LIFE TRANSFER

SEAGRASS TRANSPLANTATION FOR TRANSITIONAL ECOSYSTEM RECOVERY

Deliverable D.3 Action

Third monitoring progress report in Mar Menor lagoon (Spain)

Project responsible

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Third monitoring progress report Mar Menor lagoon

CONTENT

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EXECUTIVE SUMMARY

Action C.3 transplantation of submerged aquatic angiosperms Mar Menor lagoon was made in the areas selected by the executive project of Action A4, in spring 2022 and repeated three times a year for the year 2023. This task (C.3) was carried out by the University of Murcia partner. Transport and transplantation methods were carried out following the operating protocol learned in Action A5.1. Training of local operators (Action A5.2.), in the case of Mar Menor, was attended by researchers from the same University of Murcia research group, in collaboration with LIFE transfer project, and by University of Murcia master's degree students. This action is continuous as it is combined with the action C.3.

Monitoring sampling campaigns have been carried out following the official technical specifications. Monitoring action started in May 2022 of the different species of angiosperms, both donors and receiving stations. This third monitoring report represents an update to the third monitoring year (2024).

The different parameters measured were: monitoring of the plant growing and the rate of expansion of the newly formed meadows (transplant survival, rate of expansion of the transplant and covering of the newly formed meadows); water column parameters (including temperature, salinity, dissolved oxygen, pH, Eh, particulate matter, chlorophyll and nutrients: total ammonium; oxidized nitrogen; dissolved inorganic phosphorous; dissolved silicates, suspended solids); sediment parameters. The main characteristics of the benthic communities (macroinvertebrates and macrophytobenthos) settled particulate matter and fish fauna were also measured.

Up to the spring 2024, transplants survival reached 100% in all deep receiving stations for the case of *Cymodocea nodosa.* In the case of shallow receiving stations for the same species, there was also an increase in the survival, but not to the 100% in some cases. Just one *C. nodosa* sallow receiving station presented a decrease in survival of transplanted sods. Therefore, receiving station for *Ruppia cirrhosa* transplants maintained its percentage of sods survival in 2024, comparing with 2023 values.

Regarding to the rate of expansion of the transplants, for the case of *C. nodosa* transplantation, in 2024, highest values of expansion corresponded to deeper receiving stations. Despite this, some previously most expanded transplants corresponding to shallower stations maintained their rate and growing. The case of *R. cirrhosa* transplants resulted different. This species showed the highest values of expansion rate in this monitoring year (2024), despite its disappearance in 2022, and reinforcing via replants in 2023. However, donor natural meadows disappeared.

Covering of newly formed meadows stay similar to those from 2023. Two deeper, and two shallower stations of *C. nodosa* transplants showed a coverage higher than 50% of the total surveyed area. In the case of *R. cirrhosa* transplants, the mean percentage of covering increased across the monitoring years.

Results corresponding to the biological and environmental quality status of transplant stations, water matrix physico-chemical parameters did not show clear differences among both species transplants.

For nutrient analyses, the donor sites in the case of *C. nodosa* showed higher nutrient concentrations than the recipient sites. In *R. cirrhosa*, the concentration of both nitrite and silicate were higher compared to the *C. nodosa* stations. No differences were found in the mean values of total suspended solids and pigments except at the *C. nodosa* donor emerged station. However, the emerged *C. nodosa* areas considered as donors in this work had the highest values of chlorophyl-*a* and carotenoids in water matrix.

Sediments from the *C. nodosa* donor stations more contained in fine particules fraction than the receiving stations. No differences were found in the sediment characteristics of the *R. cirrhosa* stations. In the case of *C. nodosa*, a donor station located near port infrastructures showed the highest values of nutrient content. The nutrient contents of sediments from *R. cirrhosa* transplants were generally higher than those of *C. nodosa*.

Because of macrophyte samples area under expertise taxonomic revision, results presented in this report correspond to those recorded in Action A.2.2 (Ex-Ante monitoring program). A total of 28 species were identified, corresponding to the filums Chlorophyta, Rhodophyta, Orchrophyta and Tracheophyta. MaQI application index is under analysis due to the processing of the samples. Results of species richness and relative dominance in terms of covering are presented in this report.

Regarding to the benthic macroinvertebrates and the application of the BITS and M-AMBI indices, data presented in this report corresponds to an update for the second year of monitoring (2023). Data corresponding to third monitoring year (2024) are under analysis. For this report, a total of 87 taxa corresponding to the filums Annelida, Mollusca, Arthropoda, Cnidaria, and Porifera, were identified. The dominant filum was Annelida (48 taxa identified), and the less dominant were Cnidaria and Porifera (1 taxa each one). Across the different periods presented, the richness trended to increase in the deeper *C. nodosa* receiving stations, and in the *R. cirrhosa* receiving one. Highest values of species richness correspond to *R. cirrhosa* receiving station during the second year of monitoring (2023), which also presented the lowest richness value for the year 2021. Differences among the deeper and shallower *C. nodosa* receiving station was observed for species richness and diversity index.

The application of the BITS quality status index showed a similar dynamic than those from species richness and diversity index, for the case of *C. nodosa* receiving stations: increasing across time in the deeper areas and decreasing in the shallower ones. However, in the case of *R. cirrhosa* receiving station, despite their results in species richness and diversity, BITS index value decrease across years.

The relative dominance of the different ecological groups established by M-AMBI index is presented in the results. When application of the M-AMBI index to establish the ecological quality status of the different receiving stations for both species transplanted, differences were found when comparing those receiving stations from the different depth for the case of *C. nodosa*, and across year in *R. cirrhosa* receiving stations.

The results of the HBFI index are in the process of being analysed. For this report, the abundance data of the different species obtained for the year 2023, is presented.

PROJECT DESCRIPTION

The project targets the priority habitat 1150* Coastal lagoons. The habitat can be free of vegetation or, on the other hand with marine phanerogam associations identified as priorities for the conservation status of these habitats. The sites targeted in this project originally had part of the area covered by seagrass, and the main objective is to solve the drastic regression of submerged seagrass and the slowness with which this vegetation can colonize areas. In this sense, LIFE SeResto (LIFE 12 NAT/IT/000331) proved the feasibility and good results of transplantation techniques.

For the Mar Menor case (ES6200030 and ES0000260), changes in hydrodynamic conditions after dredging one of its inlets caused the invasion and rapid expansion of *Caulerpa prolifera* (Forsskål) J.V.Lamouroux, 1809, accumulating organic matter and silt to the detriment of the original *Cymodocea nodosa* (Ucria) Ash, meadows and its fauna. In shallow areas, seagrass meadows were kept undamaged thanks to high light irradiation in the bottom, limiting the growth of *C. prolifera*. However, dredging and placement of breakwaters on beaches altered these conditions favouring the formation of sandbanks, accumulation of detritus and the turbidity of water, reducing sandy habitats and seagrass meadows. For that, it is important to restore the hydrodynamic, the quality of substrate and the water column, to recover *C. nodosa* meadows by restoration actions.

The project specific objectives are:

- Restoring and consolidating priority habitat 1150* in 6 coastal lagoons, by transplanting submerged phanerogams to promote ecosystem self-sustainability and restoring water circulation in the lagoons; an area of 1000 m^2 will be transplanted at each of the 6 lagoons with seagrass typical of that biogeographic area.
- Contributing to achieve a good ecological state of transitional state by demonstrating the effectiveness of the proposed actions to pursue the objectives set in the (WFD 2000/60/EC Art. 4).
- Quantifying the value of ecosystem services provided by the lagoon environments and the seagrass meadows.
- Training of future trainers in this technique, targeting site managers/professionals not participating to the project to ensure transferability and replicability in other sites.

