosa* and *Ruppia maritima,* even if they are classified as freshwater aquatic plants. LIFE TRANSFER was implemented on four species *C. nodosa, Z. marina, Z. noltei* and *R. cirrhosa*. The following key is useful to identify the 5 species present in seawater and brackish waters of the European coastal areas.

1. Leaves with 7 similar veins and finely denticulated apices. Very developed reddish-pink rhizomes, not rotting and characterized by evident knots with very close annular scars at the origin of the branches. Large adventitious roots at the nodes: ....***Cymodocea nodosa (Fig. 4).***

2. Leaves with 3 more evident veins. There are no marginal indentations at the apexes which may be slightly bifid (under the microscope) due to the presence of the central rib. Blackish-white rhizomes, sometimes partially pink, but little developed, rotting distally and without scarring. Numerous adventitious roots at the nodes, very thin and frail:

    a. Plants of considerable size that can exceed one meter. Slightly curved leaves, 3-7 mm wide, with circular or hollow apex. Presence of 3 large internal ribs and 2 very evident marginals. Rhizomes with a diameter of 3-6 mm but only 5-15 cm long, distally blackish and rotting...***Zostera marina (Fig. 3)****.*

    b. Smaller plants, 10-20 cm, sometimes up to 60 cm in height. Narrow leaves, 0.7-1.5-(2) mm wide. Presence of 3 large ribs, one central and two marginals. Blunt or bifid apex. Gracile rhizomes, white-blackish, 2-3 mm in diameter, a few cm long and then rotting ....***Zostera noltei (Fig. 5)****.*

3. Leaves with only 1 central vein and less than 1mm in width:

    a. Fruits of regular shape on long 2-4-(10) cm spirally twisted peduncles, 2-3 mm swollen leaf sheaths..***Ruppia cirrhosa (Fig. 6).***

    b. Fruits of irregular shape, on short 1-2-(4) cm peduncles, narrow leaf sheaths of 0.8-1.0- (1.5) mm...***Ruppia maritima.***

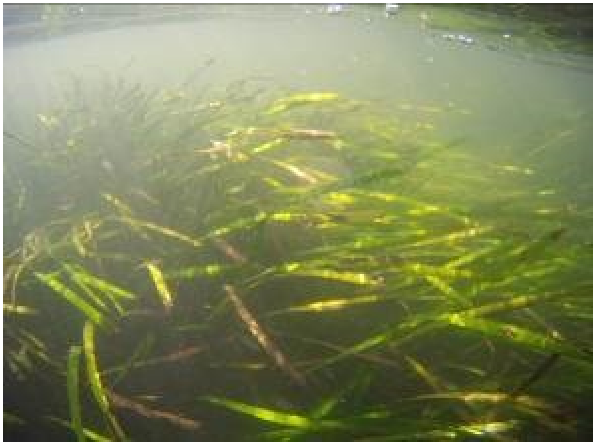
Figure 3: *Zostera marina*


Figure 4: *Cymodocea nodosa*


Figure 5: *Zostera noltei*


Figure 6: Ruppia cirrhosa

A brief description of the species transplanted within the TRANSFER project is given below.

Cymodocea nodosa thrives in environments characterized by elevated salinity levels and coarse sediments. Its average height can reach 100-120 cm, occasionally even peaking at 150 cm. This species is found in areas that have been inundated by seawater as well as in constricted regions with high salinity. However, in the latter scenario, the plants remain small and delicate due to the finer sediment grain-size. Its growth period spans from May to December, aligning with its subtropical origins. Throughout the winter months, the rhizomes persist along with the leaf bundles at their base, and at most, only 1-2 short leaves remain. The reproductive process of this species primarily relies on vegetative expansion and rhizome dispersal, as its substantial heat requirements make seed reproduction challenging. Typically, the shoots bear 2 to 4-(5) leaves, averaging around 3. These leaves exhibit varying lengths and are enclosed within tubular, membranous leaf sheaths that measure several centimetres in length.

Unlike the other species, the leaf bundles of this plant detach easily from the rhizomes. The plants are characterized by straight leaves, measuring 3-5 mm in width, and rhizomes adorned with distinct leaf scars, which form a dense network extending to approximately 30 cm in depth. These features are accompanied by a vivid orange hue. Unlike some other species, the rhizomes of this plant never develop darkened distal parts as they maintain their structural integrity throughout growth. Additionally, the roots of this species exhibit notable size and length and this is feasible due to the more oxidized nature of the coarse sediments in their habitat. In cross-section, the rhizomes exhibit a highly compact arrangement, which gives them greater resistance, alongside sizable large tubes responsible for transporting mineral salts throughout the plant. Due to these particular attributes, it stands out as the most effective plant for consolidating sediments. Its resilience is remarkable, and eradicating it becomes impractical due to the constant presence of rhizome fragments capable of initiating new shoots. Within well-established meadows, the average density of leaf bundles ranges from 1,000 to 3,000 units m^{-2}, depending on the depth. Populations in deeper waters give rise to larger yet less densely distributed plants, while the opposite is true for shallower waters.

This species can be readily distinguished by observing both its leaves and rhizomes. The leaves are characterized by rounded apexes lacking central depressions. Along the leaf's edges, there are hooked teeth (resembling ridges) that curve backward and contain numerous reddish cells abundant in tannins. Running along the length of the leaf are 7 distinct ribs, visibly connected by transverse bundles that link the primary ribs. When viewed in cross-section, the internal cells appear significantly large, while the gas-filled cavities responsible for keeping the leaves upright are smaller and ellipsoidal in shape. The cells forming the partitions that separate these cavities number a maximum of three or four. Moreover, the distinct rows of external cortical cells utilized for photosynthesis are clearly discernible due to their deeper hue and quadrangular shape. Within these cells, small chloroplasts are contained, serving as the sites for photosynthesis. Similar to other aquatic plants, stomata are absent, as gas exchange takes place directly through diffusion.

This plant species exhibits dioecious characteristics, meaning there are distinct male and female plants. While observing flowering plants is a rarity, occasional discovery of seeds does occur. The seeds stand apart from those found in the Zosteraceae or Ruppiaceae families. They are disc-shaped and flattened, featuring a hook-like structure from which the process of rooting initiates. On average, these seeds measure about 1 cm in diameter and are typically found in pairs on female plants.

A distinctive trait of this species lies in its epiphytic relationship with numerous small calcareous algae. These algae form a continuous calcium carbonate crust on the mature leaves that are no longer involved in photosynthesis. When these encrusted leaves fall and break down, they remain in the sediment, enhancing it with calcium carbonate.

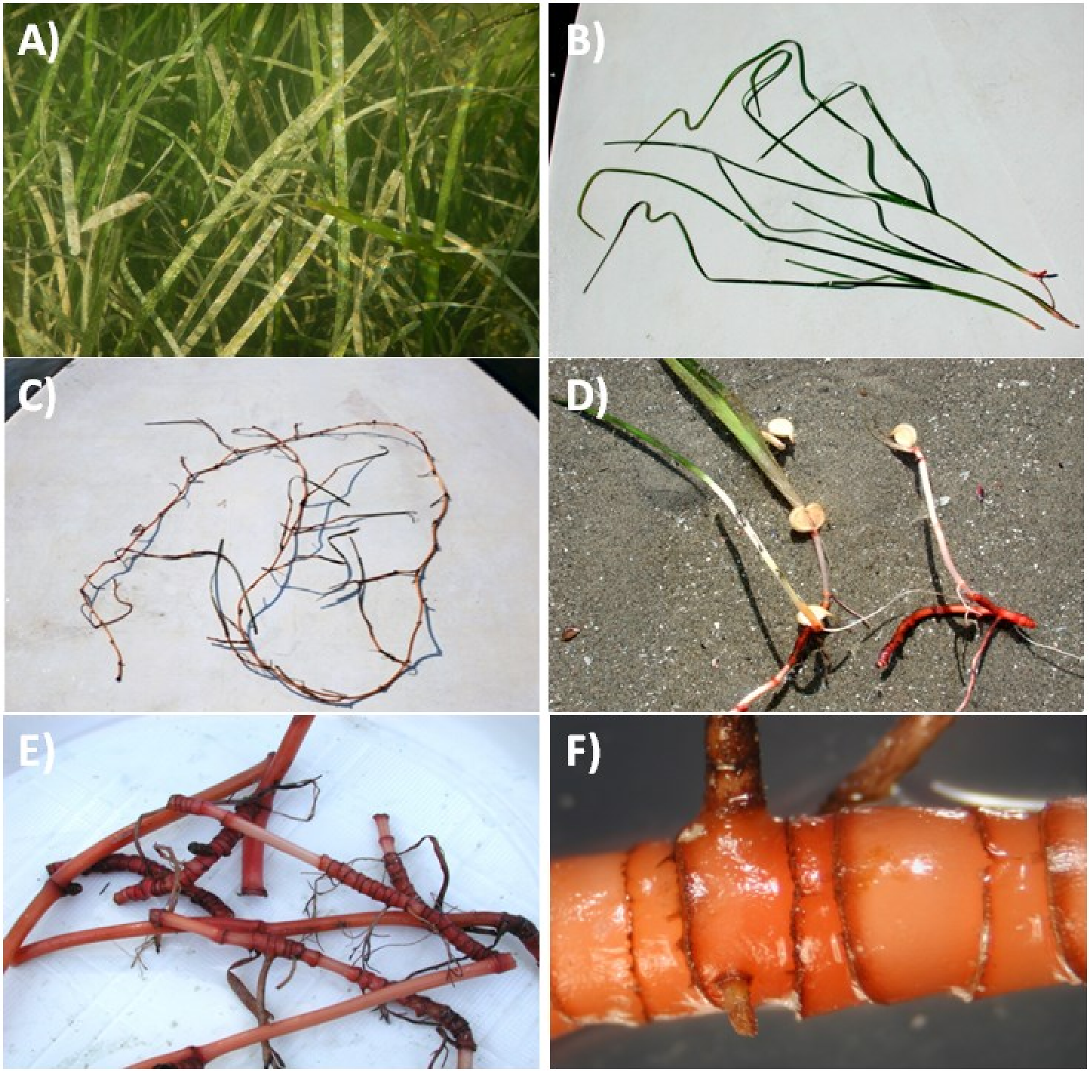

Figure 7: *C. nodosa* - A) A submerged prairie; B) Well-developed shoots; C) A rhizome with shoots and roots issued from each node; D) Seeds issued in the basal parts of the shoots; E) rhizomes with leaf scars; F) Detail of the leaf scars.

Zostera marina colonizes brackish environments showing a preference for intermediate salinity levels, typically ranging between 20 to 30 psu, and for sediments that are fine or medium-fine in texture but characterized by a good turnover because it fears high temperatures. Its growth occurs throughout the year, including the winter months, although at a considerably slower pace. During the period from April to early June, it produces flowers, seeds, and fruits, coinciding with its peak development phase.

Leaf bundles typically consist of 2 to 7 leaves (averaging around 4), varying in length and enclosed within tubular and membranous leaf sheaths measuring 5 to 15 cm in length. These leaves exhibit three primary veins. The younger and shorter leaf occupies the innermost position, while the outermost leaf falls off at intervals spanning from 28 to 99 days, depending on the season. In well-developed meadows, the average density of leaf bundles reaches approximately 600 units m^{-2}, with seasonal peaks up to over 1100 units m^{-2}. The leaves are slightly arched, measuring 5-7 mm in width and achieving heights that can surpass one meter. This species is readily identifiable through both leaf and rhizome examination. The leaves possess rounded apexes with a central depression formed by the prominent rib that traverses the center.

In the longitudinal direction, the leaf has five veins that are notably larger than the others—three central and two marginal. These veins serve as the plant's vascular system, facilitating the transport of minerals absorbed by the roots and sugars produced by the leaves during photosynthesis. Running alongside the primary veins are 6-8 slimmer bundles, which correspond to stacks of internal cells that connect the leaf's two opposite surfaces. These minor bundles encase gas-filled gaps that help maintain leaf buoyancy in the water column.

A multitude of dark, irregularly patterned transverse streaks connect the major bundles. These streaks are lateral ramifications s of the same bundles. In cross section, both the gas-filled gaps and the stacks of cells in the minor bundles are clearly visible. On the external surfaces of the leaves, a layer of quadrangular epidermal cells is present, devoid of stomata (pores for gas exchange), as aquatic plants facilitate the passage of carbon dioxide (CO_2) and oxygen (O_2) directly through their tissues.

Zostera marina produces fertile shoots that exceed the length of the leaves, reaching up to 120-130 cm. These shoots are characterized by repeated branching (3-7 times) and bear numerous flower spadices atop whitish cylindrical peduncles. The floral branches hold prominent sheaths, measuring 3-10 cm in length and extending as wide as the leaves.

The flowers, arranged in spikes, are very small. Male flowers possess unilocular anthers containing pollen, while female flowers feature an ovary with a short style and bifid stigma. Both male and female flowers are found on the same structure (monoecious plant = one house). For each female flower, two male flowers are usually observed, resulting in a total arrangement of 30 male flowers and 15 female flowers.

The fruits, measuring 3-5 mm in length, take on an ovoid-ellipsoidal form, appearing flattened and having a leathery texture.

Rhizomes, with diameters of 3-6 mm, exhibit a hue spectrum ranging from green on the surface to yellow-pink. They possess nodes and internodes that are spaced to varying degrees, and they lack leaf scars. At a certain distance from the leaf's growth region (meristem), the rhizomes become dark and deteriorate, limiting their length to merely 10-15 cm. Consequently, they penetrate the sediment to a depth of only 5-10 cm and are susceptible to easy removal. Numerous thin and delicate rootlets emanate from the nodes. When examined in cross section, the rhizomes appear compact and feature vascular bundles, as well as thick-walled cells that are partially lignified and abundant in reserve substances like starches.

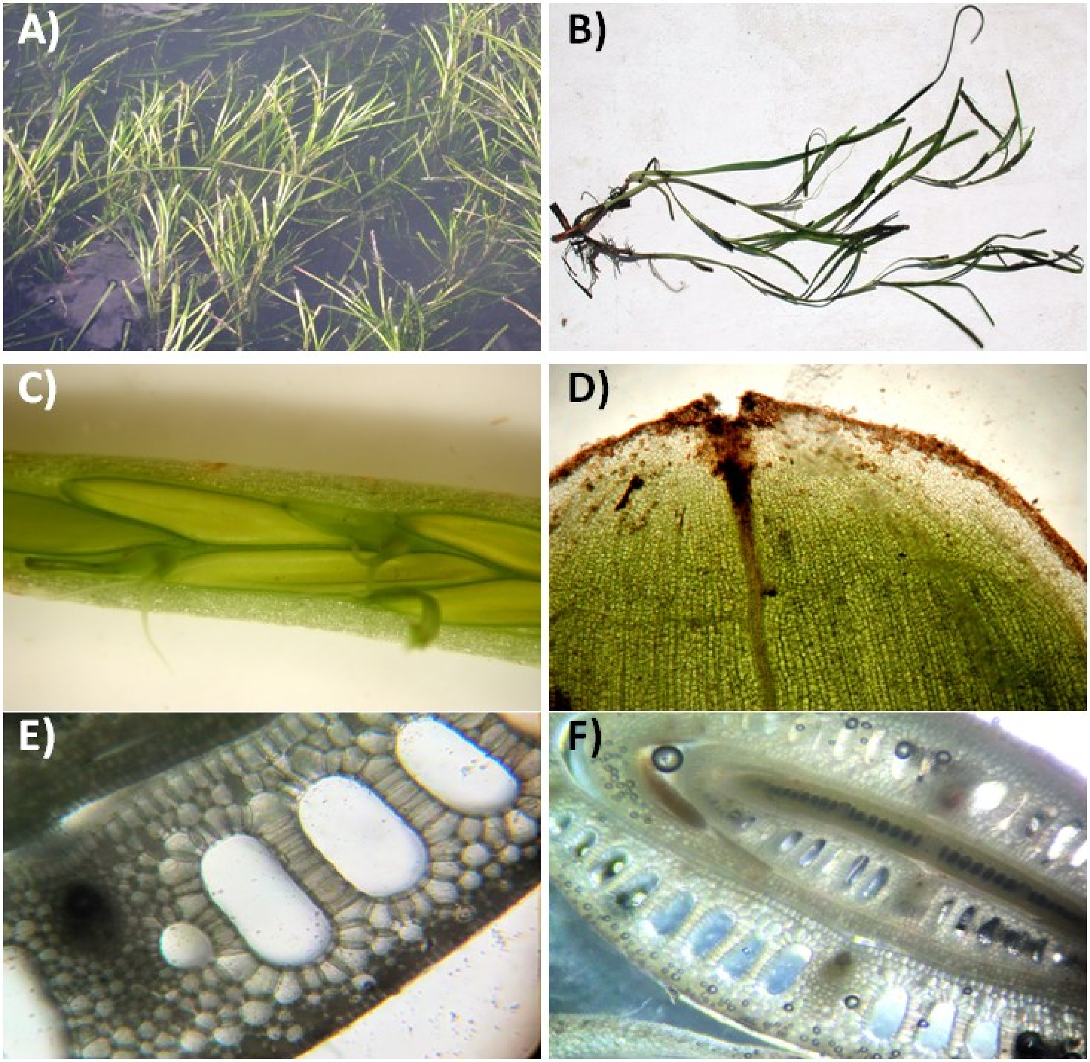

Figure 8: *Z. marina* - A) Plants with flower spadices; B) Plants with leaves and fertile shoots; C) Male flowers and smaller dark green female flower with bifid stigma; D) apex of a leaf with the central depression; E) Leaf cross section with large gas-filled gaps; F) Cross section in the basal region of a leaf.