In the case of the Mar Menor lagoon, transplantation will be carried out in the action C.3 for the species *Ruppia cirrhosa* and *Cymodocea nodosa*. Prior to this, sites were selected and characterized their environmental status in the action A.2.2 Ex-ante monitoring in Mar Menor. All the transplants will be monitored and their environmental parameters in action D.3. in order to address and test the effectiveness of the actions and to verify the results expected.

Direct expected results of the project are rooting and propagation of transplanted sods, with a success over 80% after 4/5 years. An improvement of conservation status of habitat 1150* and lagoon biodiversity and of the ecological quality of water bodies.

Referring to the mid-long-term results, over 10 years it is expected a well-structured seagrass meadow of the targeted water body surface. Therefore, a good ecological status it is expected.

TRANSPLANT MONITORING OBJECTIVES

To order to evaluate the habitat 1150* improvement and to quantify the benefits derived from transplantation of angiosperms and on the Biological Quality Elements (sensu 2000/60/EC – macrobenthos, macroalgae and fish fauna).

Monitoring is necessary both for the quantification of the results in terms of expansion of the new meadows, and to verify the possible need for corrective interventions. Meadow growth is expected to become effective the 1st year after transplantation, after the plants have adapted well to the new environment. In case of partial decay, the sods will be replaced with other new sods. If instead the failure concerns the entire station, the causes will be analysed and another area will be selected with chances of success may be greater, without additional costs for the project.

Sub Action D.3.1 Monitoring angiosperm growth:

Monitoring of the plant growing and the rate of expansion of the newly formed meadows will be carried out. Evaluated parameters will be survival of transplanted sods; rooting of the rhizomes; rate of expansion of each transplanted sod; estimate of the coverage of the newly formed meadows. Angiosperm growth will be carried out measuring the diameter of each sod. At each measurement campaign the total sod coverage will be referred to each transplant area of 10x 10 m as per cent coverage. In the successive years, the new sods naturally produced by seeds or single rhizomes will also be included in the measures. The sub-action will be documented through the preparation of maps and photo shoots and monitoring reports.

Sub Action D.3.2 Monitoring biodiversity and the environmental quality status:

In this sub-action, the monitoring of the environmental quality status through the use of the Biological Quality Elements (BQE) required by the EU Directive 2000/60 will be carried out, as well as in terms of biodiversity (habitat 1150 *) and at the ecosystem level in general. This sub-action involves both the analysis of physico-chemical parameters in water samples, sediments and particulate matter and the collection of macrophytes, benthic macroinvertebrates and fish fauna (biological elements) for the application of ecological quality indexes at all the stations monitored in Mar Menor lagoon. Physico-chemical parameters in water sand traps will be analyses every month in one station during the 1st year and the last year to compare results before and after angiosperm meadows rooting. In surface sediment analyses will occur twice a year. The biological elements will be monitored twice a year at each of 8 stations.

The action includes the implementation of the monitoring based on the indications established by the protocol. The following parameters will considered: calculation of the MaQI index(macrophytes), following Sfriso et al. (2011, 2014); calculation of diversity index, the BITS and M-AMBI indices (macrobenthos), following Mistri & Munari (2008) and Muxika et al. (2007); calculation of the HFBI, (Habitat Fish Bioindicator Index), following Franco et al. (2009); estimation of relative abundance of fish fauna of conservation interest and of commercial interest (to support action D6); estimation of the total plant biomass produced in the sites, for the quantification of the CO2 sequestration (to support the action D6).

The following abiotic parameters will also be analysed: Water matrix: Nutrients: total ammonium ($N\text{-}NH_4^+$); oxidized nitrogen ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$), dissolved inorganic phosphorus (SRP), dissolved silicates (SiO_4^{2-}); Suspended solids (TSS); Sedimentation rates by traps (SPM = Settled Particulate Matter); Other hydrological parameters: transparency (Tr); temperature (t); dissolved oxygen (DO); pH; Eh; salinity (S); depth (D), Chlorophyll-a and phaeopigments. Sediment and particle matrix deposited in sedimentation traps: total, inorganic and organic carbon (TC, IC, OC); total nitrogen (TN); total, inorganic and organic phosphorus (TP, IP, OP); wet and dry density (Dsed); humidity, porosity; percentage of fine fraction <63 µm (fines).

MATERIAL AND METHODS

Monitoring location sites

Monitoring had been carried out by UMU (University of Murcia) at all transplant stations of Mar Menor, for a total of 8 receiving of *C. nodosa* stations and 1 receiving stations of *R. cirrhosa*. Provisioning meadows included 5 different stations (4 for *C. nodosa*, and 1 for *R. cirrhosa*).

For *C. nodosa* transplants, Los Urrutias location was proposed both for donor and receiving stations. Within the donor station, 4 different locations were monitored considering different threats under study: Port (LUDP), watercourse affected by heavy metals (BEDW), emersion risk (LUDS) and natural meadows nearby (LUDN). Regarding to receiving station (LUR), a total of 8 sites has been monitored (**Figure 1**).

For *R. cirrhosa*, 1 transplant location has been monitored in the Perdiguera island (IPR), and the same for donor site in Encañizadas (ENDN), considering the last one as a natural donor meadow (Figure 1)

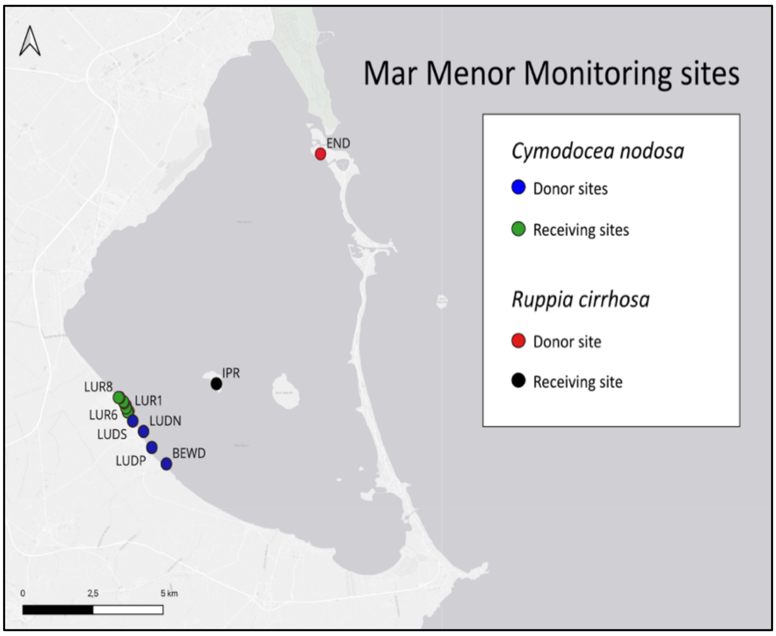

Figure 1. Location of C. nodosa and R. cirrhosa donor and receiving meadows in the Mar Menor coastal lagoon monitored included in this report.