Zostera noltei prefers environments with low water turnover. It grows on mudflats and on the edges of salt marshes on loosely compact and fine-grained substrates. The rhizomes have a light or pinkish colour which becomes blackish in anoxic environments. Generally, rhizomes have few branches and limited dimensions and do not sink deeper than 5 cm in the sediments, so they are easily eradicated. The nodes and internodes are variously spaced and do not have leaf scars. At the level of the nodes, leaf bundles and numerous rootlets are emitted, which, in turn, emit thin root hairs. The leaves are ribbon-like, varying in number between 2 and 5, wide 0.7-1.5 mm and long 10-20-(60) cm (in deeper waters). They have a central rib and an apical depression. The leaf bundles consist of 2 to 5 leaves (averaging at 2.2), each varying in length, contained within a compact, tubular, and membranous leaf sheath. The younger and shorter leaf occupies the innermost position, while the outer leaves are shed at varying intervals based on the prevailing season. In thriving grassland areas, the average density of these leaf bundles reaches approximately 6,000 to 7,000 units m^{-2}, with seasonal highs surging to 15,000 units m^{-2}. It grows all year round, including the winter months, albeit much more slowly, and produces flowers, seeds and fruits between June and August. In this period it also reaches its maximum development. It is a noticeably smaller species compared to *Zostera marina*, making it easily distinguishable from the latter. Similarly, its leaves exhibit rounded apexes and a distinct central depression formed by the prominent rib that runs down its center. In the lengthwise direction, the leaf display three noticeably larger veins compared to the others—one central and two marginals. These veins serve as the plant's vascular system, facilitating the transport of minerals absorbed by the roots and sugars produced by the leaves during photosynthesis. Running parallel to the primary ribs are 4 to 6 thinner bundles, corresponding to stacks of internal cells that connect the two opposite faces of the leaf. These minor bundles encase pockets filled with gas, allowing the leaves to remain upright in the water column. In this species, much like the major bundles, dark, irregularly shaped transverse streaks connect the primary bundles; these streaks represent lateral branches of the major bundles.

Both the gas-filled gaps and the cell stacks of the minor bundles are clearly visible when looking at a cross-sectional view of the leaf. Eventually, reddish-brown cells rich in tannins are recognizable preserving the leaves from grazing by herbivorous organisms and making them less palatable and digestible.

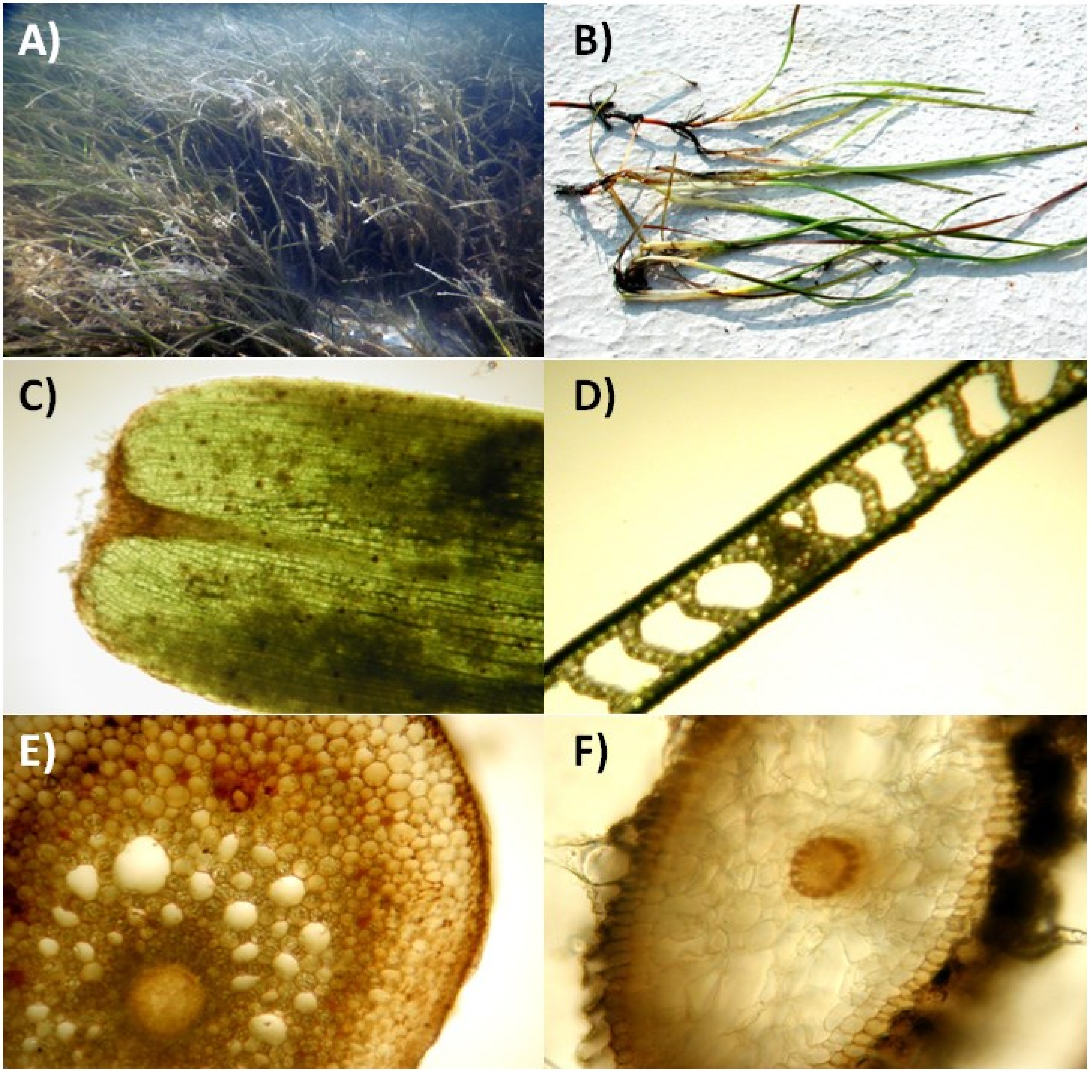

Figure 9: *Z. noltei* - A) Well-developed summer meadow; B) Whole plants; C) Apical leaf view with central depression; D) Leaf section with large gas-filled gaps; E) Rhizome section; F) Root section.

Ruppia cirrhosa colonizes extremely confined environments, mostly characterized by low salinity and muddy oxygen-depleted sediments in which the rhizomes form underground networks that are easy to eradicate. The stem can exceed 100 cm in height and the rhizomes, whose diameter varies between 0.8 and 1.2 mm, are provided with many yellowish nodes and internodes. The herbaceous stems bear packets of 3-5 leaves 15 to 17 cm long and 0.8 to 1.0 mm wide which progressively taper down to 0.5 - 0.6 mm at the apex. The leaves possess only one central rib. The terminal sections exhibit regular serration, featuring numerous apical denticles presenting 2-3 prominent cells. Along the entirety of the upper edges of the leaflets, single spiny teeth face upwards, spaced evenly at regular intervals. These teeth, measuring 30-50 µm in length, are composed of 3-6-(12) stacked cells with diameters of 10-12 µm. The apical portion of the leaflets, and in general, much of the leaf surface, contains several reddish-brown tannin-rich cells. These cells, which are typically larger than the epidermal cells, measure around 15-20 x 25-50-(75) µm. Similar cells are also found in the stems and basal sheaths, where they appear elongated and slender, measuring 8-12-(14) x 300-400-(500) µm. The lower section of the leaf, which connects to the rhizomes, is enveloped by an inflated leaf sheath measuring 2.0-2.5 mm in width and 12-15 mm in length. The upper part of this sheath features two obtuse ears that measure 700-800 µm in width. When examining a cross-sectional view of the leaf, it becomes evident that it is flat and elliptical, considerably more elongated compared to *R. maritima*. It features a central vascular bundle along with two small, strongly flattened lateral lacunae with a diameter surpassing 70-80- (100) µm. The epidermal layer of the leaf is made up of rounded-quadrangular cells, each measuring about 15-20 µm in diameter. Adjacent to this layer are 1-3 layers of medullary cells, positioned along the sides of the gas-filled gaps. In the center of the leaflet, vascular bundles are situated. The cross-sectional thickness measures approximately 170-200 µm. Reproduction takes place both through vegetatively, involving the growth and spread of rhizomes, and sexually, involving the production of flowers, fruits, and seeds. The inflorescences are carried by lengthy flower stalks, measuring 10-15-(20) cm in length and 0.7-1.0 mm in diameter. These stalks are coiled in a distinctive spiral pattern similar to tendrils and give rise to (3)-5-10 female flowers (carpels), each with its own stalk, which then develop into fruits. Often the fruits are not pedunculated and are aborted. Ripe fruits are symmetrically shaped, ellipsoidal, with average dimensions of approximately 2 mm in length by 1.0-1.2 mm in width, featuring a rostrate apex. The male flowers are compact and bear numerous globose pollen sacs (anthers) measuring 0.8-1.1 x 1.3-1.6 mm, resembling coffee beans. These anthers contain tannin-rich cells. The anthers have short peduncle and remain enclosed within the leaf sheaths.

The rhizomes are notably slim, with an average diameter of 0.8-1.2 mm, and are characterized by a series of nodes and internodes. The internodes, which tend to be rather short, average around 0.7-1.0 cm in length and exhibit a yellowish hue. Emerging from these nodes are both herbaceous stems and leaf bundles, along with numerous rootlets measuring a few centimetres long and having a diameter of 300-330 µm. In cross section, the rhizomes display a distinctive central vascular structure, featuring partially lignified vessels with a reddish-brown coloration. Additionally, numerous large gaps contribute to a trabecular appearance within the section. These rhizomes extend into the sediment by up to 5-7 cm and can be easily uprooted. Similar to *Z. noltei*, the rhizomes of this species also exhibit limited growth, turning dark and deteriorating after short distances. The rootlets associated with these rhizomes are delicate and thin.

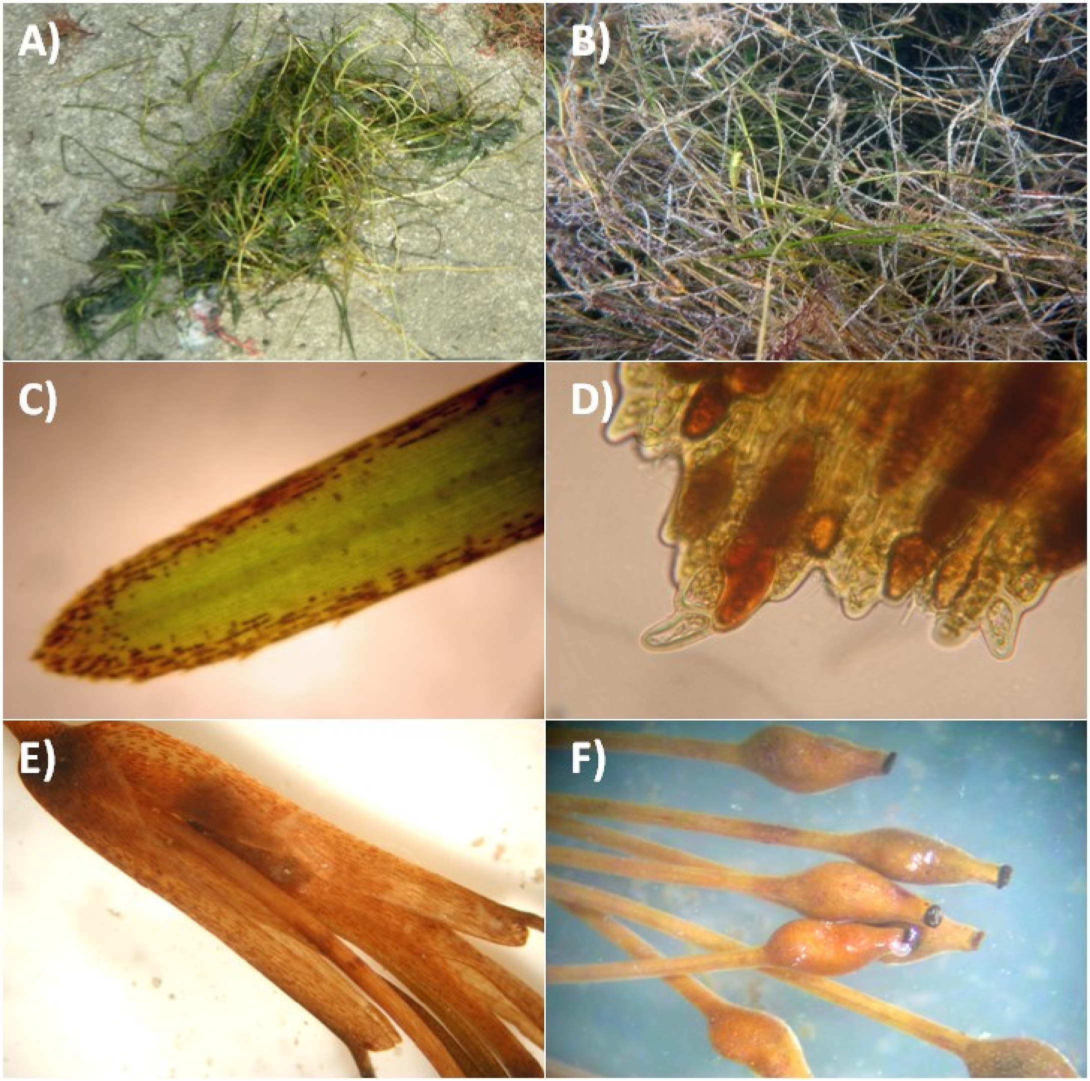

Figure 10: A) A bunch of plants; B) Submerged dense prairie with a male flower; C) Apical region of a leaf with apical denticles and many tannic cells; D) Detail of apical denticles and tannic cells; E) View of the large sheats (4mm); F) Detail of the symmetric fruits.

Seagrass in lagoons and habitat 1150* Coastal lagoons

Habitat 1150* (Coastal Lagoons) stands out as a prioritized habitat under Annex I of the Habitat Directive (92/43/CEE).

The Habitat Coastal lagoons encompass coastal aquatic ecosystems characterized by saline or brackish, shallow waters. This habitat exhibits significant seasonal fluctuations in salinity and depth due to factors like water sources (marine or continental waters), precipitation, and temperature, all of which influence evaporation. It can be wholly or partially separated from the sea by sand banks or shingle, or, less frequently, by rocks. Salinity levels can span from brackish to extremely saline waters, influenced by rainfall, evaporation, and influx of marine waters during storms, as well as temporary inundation by seawater in winter or tidal exchanges.

The habitat is facing significant threats and in the assessment for the period 2013-2018 it was reported in "Unfavourable" status in all Biogeographical regions except the Black Sea. The lagoon environment can appear devoid of seagrasses or feature diverse aquatic angiosperms such as *Potamogeton pectinatus*, *R. maritima*, *R. cirrhosa*, *Z. noltei*, *Z. marina*, *C. nodosa*, and numerous species of macroalgae (Ulvaceae, Gracilariaceae, Cladophoraceae, Solieriaceae, Caulerpaceae, etc.). The composition of aquatic vegetation varies across different lagoon contexts, sometimes with different dominant plant species. In pristine areas, several researchers (Orfanidis, 2003; Viaroli et al., 2008; Sfriso et al., 2009) affirm that under natural conditions, aquatic angiosperms are typically the primary colonizers of this habitat.

Aquatic vegetation includes both aquatic plants and macroalgae. Typically, a mix of various species can be found, displaying remarkable adaptability to the specific environmental characteristics of each location. These traits mainly vary based on factors such as the geographical features of the area, the composition of the substrate (whether rocky, sandy, or muddy), hydrodynamics, confinement levels, fluctuations in parameters like temperature and salinity, access to light and nutrients, and the presence of stressors like pollutants, and activities that lead to sediment resuspension and water cloudiness. Aquatic plants and macroalgae are aquatic organisms capable of photosynthesis converting solar energy and fixing CO_2 in organic matter and releasing O_2 as a byproduct. Therefore, they contribute as primary producers to build the base of the food chain in coastal environments and structure the type of ecosystem that can be established.