Sub-action D.3.1. Monitoring angiosperm growth

According to the general plan of the project monitoring of the plant growing and the rate of expansion of the newly formed meadows must be carried out once a month for the first 6 months after transplantation; from the 7th month, 1 sampling campaign in early spring, 1 in early summer, and 1 in early autumn of each year. Monitoring will be carried out at all transplant stations for *C. nodosa* and *R. cirrhosa,* for a total of 14 stations (8 receiving + 4 donor for *C. nodosa*, and 1 receiving + 1 donor for *R. cirrhosa*).

Angiosperm growths were carried out measuring the diameter of each sod. At each measurement campaign the total sod coverage was referred to each transplant area of 10 x 10 m as per cent coverage (%).

Parameters determined:

- Transplant survival.
- Rate of expansion of the transplant.
- Covering of meadows.

Sub-action D.3.2. Monitoring biodiversity and the environmental quality status

Water matrix and physico-chemical parameters

According to the general plan of the project monitoring of water analyses and other physicochemical parameters were carried out once a month for the first 6 months after transplantation; from the 7th month, 1 sampling campaign in early spring, 1 in early summer, and 1 in early autumn of each year.

Parameters determined:

- Nutrients: total ammonium (N-NH_4^+); oxidized nitrogen (N-NO_2^-, N-NO_3^-); dissolved inorganic phosphorous (SRP); dissolved silicates (SiO_4^{2-}); suspended solids (TSS).
- Transparency (Tr), temperature (t), dissolved oxygen (DO), pH, Eh, salinity (S), Chlorophyll-a and phaeopigments.

Physicochemical parameters corresponding to: Transparency (Tr), temperature (T), dissolved oxygen (DO), pH, Eh, Salinity (S) and depth (D) were measured with a multiparametric sonde YSI- EXO2. Nutrients corresponding to total ammonium (N-NH_4^+); oxidized nitrogen (N-NO_2^-, N-NO_3^-); dissolved inorganic phosphorous (SRP) and dissolved silicates (SiO_4^{2-}) were analysed by spectrophotometric and colourimetry method with the SEAL AutoAnalyzer 3 HR following Parsons et al (1984), chlorophyll-a (Clh *a*) and phaeopigments were estimated via spectrophotometric method and suspended solids (TSS) by weight difference, following Parsons et al. (1984).

Sediment

According to the general plan of the project sediment sampling were carried out twice a year (spring and autumn) in each of the donor and receiving sites proposed for both species under study, taking 3 replicates by site. Samples were taken using a 10 cm diameter corer from the first 5 cm of sediment. Collected material was transferred to their respective containers and frozen to their posterior analysis in the laboratory.

Parameters to determine:

- Humidity, porosity and wet and dry density.
- Percentage of fine fraction <63 μm.
- Total, inorganic and organic carbon (TC, TIC, TOC).
- Total nitrogen (TN).
- Total, inorganic and organic phosphorous (TP, IP, OP).

Sediment nutrients (TC, IC, and TN) were analysed by Elemental Analyzer LECO CN 828 and total organic carbon (TOC) by Elemental Analyzer LECO CHNS 932 with HCl addition. The standard used for total nitrogen and total carbon determination is the Leco Soil LCRM 502-697 (lot 1001) and the standard used for organic carbon is the Leco Soil LCRM 502-308.

Total, inorganic and organic phosphorous were analysed following Aspila et al. (1976) and by colourimetry method following Murphy & Riley (1962) and Parsons et al. (1984). Humidity, porosity, and wet and dry density were obtained by drying at 110 °C in crucibles of known volume and weight. Humidity (the values is expressed as a percentage by the ratio: ml water/g wet sediment); Porosity (ml water/ml wet sediment); Wet and dry density (wet weight/sediment volume and dry weight/sediment volume, respectively). The percentage of fine fraction <63 µm in the surface sediment was obtained by wet sieving approximately 50 g of dry sediment through sieves previous separation of the <1 mm shell fraction.

<u>Settled particulate matter.</u>

Sedimentation traps (base: 20 x 20 cm; mouth: 15 x 15 cm; height 10 cm) have been placed and collected every 7-28 days during the first year (2022) in both receiving and donor sites.

Methodology for determination of physico-chemical parameters and nutrients is similar to sediment procedures. Sedimentation rate is being estimated as dry weigh of sediment deposited (Kg)/m^2/day.

Parameters determined:

- Settle particulate matter (SPM)
- Humidity, porosity and wet and dry density.
- Percentage of fine fraction <63 µm
- Total, inorganic and organic carbon (TC, TIC, TOC).
- Total nitrogen (TN).
- Total, inorganic and organic phosphorous (TP, IP, OP).

<u>Macrophyte monitoring and MaQI application.</u>

According to the general plan of the project macrophyte sampling campaigns area carrying out twice a year (spring and autumn) in each donor and receiving sites proposed for both species under study, taking 3 replicates by site.

Parameters to determine:

- Species of macroalgae present.
- Total covering of macroalgae.
- Relative abundance of macroalgae divided in high ecological value taxa (score 2) and Rhodophyta and Chlorophyta with score 0-1.
- Species of phanerogams presents and percentage of covering within the sites.

For macroalgae total covering, all species presented in a 400 cm^2 surface were collected, and covering were referred to this area in term of percentage (%). Macroalgae collected were fixed in 4% formaldehyde in sea water for its posterior taxonomic identification in the laboratory of Universidad Complutense of Madrid (Spain), with the collaboration of experts (Isabel María Pérez-Ruzafa).

Relative abundance of macroalgae will be determined as the number of species within the study site and classified according to the punctuation proposed by Sfrisso (2010): species with punctuation 2 (high ecological value) and species with punctuation 0-1 were subdivided in Chlorophyta and Rhodophyta. Wet weigh of all the classes will be measured using an electronic balance.

All the parameters mentioned before are necessary to determine the application of Macrophyte Quality Index (MaQI) (Sfriso, 2010).

<u>Benthic macroinvertebrates</u>

According to the plan of the project, benthic macroinvertebrates were sampled in each of the donor and receiving sites proposed for both species under study, taking 3 replicates by site in spring and autumm. Samples were taken by mean of a 13 cm diameter corer from the land. These sample were sieved with a 0.5 mm sieve bag. Collected material was transferred to their respective containers with sea water to their posterior preservation and identification in the laboratory. Abundance and specific richness will be determined for the application of the biological indices BITS (Michele & Munari, 2008) and M-AMBI (Muxika et al., 2007).

Parameters to determine:

- Taxonomic identification to species level, when possible, for benthic macroinvertebrates.
- Abundance and number of species.

These parameters are necessary for the calculation of the BITS and M-AMBI indices (Muxika et al., 2007 and Mistri & Munari, 2008).

<u>Fish fauna</u>

Fish fauna monitoring and HBFI application will be studied twice a year (spring and autumn) at each location during the monitoring, with a total of 6 samples a year. The campaigns were carried out using a beach net trawl survey to assess the state of the fish community through the HBFI index, as an indicator of the ecological quality of the area.

The parameters to be determined are those necessary for the application of the HBFI Index and for the assessment of the presence/absence of species of conservation interest. The analyses correspond to:

- Taxonomic identification of the individuals identified at species level, carried out on the basis of the identification key and available manual.
- Abundance of all individuals fished.
- For each species, in samples with less than 100 individuals, the length (total length in mm) of each individual will be measured. For very large samples, the measurement shall be made on 100 individuals chosen by chance.