Macroalgae (the seaweeds) include all multicellular or cenocytic organisms (comprising a single large cell with numerous nuclei). These organisms possess a structure called "thallus" that lacks differentiation into roots, stems/rhizomes, and leaves although structures mimicking these tissues may be present in some species. Additionally, algae lack flowers, fruits, seeds, and a vascular system for nutrient transport between roots and leaves. This classification comprises three major groups: green algae (Chlorophytes), red algae (Rhodophytes), and brown algae (Phaeophytes). Even within these groups, algae exhibit a wide range of colours and shapes (such as filamentous, tubular, ribbon-like, laminar, encrusting, calcareous, etc.), and proper identification often necessitates an examination of their reproductive structures. In cases of morphological convergence, genetic-molecular analyses might be required for accurate identification.

Figure 11: . Macroalgae – Green algae (in photo Ulvaceae) , red (in photo Solieriaceae) and brown algae (in photo Fucaceae).

Aquatic angiosperm meadows (the seagrass), including species within the genus *Ruppia* that aren't exclusively confined to freshwater habitats and are classified as aquatic plants (Short, 2003), are frequently considered indicators of the ecological well-being of coastal and transitional water systems. They serve as prominent markers of nearly pristine conditions. Aquatic angiosperms encompass well-structured photosynthetic organisms, characterized by distinct tissue differentiation into roots, stems or rhizomes, and leaves. They possess a vascular system (bundles or internal ribs for transporting mineral salts and sap) and, during the reproductive season, develop flowers, fruits, and seeds. Their rhizomes or creeping stems consist of nodes and internodes of varying lengths, producing leaf bundles upwards and roots downwards at each node.

Figure 12: Seagrass meadows of *Z. marina* and *C. nodosa*

Seagrass benefits

Biodiversity conservation

Seagrasses play a fundamental ecological role supporting a wide range of ecosystem services by creating sheltered areas, feeding grounds, and safe nurseries for various marine and terrestrial organisms (United Nations Environment, 2020). Numerous marine species utilize seagrasses as nursery habitats and support early development stages of marine migrant fish (Andolina et al., 2020) aiding protection from predatory birds as the European shag *Gulosus aristotelis.* Some of these marine species are classified as Threatened, Endangered, or even Critically Endangered by the International Union for Conservation of Nature (IUCN) as indicated by Lefcheck et al. (2019) and include noteworthy examples such as the European eel (*Anguilla anguilla*). Among these fish species, seahorses stand out as they spend a substantial portion of their time clinging to seagrasses with their tails, using these habitats to search for food. Within this environment, various small lagoon fish seek shelter, including protected species such as: the lagoon goby (*Knipowitschia panizzae*), the gray goby (*Pomatoschistus canestrinii*), the Mediterranean banded killifish (*Aphanius fasciatus*) and the "Needle fish" (*Syngnathus acutus, Syngnathus abaster, Syngnathus typhe*). Moreover, numerous species of birds are to be accounted that feed in these habitats. From a taxonomic point of view, the main invertebrate groups of fauna associated with seagrass systems belong above all to molluscs, crustaceans, polychaetes, annelids, and to a lesser extent nematodes, flatworms, echinoderms, cnidarians, bryozoans, hydroids and anthozoans creating a varied and rich environment (Gambi & Morri, 2008).

Sediment stabilization

Seagrasses play an active role by removing nutrients, particles, and pollutants. They enhance water quality by effectively filtering, cycling, and retaining nutrients, suspended particulates and harmful substances through absorption by their leaves and roots facilitating settlement and consolidation of sediments by intricate mat of roots and rhizomes and promoting calcareous concretions and thanatocenoses (East et al., 2023).

CO_2 Sink

Seagrass meadows have a substantial global role as carbon sinks, demonstrating a remarkable capacity to absorb and store carbon within sediment, commonly referred to as 'blue' carbon (Nellemann et al., 2009). At a global scale, the estimated organic carbon stock in seagrasses is substantial, reaching up to 19.9 Petagrams (Pg = 10^{15} g = billion tonnes) (Fourqurean et al., 2012) and are estimated to bury 27.4 Teragrams C yr^{-1} (Tg = 10^{12} g = million tonnes), roughly 10% of the yearly estimated organic carbon burial in the oceans despite covering no more than the 0.2% of the area of the world’s ocean.

Figure 13: : *Z. marina* leaf encrusted with calcareous epiphytic macroalgae.

Carbon sequestration and storage within seagrass meadows occur in two primary ways: through the seagrass biomass itself (autochthonous Corg) and by hosting small calcareous epiphytic macroalgae (such as *Hydrolithon*, *Pneophyllum*, *Melobesia*) and capturing organic particles from adjacent ecosystems (allochthonous Corg), sometimes leading to the creation of extensive carbon deposits within sediments, enduring for millennia if left undisturbed. Notably, carbon stored in above-ground living biomass, such as leaves, is susceptible to grazing, export, or decomposition, rendering it a short-term carbon sink. Seagrasses capacity for carbon sequestration varies among species, meadow characteristics, degree of confinement and environmental conditions. Diminishing seagrass meadows lead to a reduction in carbon sequestration and storage capabilities, triggering increased CO_2 emissions due to the remineralisation of sedimented Corg deposits. The ongoing rate of seagrass loss could potentially result in the release of up to 299 TgC yr^{-1} (Fourqurean et al., 2012). Similar to the deterioration of terrestrial carbon reservoirs, the degradation of seagrass ecosystems could significantly contribute to anthropogenic CO_2 emissions, accelerating climate change. To the aforementioned organic carbon fixed and sequestered at the level of the sediment by the seagrass is added the inorganic carbon precipitated locally in the form of carbonate by numerous shellfish such as grazers and filter feeders and the crustose coralline algae (Figure 13), epiphytic microcalcareous seaweeds growing on the leaves of the prairies which, under optimal ecological conditions, lead to the precipitation of 160-560 g dw m^{-2} yr^{-1} of calcium carbonate (Sfriso et al., 2020).

Despite their substantial role as carbon sinks and the potential CO_2 emissions from degradation, seagrass meadows have traditionally been disregarded in greenhouse gas emission assessments and consequently omitted from climate change mitigation strategies, which often concentrate on terrestrial ecosystems (e.g., the United Nations Programme on Reducing Emissions from Deforestation and Forest Degradation in Developing Countries). Noteworthy, reports by Nellemann et al. (2009) and Laffoley and Grimsditch (2009) underscored the potential for climate change mitigation through the restoration and conservation of seagrass meadows, in addition to mangroves and salt-marshes, collectively termed as "blue carbon strategies". Therefore, the Intergovernmental Panel on Climate Change (IPCC) has issued guidelines supporting the inclusion of greenhouse gas emissions or sequestration from seagrass meadow conversion and rehabilitation in national inventories (IPCC, 2013). Additionally, carbon standards have been introduced to enable restoration initiatives to benefit from carbon credits, such as the Verified Carbon Standard (Needelman et al., 2018).

Their elevated productivity significantly influences the carbonate chemistry of the surrounding seawater, primarily due to the substantial uptake of dissolved inorganic carbon during the process of photosynthesis. Consequently, seagrasses tend to elevate the pH of the seawater during daylight hours, potentially counteracting the harmful consequences of the escalating anthropogenic CO_2 levels within the seawater. This phenomenon offers potential benefits to marine organisms, especially those that engage in calcification, such as crustose coralline algae, and shellfish, residing within or in close proximity to seagrass habitats. These organisms can discover a local sanctuary from the effects of ocean acidification in seagrass meadows. Healthy seagrass meadows have the potential to bolster the resilience of the most fragile species to ocean acidification in the short term (Wahl et al. 2017).

Figure 14: *C. nodosa* view.

Experience of LIFE TRANSFER seagrass transplantation in lagoons

LIFE TRANSFER was implemented in six different lagoons of three Member States, four lagoons in Italy, one lagoon in Greece and one lagoon in Spain.

The LIFE TRANSFER project is the upscaling of LIFE SERESTO (LIFE12 NAT/IT/000331) carried out between 2014 and 2017 in the Venice Lagoon (Italy) where it successfully accomplished the recolonization of angiosperms in the SIC IT3250031 “Northern Venice Lagoon”. This goal was achieved by transplanting *Z. marina*, *Z. noltei*, *C. nodosa*, and *R. cirrhosa* to small designated locations. The transplantation process involved manual intervention and the transfer of a limited number of plants. This method proved advantageous due to its cost-effectiveness and minimal impact on the donor sites. As a result of the project, seagrass meadows naturally expanded into the surrounding areas. This expansion facilitated seed establishment and aided the growth of new rhizomes produced by the transplanted specimens. During LIFE SERESTO critical issues have also been observed in transplant operations related to the marking of stations with poles or buoys or to the site protection with sheltering devices (e.g., wooden fascines) that often act as collection points for fast growing macroalgae such as Ulvaceae suffocating the nearby transplanted seagrass. Eventually, pleustophytic (floating) fast growing macroalgae (such as Ulvaceae, Gracilariaceae and Soleriaceae) can be a dangerous obstacle to the growth of aquatic plants as they can quickly colonize a shallow water column (in few weeks) in highly trophic areas during late spring-early summer, suffocating the underlying seagrass transplants. Also on field experience proved there are no advantages in transplanting big sods of 30 cm diameter that where abandoned in favour of sods of 15 cm of diameters.

Successful transplant operations must also take into account the seasonality and life cycle of the different species which influence the optimal periods for transplanting rhizomes and sods in the recipient sites. The ideal seasons identified in the Venice Lagoon in the framework of the Life Seresto project were autumn for *Z. marina*, late spring and autumn for *Z. noltei*, late spring and early summer for *C. nodosa* and end summer-autumn for *R. cirrhosa*.

LIFE TRANSFER took advantage of the experience developed using the same methodology avoiding critical issues such as protection of transplant with any kind of shelter as well as the use of sods of 30 cm of diameter. Anyhow the project tackled new challenges such as applying the methods to six different lagoons scattered into the Mediterranean presenting different conditions, Figure 15. For instance, in the Amvrakikos lagoons in Greece was recorded a decrease in above ground biomass of *Z. noltei* in Autumn which may indicate a difference in growth cycles with other partner countries. In Mar Menor in Spain salinity range from 38 to 42 PSU (Practical Salinity Unit), value never reached in Italian lagoons where salinity range was from 17 to 26 PSU.

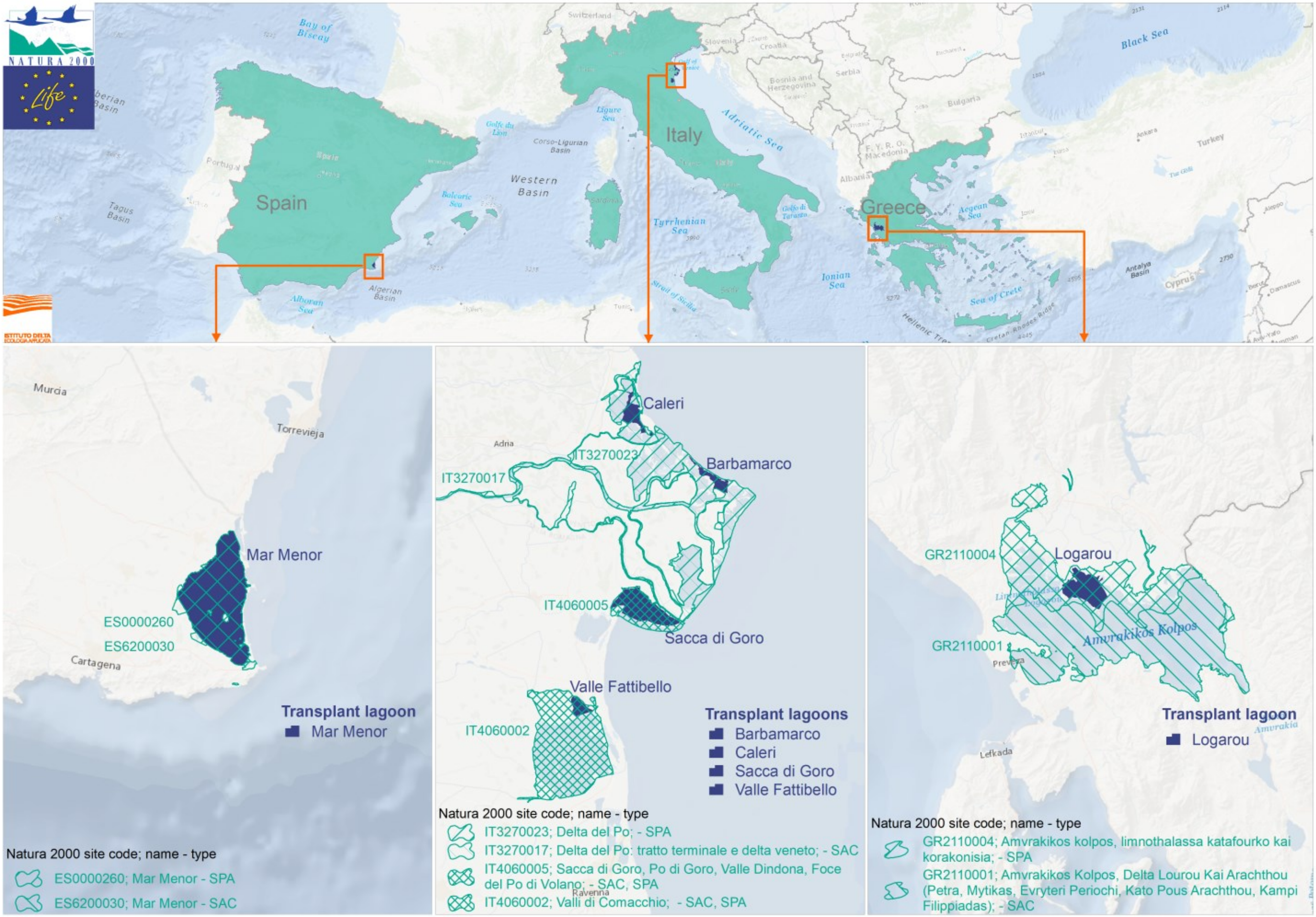

Figure 15: The six lagoons in which LIFE TRANSFER was implemented.

Transplant techniques

Explantation and transplantation of sods

The technical operations of explantation and implantation are preferably performed from the boat. This is particularly important because the areas designated for these actions typically consist of delicate, low-density sediments that are easily prone to resuspension. When operators walk on the seabed during subsequent transplantation activities, there is a risk of causing harm to existing transplants and disturbing the fine sediment, leading to elevated water turbidity. Alternatively, transplant actions can be carried out optimally by scuba or snorkel operators, minimizing the impact on the areas of intervention. The explantation of small species such as *Ruppia cirrhosa* and *Zostera noltei* usually takes place by 15 cm diameter steel serrated corers (Figure 16A) collecting the first 15-20 cm of sediment with rhizomes of the selected species. All this is done taking care to insert the leaves vertically inside the corer to prevent them from being cut when they are longer. The sods are placed inside perforated buckets which are in turn immersed in basins with sea water to keep them moist during transport until the moment of transplanting. The ideal time between explanting and implanting ranges from a few hours to 24 hours in the coldest seasons in order to minimize the stress on the explanted seagrass. In the recipient stations the corer is then used to create a hole in the sediment into which the sods are inserted. The sods should be carefully positioned, ensuring it is situated at a depth of no less than 30-40 cm below the average tide level (Figure 17). This methodology aims to ensure the integrity and vitality of the transplanted seagrasses.

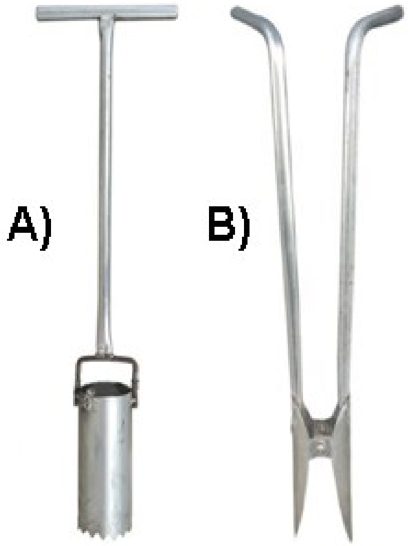

Figure 16: . A) A serrated corer and B) a double handle duckbill shaped transplanter.