Table 1. Number of samples collected per station for Action D.3.1 Monitoring of angiosperm growth, and D.3.2 Monitoring biodiversity and environmental parameters, are showed.

Action		2022												2023												2024					
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	III	IV	V	VI
D3.1		**Monitoring angiosperm growth**																													
D3.1	Transplant survival						9	9	9		9	9	9			9			9			9						9			9
D3.1	Rate of expansion.						9	9	9		9	9	9			9			9			9						9			9
D3.1	Covering.						9	9	9		9	9	9			9			9			9						9			9
D.3.2		**Monitoring biodiversity and environmental**																													
D.3.2	Water matrix						14	14	12		14	14	14			14			14			14						14			14
D.3.2	Settled particulate matter							5	6		9	7	7																		
D.3.2	Sediment						42				6	36							42			42									42
D.3.2	Macrophyte MaQI						14				6	36							42			42									42
D.3.2	Fish						3	1			1	3					4					4								4	
D.3.2	Macrobenthos AMBI/BITS						42				6	36							42			42									42

RESULTS

Sub-Action D.3.1. Monitoring angiosperm growth

For the transplants from spring 2022, first angiosperm growth monitoring data were recorded in June 2022, and continued monthly during the same year. Monitoring of angiosperms growth continues in 2023: 1 campaign in March, 1 campaign in June, and 1 campaign in October. Another campaign in spring 2024 was carried out. At each campaign, a total of 9 measurements (8 for *C. nodosa* + 1 for *R. cirrhosa*) were done at each campaign (**Table 1**). For this report, results are shown as an updated state of the sub-action (up to spring 2024). Transplant survival (**Figure 2**) reached 100% in all deep receiving stations (LUR1, LUR5, and LUR8), for the case of *C. nodosa*, and in LUR4, and LUR6 (shallow receiving station). Other shallow receiving stations also increased their survival but not reached a 100% (LUR3, and LUR7). For the case of LUR2 (shallow), survival decreased. Receiving station for *R. cirrhosa* transplants increased its survival in 2023 (90% of sods transplanted survived) and maintained relatively similar values in the last campaign in spring 2024 (80% of sods).

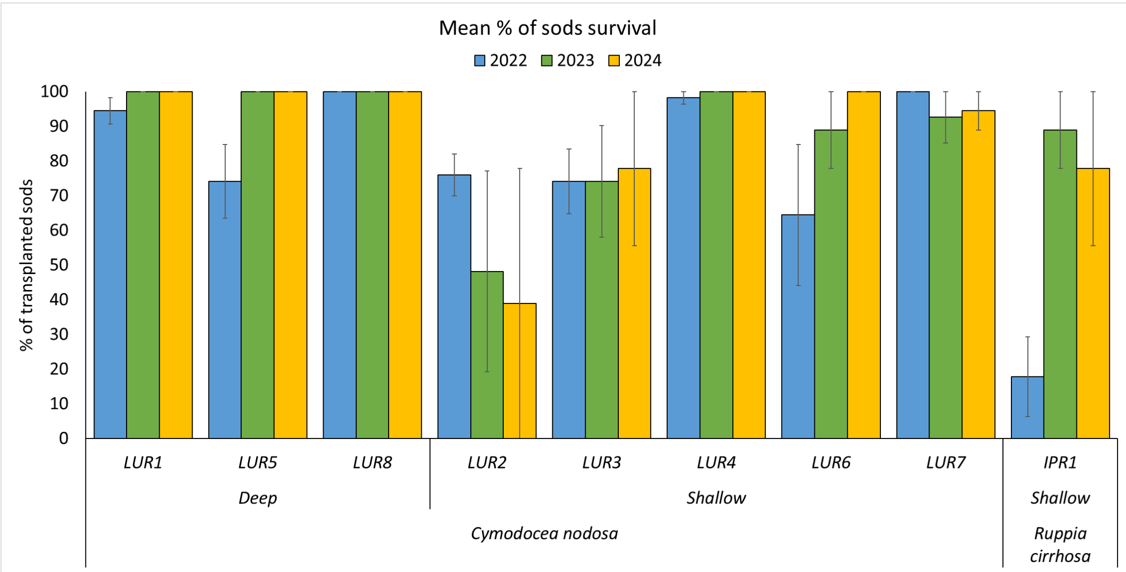

Figure 2. *Cymodocea nodosa* and *Ruppia cirrhosa* mean percentage of transplanted sod survival for the different years of monitoring. Survival measured as the percentage of presence or absence of the 9 sods in each transplant within the 10 x 10 m area.

The highest values of rate of expansion of *C. nodosa*, in the first monitoring period was shown in LUR4, and LUR1 (**Figure 3**). However, in 2023, highest mean values of rate of expansion was shown in LUR1, and LUR5. In both cases, the maximun rate value corresponded to June 2023. The rest of receiving sites did not show mean values greater than 1. A decreases in the rate of expansion was shown in LUR4 for the year 2023, respect to 2022. During the current year of monitoring (2024), despite the previous highest values recorded corresponded to LUR1 and LUR5, in this period, higest vaues of rate of expansion was measured in LUR8. Within the sahllow stations, LUR3, and LUR6 presented similar mean values of rate of expnsion.

The case of *R. cirrhosa* resulted different in terms of rate of expansion. This species transplantation desapeared in August 2022, and was transplanted as a reinforcement in June 2023, with a decrease in its surface to October 2023. However, in 2024 the situation was turned inverse: a great expansion was observed in June 2024 for the receving sites, whereas donor natural meadows disapeared.