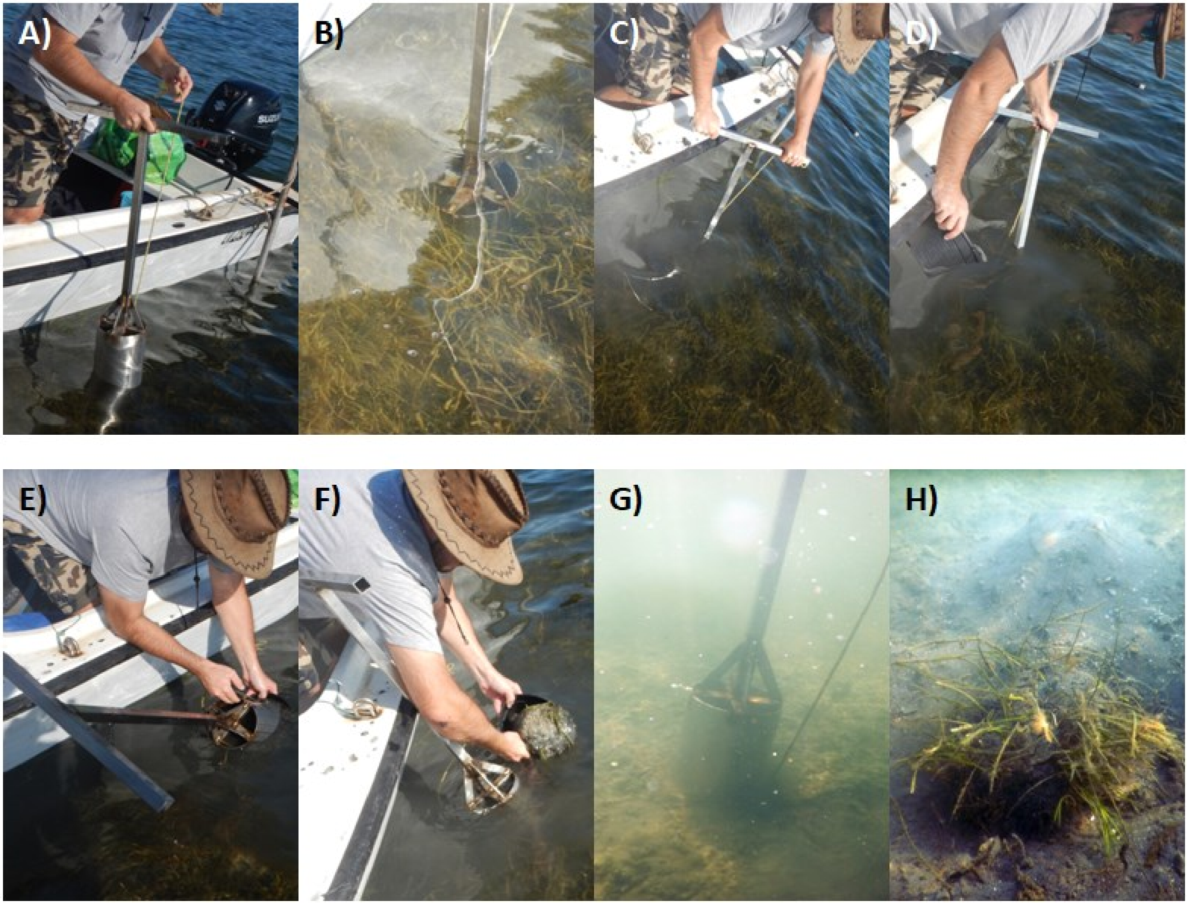

Figure 17: Sod transplantation by serrated corer in low tide. A-F) Explant; G-H) Inplant

During the implementation of LIFE TRANSFER further improvement were made for the transplant in case of limited water transparency. In lagoons, the transparency of the water varies over the course of the seasons, even wind and tide can also reduce transparency to the point of making the hole made with the corer invisible. In order to tackle such condition HCMR (Hellenic Centre for Marine Research) developed a novel transplanter tool (Figure 15B) that overcome the visibility problem. The sod is inserted inside the transplanter, then from the boat the transplanter in one movement opens a hole and release the sod into the sediment. The use of the novel transplanter is visible in Figure 18the pictures sequence

Figure 18. One sod is introduced in to the transplanter A-C) the tool is inserted into the sediment and the closure of the handle release the sod D-F).

Explantation and transplantation of rhizomes

Regarding the transplantation of bigger seagrasses as *Z. marina* and *R. cirrhosa* (but sometimes also the smaller *Z. noltei*), it is preferable to employ the rhizome dispersion method. Based on the experience gained from the Life SERESTO it has been observed that this approach is highly effective and ensures excellent survival results for these species. This technique undoubtedly requires less effort compared to sod transplants because both the extraction and planting phases do not necessitate the use of trasplanters or corers. The rhizomes (along with their leaf bundles) can be gathered using a rake directly from a boat and subsequently sorted and cleaned of mud residues or can be collected by snorkel operators. The rhizomes are then loosely tied in bunches usually of 50-150 shoots with a string placed basins filled with seawater and transported to the chosen transplant site. Planting operations can be performed using a pair of telescopic grabber tongs (**Figure 19**), or manually by snorkel operators. This allows for the insertion of the rhizome into the sediment at a depth of 5-10 cm below the surface. In the case of very low tides and consequently shallow depths, this operation can be performed manually from the boat, enhancing control during rhizome transplantation. As previously described, planting ten *Z. marina* rhizomes in a closely spaced area is equivalent to planting a single sod of 15 cm in diameter. The rhizomes should be positioned at least 30-40 cm below the mean tide level.

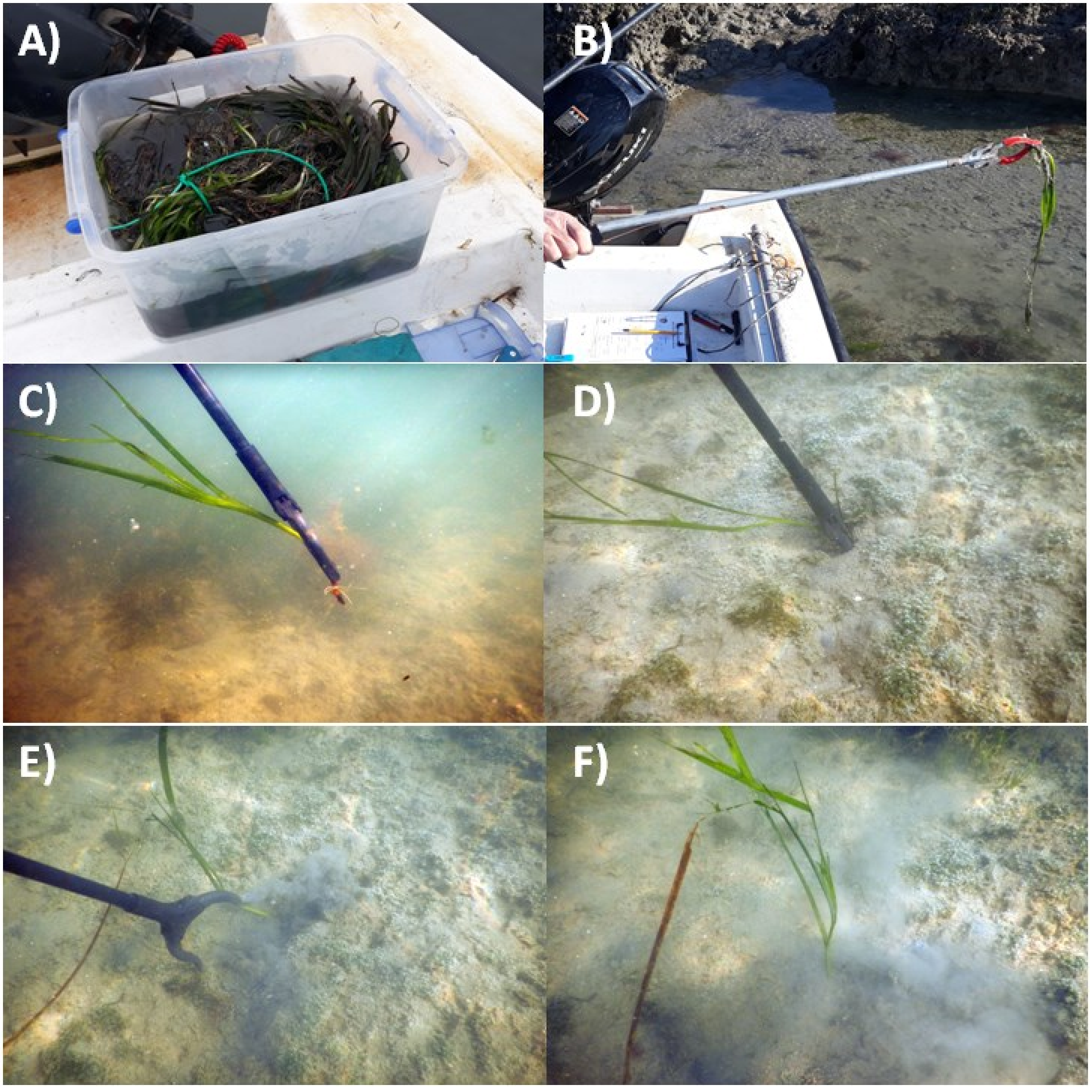

Figure 19: Single rhizome transplant. Rhizomes collected by a rake are placed in a basin and transplanted by a pair of telescopic grabber tongs.