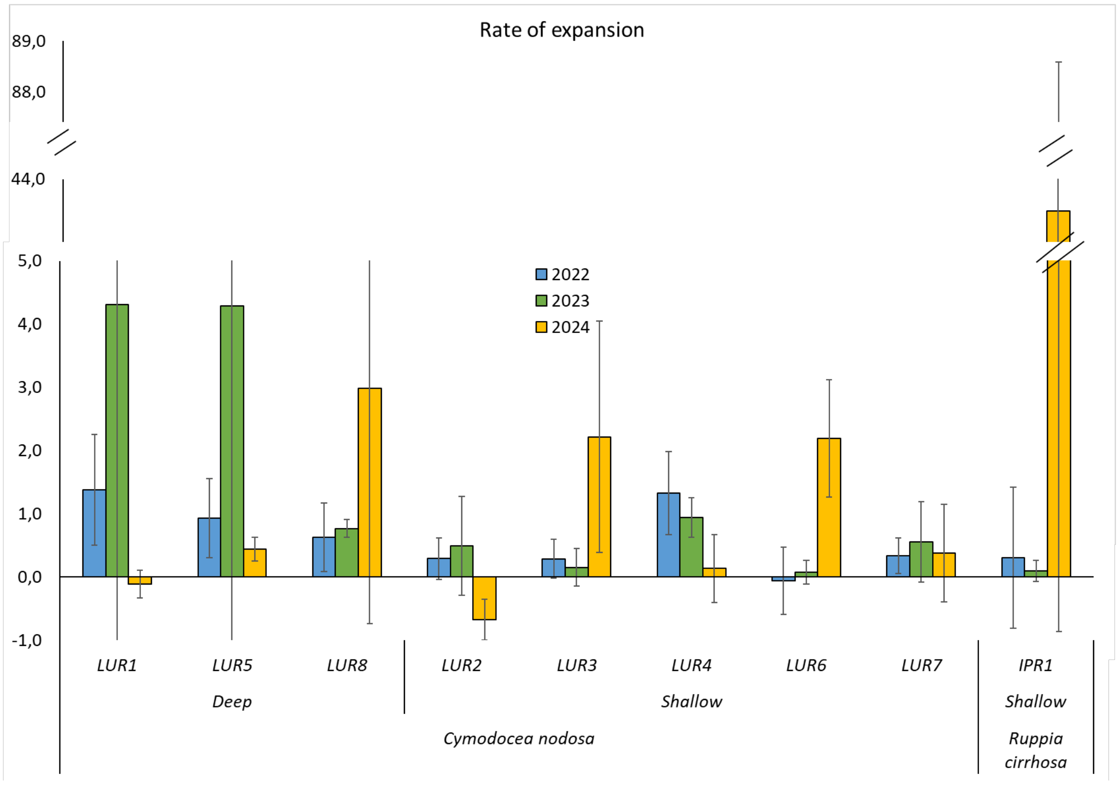

Figure 3. Mean values of rate of expansion of the transplants for the different years of monitoring for both species: *Ruppia cirrhosa* (orange), and *Cymodocea nodosa* in shallow and deeper areas. Rate of expansion ratio (cm^2) measured as the difference between the initial surface area and the final surface area divided by the final surface area.

First year of monitoring of *C. nodosa* transplants, the highest value of covering was reached in LUR4 (shallow area), which correspond to the highest value of rate of expansion in the first year (2022) (**Figure 4**). Lowest values were observed in other shallow stations (LUR2, and LUR3). During the second year of monitoring (2023), greater values of coverage was reached by almost all receiving stations. The highest value corresponded to LUR4 (>50%), followed by the deepest stations LUR1, LUR5, and LUR8. Shallow stations corresponding to LUR2, LUR3, LUR6 and LUR7 presented the lowest values of coverage. The dynamic stay similar during the third year of monitoring (2024). However, whereas for the case of the shallow station LUR6, in 2023 did not cover most of the 10%, in this last year of monitoring, covering of newly meadows reached values of almost 50% of the total area surveyed. In this year, receiving stations with a coverage higher than 50% of the total area surveyed was those from deeper area (LUR1, and LUR5), and the shallow stations LUR4, and LUR6.

For the case of *R. cirrhosa* transplants (IPR1) (**Figure 4**), the mean percentage of coverage increased across the monitoring years. During the first year of monitoring (2022), total covering was almost zero, because of its disappearance during the second month of monitoring. In the year 2023, thanks to the reinforcement of this species transplantation, mean coverage reached values of 11%. During the last year of monitoring (2024), as mentioned, the case of receiving site of *R. cirrhosa* experienced a suddenly increase in their surface, reflected both in the rate of expansion mentioned (**Figure 3**), and its mean coverage (**Figure 4**).

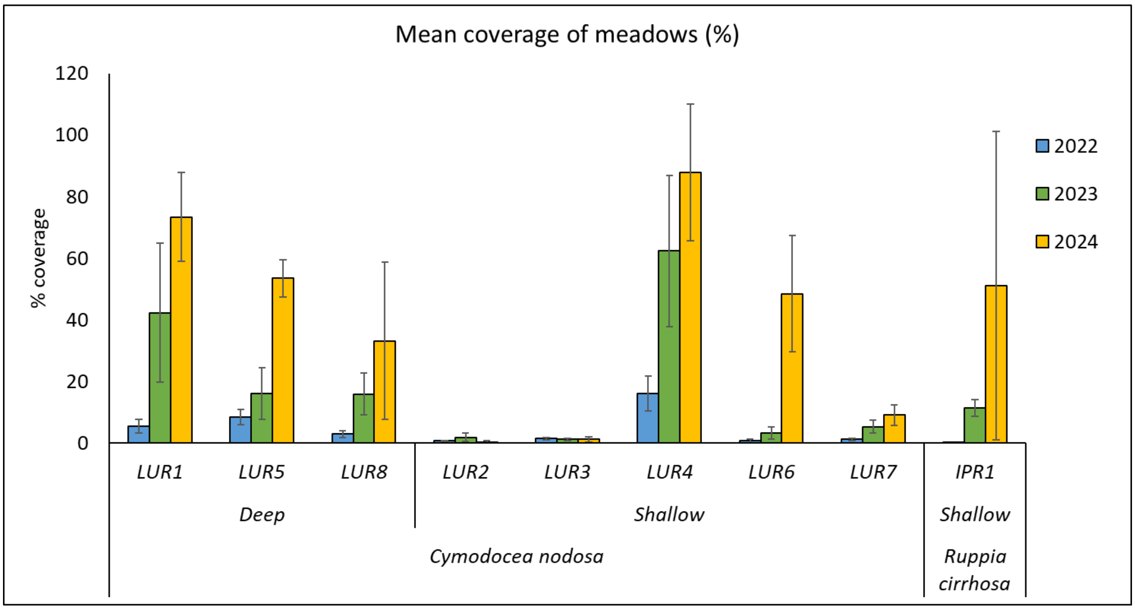

Figure 4. Mean coverage of meadows (%), for both species: *Ruppia cirrhosa*, and *Cymodocea nodosa* in shallow and deep areas, for the tree years of monitoring. Percentage of coverage is based on the sum of the coverage of the 9 sods in each 10 x 10 m transplanting area.

Sub-action D.3.2. Monitoring biodiversity and the environmental quality status

Monitoring of the water matrix and physico-chemical parameters, and settled particulate matter presented in this monitoring report correspond to the second year of monitoring (2023). Periodicity, and total number of samples collected for each variable is presented in **Table 1**.

<u>Water matrix and physico-chemical parameters</u>

For this report, these parameters were sampled every three months, in 12 stations for the case of study of *C. nodosa* (8 receiving stations + 4 donor stations) and 2 for *R. cirrhosa* stations (1 receiving + 1 donor station). Monitoring has been continued during 2024 in March and June but only the results for 2021, 2022 and 2023 are shown.

Results of the physico-chemical parameters are presented in **Table 2**, nutrients result in **Table 3**, suspended solids in **Figure 5** and pigments in **Figure 6.**

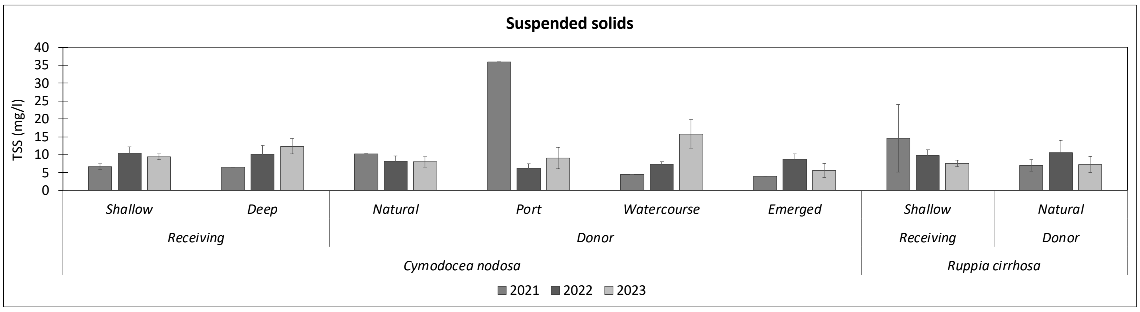

Figure 5. Mean suspended solids in water (mg/l) from the different transplant stations for both donor and receiving *C. nodosa* and *R. cirrhosa* sites for the Ex-Ante monitoring (2021) and first and second year of monitoring (2022 and 2023). Donor stations code: BEDW (Beal watercourse); LUDN (C. nodosa natural meadow); LUDP (Port); LUDS (Emersion risk); ENDN (R. cirrhosa natural meadow).

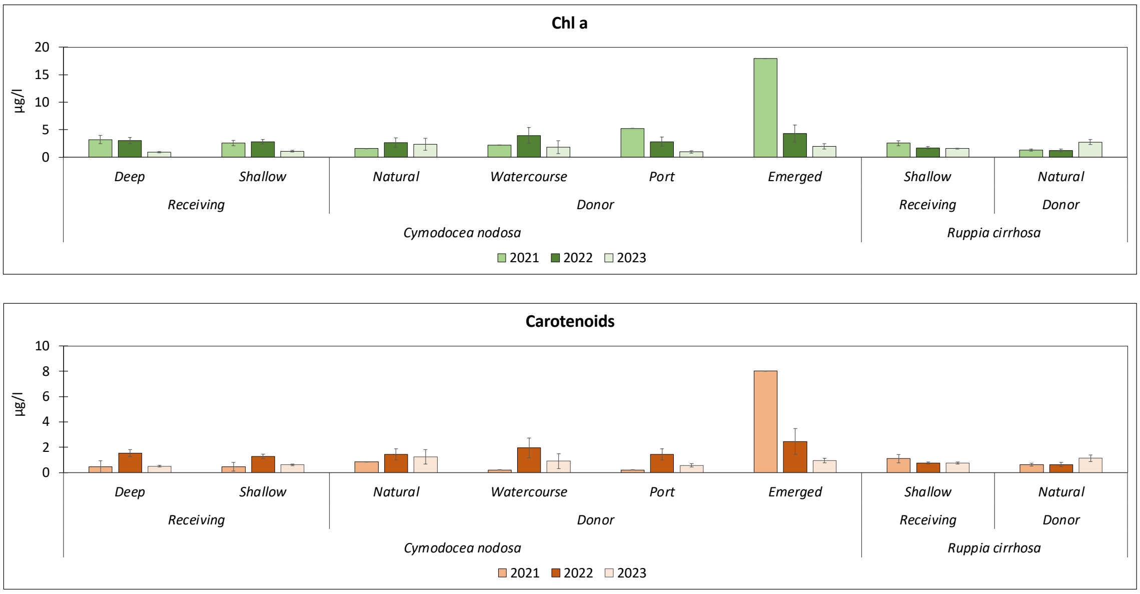

Figure 6. Mean pigments (chlorophyll a, and carotenoids) in water (µg/l) from the different transplant stations for both donor and receiving *C. nodosa* and *R. cirrhosa* sites for Ex-Ante monitoring (2021) and first and second year of monitoring (2022 and 2023). Donor stations code: BEDW (Beal watercourse); LUDN (*C. nodosa* natural meadow); LUDP (Port); LUDS (Emersion risk); ENDN (*R. cirrhosa* natural meadow).

Table 2. Mean values and standard error (SE) for physico-chemical parameters measured in all stations considered in the Ex-Ante Monitoring (2021) and first and second year of transplant (2022 and 2023).

Year	SpTransp	Receiving Donor	Zone	ODO (mg/L)	Temp C	pH	Eh (mV)	Salinity (PSU)
2021 (Action A.2.2)	*Cymodocea nodosa*	Receiving	Shallow	5.19 ± 0.09	28.77 ± 0.05	7.99 ± 0.02	73.91 ± 1.85	42.84 ± 0.02
			Deep	5.53 ± 0.18	28.98 ± 0.04	8.11 ± 0.04	81.48 ± 4.79	42.65 ± 0.02
		Donor	Emerged	3.58 ± 0.13	26.99 ± 0.01	7.41 ± 0.01	151.77 ± 0.62	43.45 ± 0.00
			Natural	6.61 ± 0.06	27.98 ± 0.01	7.86 ± 0.00	74.23 ± 0.31	42.66 ± 1.08
			Port	6.81 ± 0.09	28.06 ± 0.03	7.97 ± 0.01	52.45 ± 0.21	42.37 ± 1.38
			Watercourse	8.54 ± 0.28	29.64 ± 0.32	8.19 ± 0.01	47.57 ± 0.35	40.44 ± 1.64
	Ruppia cirrhosa	Receiving	Shallow	7.15 ± 0.09	28.90 ± 0.02	7.85 ± 0.00	79.89 ± 0.32	42.68 ± 0.01
		Donor	Natural	11.17 ± 0.08	30.24 ± 0.01	9.05 ± 0.03	43.94 ± 1.67	40.17 ± 0.02
2022	*Cymodocea nodosa*	Receiving	Shallow	6.75 ± 0.04	20.88 ± 0.15	8.18 ± 0.00	7.98 ± 2.57	41.68 ± 0.12
			Deep	7.07 ± 0.05	20.87 ± 0.19	8.20 ± 0.01	27.06 ± 3.07	41.33 ± 0.21
		Donor	Emerged	7.04 ± 0.11	19.58 ± 0.41	8.20 ± 0.01	9.50 ± 7.37	41.84 ± 0.33
			Natural	7.89 ± 0.10	21.48 ± 0.46	8.28 ± 0.01	3.33 ± 6.54	41.19 ± 0.50
			Port	7.08 ± 0.07	17.84 ± 0.43	8.80 ± 0.36	32.81 ± 8.23	42.03 ± 0.32
			Watercourse	6.25 ± 0.05	20.19 ± 0.37	8.10 ± 0.01	64.01 ± 6.78	41.59 ± 0.15
	Ruppia cirrhosa	Receiving	Shallow	7.29 ± 0.08	21.47 ± 0.33	8.24 ± 0.01	-26.25 ± 4.90	41.73 ± 0.38
		Donor	Natural	6.86 ± 0.19	26.77 ± 0.48	8.08 ± 0.01	1.46 ± 3.93	40.40 ± 0.10
2023	*Cymodocea nodosa*	Receiving	Shallow	7.68 ± 0.05	24.34 ± 0.13	8.33 ± 0.00	-91.40 ± 1.11	43.86 ± 0.03
			Deep	7.05 ± 0.05	25.26 ± 0.11	8.32 ± 0.00	-94.41 ± 0.95	43.88 ± 0.04
		Donor	Emerged	7.71 ± 0.22	25.48 ± 0.36	8.32 ± 0.01	-89.57 ± 3.19	44.13 ± 0.07
			Natural	7.41 ± 0.16	24.86 ± 0.25	8.33 ± 0.02	-98.31 ± 2.56	43.53 ± 0.19

			Port	7.85 ± 0.03	22.93 ± 0.22	8.42 ± 0.00	-110.52 ± 1.53	43.02 ± 0.11
			Watercourse	4.88 ± 0.12	25.97 ± 0.24	8.08 ± 0.03	-80.20 ± 3.13	43.35 ± 0.07
	Ruppia cirrhosa	Receiving	Shallow	6.76 ± 0.14	26.16 ± 0.33	8.16 ± 0.01	-98.32 ± 2.02	39.32 ± 0.69
		Donor	Natural	3.98 ± 0.03	25.96 ± 0.02	8.07 ± 0.00	-94.25 ± 1.29	39.09 ± 0.09

Table 3. Results of annual mean nutrients concentrations in the years 2021, 2022 and 2023 in water matrix samples from the different species transplant stations both donor and receiving. BDL (Below detection limit).