Transplantation patterns

Transplant monitoring sometimes can be challenging because of tide constrains o during summer months when waters are murky and the plants are difficult to observe. Therefore, transplantation patterns are usually implemented by operators, in order to make transplants more recognizable and to promote a rapid fusion of the patches forming from transplanted units at each recipient site. In the Life SERESTO a triangular shaped scheme for sod transplants was used (Figure 20A), and in LIFE TRANSFER also a cross shaped scheme was implemented (Figure 20B). During the latter at each campaign, transplantations follow an operational plan that involves placing 2 groups of sods approximately 5 meters apart. Each group consists of 5 sods spaced 50 cm apart from each other, arranged in a square pattern with one clump positioned centrally in relation to the others. In the case of *Z. marina*, groups of 10 transplanted rhizomes may be used instead of sods, placed in close proximity to each other. The transplant areas can be marked with visible poles or reeds, with each series of transplants marked for each year of activity, to facilitate the subsequent study of sod growth. However, these must be positioned with attention at least one meter away from the transplants to prevent macroalgae from gathering around the poles, suffocating the nearby transplants and should the recipient site be subject to occasional macroalgal blooms in late spring, marking poles should be avoided altogether.

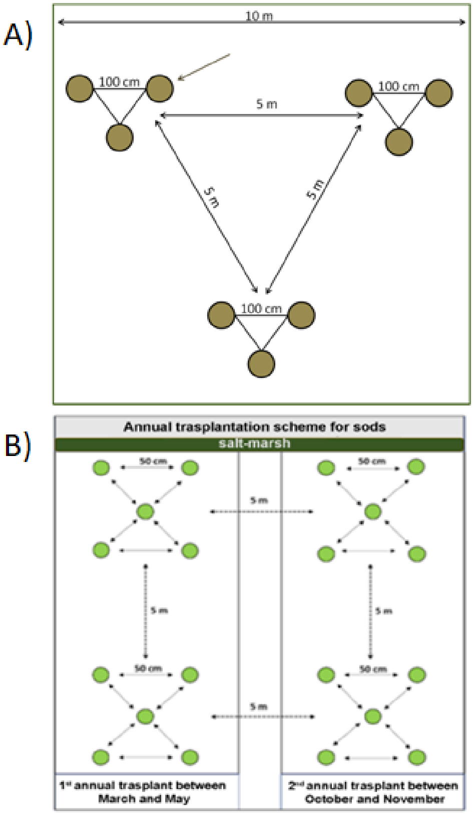

Figure 20: Transplant patterns in A) LIFE SERESTO and B) Life TRANSFER

Other seagrass transplant experiences

A literature survey of the last decades reveals how numerous initiatives have been so far undertaken for the transplantation of *C. nodosa, Z. marina, Z. noltei* and *R. cirrhosa* in the world and especially in Europe. The locations and experiences acquired on seagrass transplantation are summarized in Figure 21. Different authors have undertaken many transplantation strategies focusing on different parts of the donor site for transplantation of: sods, rhizomes, shoots and germinated seedling. Numerous arrangements and transplant schemes were also tested although no one was identified as being more effective than the others in ensuring plant survival. Globally, transplant units can be sediment-intact (sods) or sediment-free (shoot/rhizomes/seed/seedlings). Although the explantation and transplantation actions can be carried out both manually and mechanically, the manual approach has been often privileged from the authors, especially in the planting phase which is usually carried out manually by snorkel or scuba divers (especially for sediment-free transplant units). Usually, the sods are privileged in areas with high water hydrodynamics as they guarantee better anchorage and an intact root system however in lentic areas with low to moderate hydrodynamics and good light availability, the rhizomes showed higher growth and expansion rates which in favourable conditions (Sfriso, et al., 2021) can lead to the formation of patches extended up to a few meters over the course of one year with a survival that can fluctuate from 39 to 95%. To this should be added the higher number of rhizomes or shoots that can be transplanted compared to the sods and therefore the greater dispersion of the transplants and a greater possibility for a successful taking root. Most of the reported transplant experiences concerned sods and rhizomes/shoots but seedlings transplants were only implemented by few authors especially for *Z. marina* and *C. nodosa*. Additionally, the transplants of *R. cirrhosa* have been previously implemented only in the Venice lagoon (Italy) by sod, and there is little information on this species although it has shown higher tolerance to moderate to high trophic conditions which can be critical for other species survival.

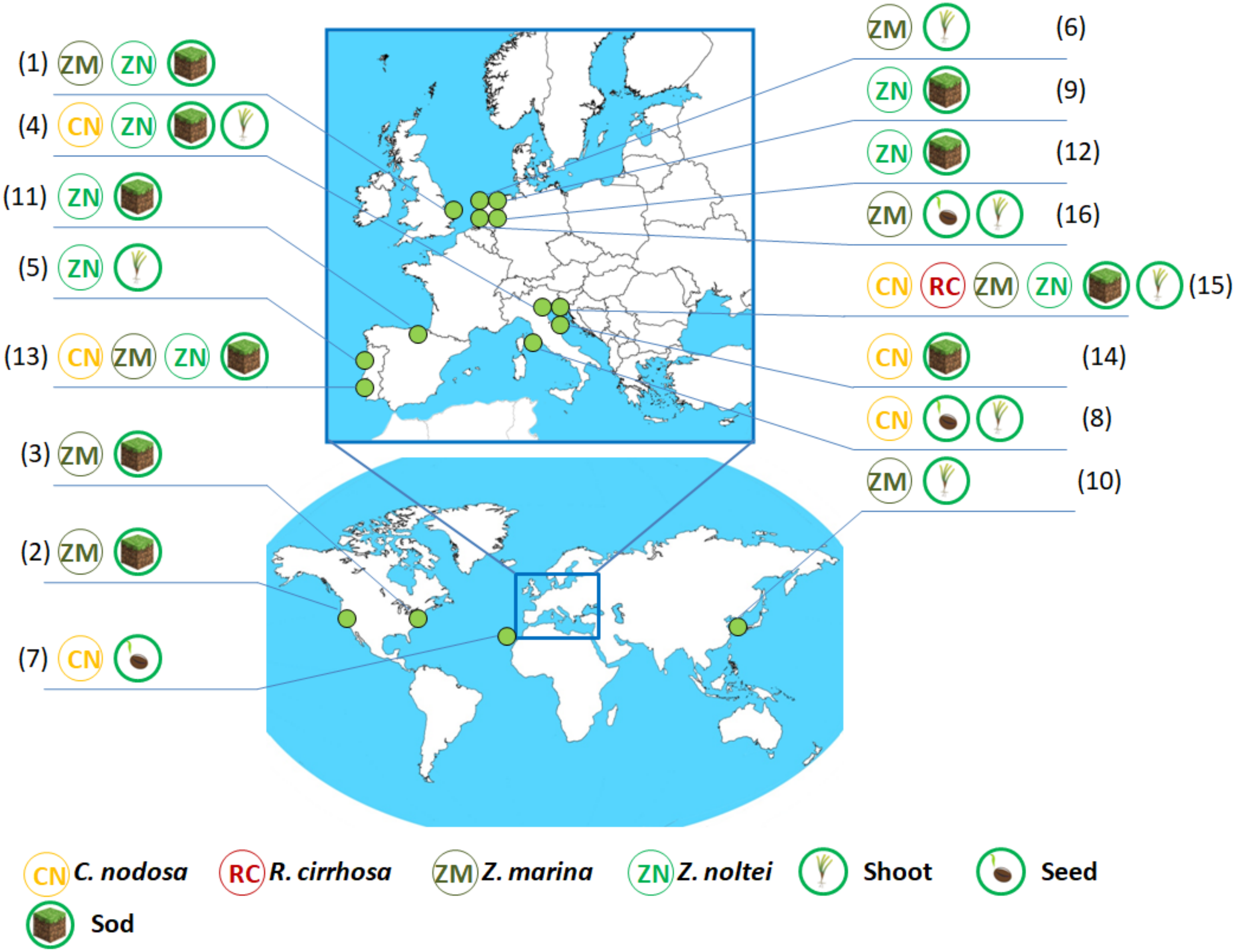

Figure 21: previous seagrass transplant experiences, species involved and approaches used, adapted from a figure presented in Sfriso et al. (2023). Legend: (1) Ranwell et al., (1974), (2) Zimmerman et al. (1995), (3) Davis et Short (1997), (4) Curiel et al. (2003), (5) Martins et al. (2005), (6) Bos et Van Katwijk (2007), (7) Zarranz et Gonza (2010), (8) Balestri et Lardicci (2012), (9) Suykerbuyk et al. (2012), (10) Li et Lee (2010)/Li et al. (2014), (11) Valle et al., (2015), (12) Suykerbuyk et al., (2016), (13) Paulo et al., (2019), (14) Da Ros et al. (2021), (15) Sfriso et al., (2021), (16) Cronau et al. (2023).

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LIFE TRANSFER

SEAGRASS TRANSPLANTATION FOR TRANSITIONAL ECOSYSTEM RECOVERY