Year	SpTransp	Receiving Donor	Zone	N - NO3 µg at /l	N - NO2 µg at /l	N - NH4 µg at /l	Si - SiO2 µg at /l	P - PO4 µg at /l
2021 (Action A.2.2)	*Cymodocea nodosa*	Receiving	Shallow	7.25 ± 4.40	0.96 ± 0.47	1.69 ± 1.12	3.99 ± 0.67	0.19 ± 0.01
			Deep	7.61 ± 1.87	0.99 ± 0.16	0.90 ± 0.14	3.26 ± 0.37	0.20 ± 0.01
		Donor	Emerged	BDL	0.15 ± 0.00	1.30 ± 0.00	2.81 ± 0.00	0.33 ± 0.00
			Natural	BDL	0.13 ± 0.00	BDL	6.22 ± 0.00	0.12 ± 0.00
			Port	BDL	0.13 ± 0.00	BDL	2.38 ± 0.00	0.21 ± 0.00
			Watercourse	3.13 ± 0.00	0.29 ± 0.00	BDL	1.77 ± 0.00	0.20 ± 0.00
	Ruppia cirrhosa	Receiving	Shallow	0.09 ± 0.13	0.19 ± 0.01	0.89 ± 0.19	15.29 ± 0.30	0.42 ± 0.11
		Donor	Natural	0.31 ± 0.21	0.13 ± 0.01	0.50 ± 0.17	2.06 ± 0.29	0.16 ± 0.02
2022	*Cymodocea nodosa*	Receiving	Shallow	6.65 ± 1.20	0.57 ± 0.09	1.95 ± 0.32	10.37 ± 1.99	0.18 ± 0.03
			Deep	6.55 ± 0.90	0.63 ± 0.09	2.28 ± 0.32	11.82 ± 1.99	0.17 ± 0.02
		Donor	Emerged	5.37 ± 1.62	0.56 ± 0.17	2.91 ± 0.22	11.24 ± 4.03	0.15 ± 0.03
			Natural	5.80 ± 1.95	0.49 ± 0.12	2.05 ± 0.86	9.73 ± 2.82	0.19 ± 0.03
			Port	7.21 ± 1.39	0.73 ± 0.09	6.71 ± 1.62	11.89 ± 4.09	0.22 ± 0.05

			Watercourse	200.46 ± 180.55	2.31 ± 0.91	9.82 ± 3.60	20.13 ± 11.18	0.26 ± 0.05
	Ruppia cirrhosa	Receiving	Shallow	1.13 ± 0.56	0.16 ± 0.03	1.52 ± 0.50	12.55 ± 3.91	0.16 ± 0.05
		Donor	Natural	0.27 ± 0.10	0.07 ± 0.01	4.61 ± 2.53	3.52 ± 0.61	0.14 ± 0.03
2023	*Cymodocea nodosa*	Receiving	Shallow	0.004 ± 0.02	0.11 ± 0.02	0.63 ± 0.46	7.50 ± 1.32	0.12 ± 0.02
			Deep	0.09 ± 0.05	0.16 ± 0.04	0.40 ± 0.10	7.31 ± 1.03	0.13 ± 0.01
		Donor	Emerged	0.02 ± 0.05	0.12 ± 0.03	1.61 ± 1.17	7.59 ± 2.03	0.17 ± 0.05
			Natural	BDL	0.24 ± 0.12	1.04 ± 0.75	6.19 ± 1.13	0.24 ± 0.04
			Port	0.21 ± 0.09	0.11 ± 0.03	0.77 ± 0.35	8.73 ± 2.92	0.16 ± 0.04
			Watercourse	1.17 ± 0.51	0.21 ± 0.09	3.43 ± 1.85	10.09 ± 4.42	0.24 ± 0.06
	Ruppia cirrhosa	Receiving	Shallow	0.04 ± 0.04	0.10 ± 0.03	3.39 ± 3.23	11.20 ± 3.62	0.21 ± 0.07
		Donor	Natural	0.13 ± 0.06	0.15 ± 0.03	6.50 ± 4.49	9.58 ± 3.75	0.31 ± 0.07

Settled Particulate Matter (SPM) rates

Sedimentation traps (base: 20 x 20 cm; mouth: 15 x 15 cm; height 10 cm) have been placed and collected every 7-28 days during the first year (2022) in both receiving and donor sites. Results of the physico-chemical parameters determined in sediments are presented in **Table 4** (porosity is under laboratory analysis) and total carbon in **Figure 7**, total nitrogen in **Figure 8** and total phosphorous in **Figure 9**. In terms of the nutrients analysed, both for carbon, nitrogen and phosphorus, the receiving area of *R. cirrhosa* show a higher concentration. In the case of *C. nodosa* it is the donor areas that show the highest concentration.

Table 4. Physico-chemical parameters determined sediments collected from settled particulated matter traps. Mean values correspond to first year (2022) of transplants. < 0.063: fine particle percentage.

	Donor/Receiving	Humidity	Wet Density (g/cm^3)	Dry Density (g/cm^3)	< 0,063(%)
Cymodocea nodosa	Donor	1.37 ± 0.44	1.15 ± 0.06	1.18 ± 0.06	42.01 ± 7.45
	Receiving	0.35 ± 0.07	1.54 ± 0.02	1.54 ± 0.01	4.94 ± 0.76
Ruppia cirrhosa	Donor	1.94 ± 0.81	0.95 ± 0.19	0.97 ± 0.21	60.22 ± 7.01
	Receiving	1.76 ± 1.20	0.95 ± 0.08	0.94 ± 0.07	65.60 ± 11.71

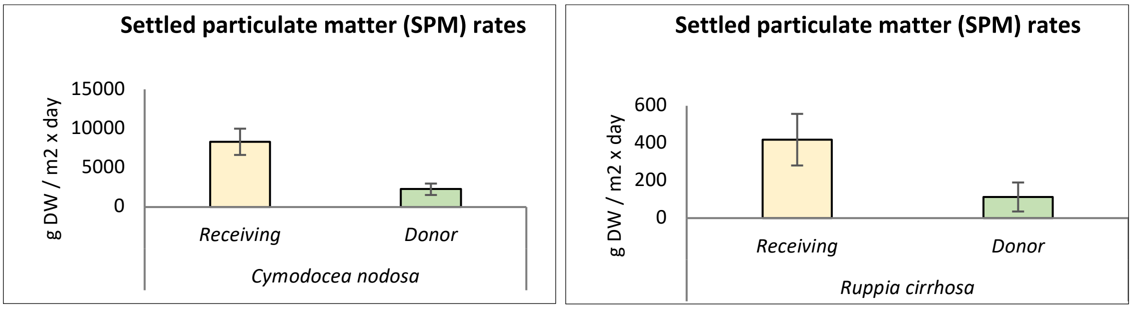

Figure 7. Mean Settled particulate matter (SPM) rates in the study areas in the first year (2022) of transplants.

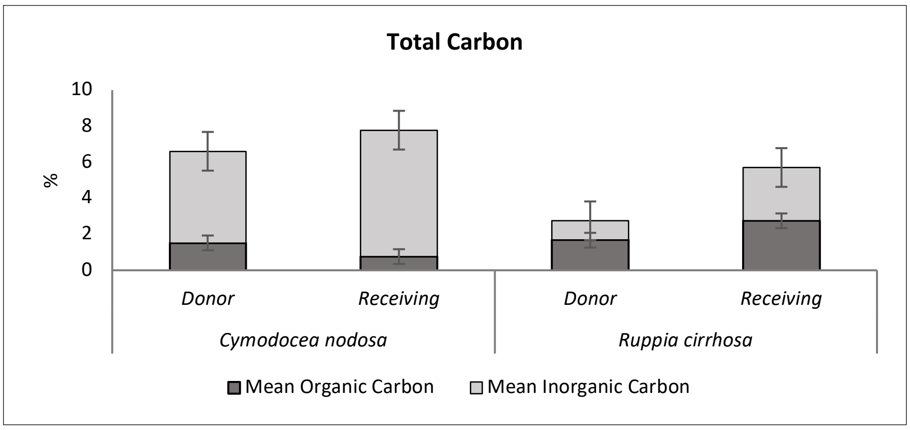

Figure 8. Total carbon mean values determined sediments collected from settled particulate matter traps for the first year (2022) in both species transplanted stations: receiving and donor.

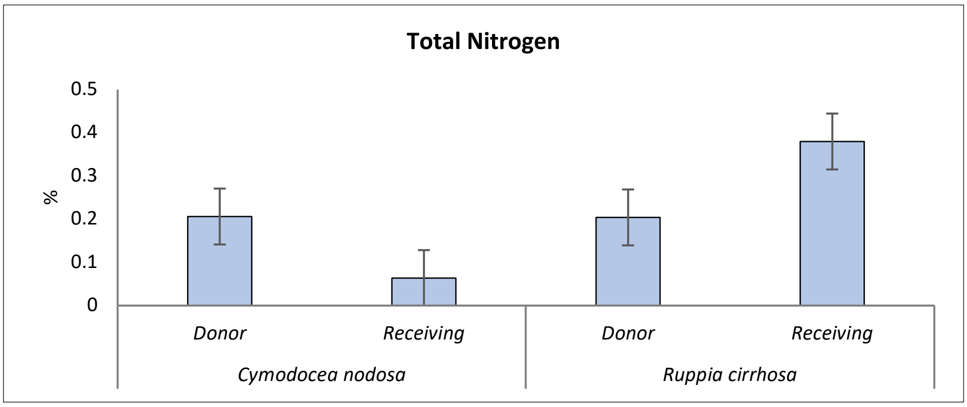

Figure 9. Total nitrogen mean values determined sediments collected from settled particulate matter traps for the first year (2022) in both species transplanted stations: receiving and donor.

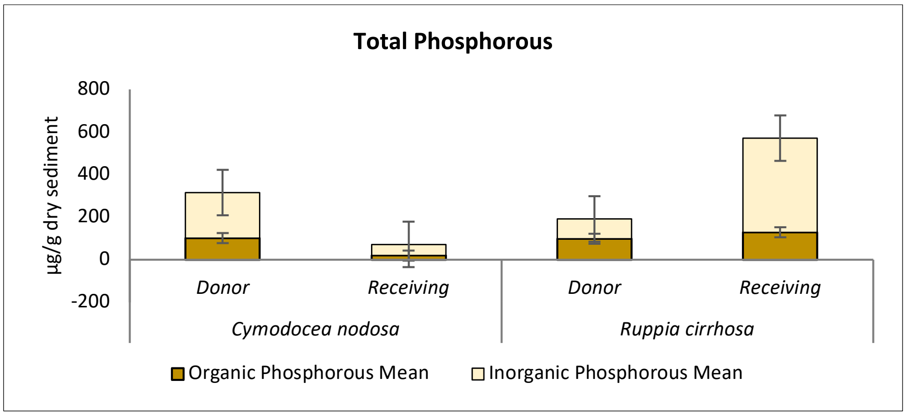

Figure 10. Total phosphorous mean values determined sediments collected from settled particulate matter traps for the first year (2022) in both species transplanted stations: receiving and donor.

<u>Sediment</u>

For this report a total of 36 samples will be taken for *C. nodosa* sites, and 6 for *R. cirrhosa* case of study in June 2023 and October 2023, but in the case of nutrients, only the results of the June campaign are shown. Sediment samples from October 2023 are being analysed. Monitoring has been continued during 2024 in June. Results of the physico-chemical parameters determined in sediments are presented in **Figure 11**, **Figure 12**, **Figure 13**, and **Table 5**.

In the case of the physical-chemical parameters, a higher concentration of organic matter and a higher percentage of fine fraction was obtained in the *Cymodocea nodosa* donor zones (LUDP and BEDW). These are harbour and watercourse areas respectively, with sandy-muddy sediment and a high biomass of macrophytes. In the case of the receiving areas the concentration is lower, but the values are in the appropriate range. However, in both the donor and receiving areas of *Ruppia cirrhosa* the concentration of both organic matter and fine particulate matter, as well as nutrients (carbon, nitrogen and phosphorus) is high due to the characteristics of the sediment, which is muddy, and the hydrodynamic characteristics of both areas. In terms of nutrients, a higher concentration of total carbon in the receiving areas of C. nodosa has been obtained in 2022 and 2023 compared to 2021, while the concentration of nitrogen and phosphorus has been lower in 2022 and 2023 than in 2021.

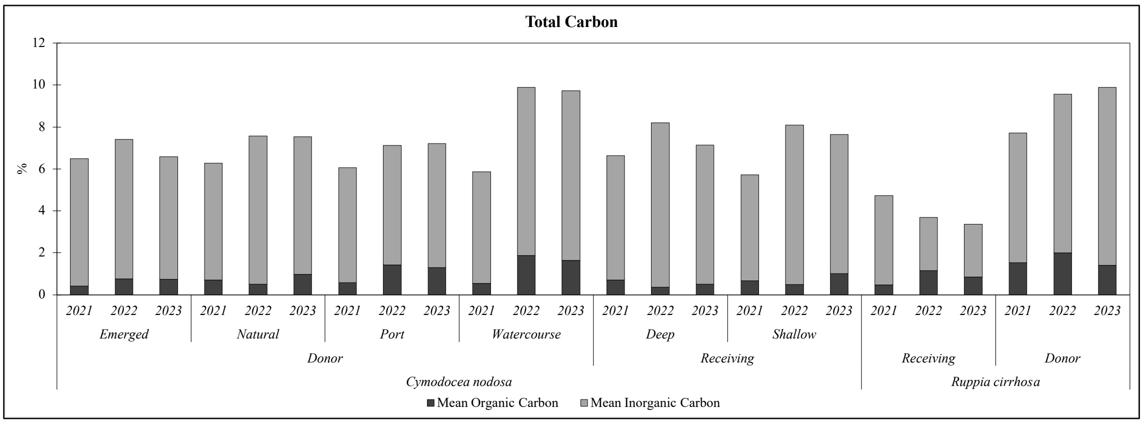

Figure 11. Total carbon mean values of parameters measured in surface sediments for the Ex Ante monitoring (2021), first year of monitoring (2022) and second year (June 2023) in both species transplanted stations: receiving and donor.

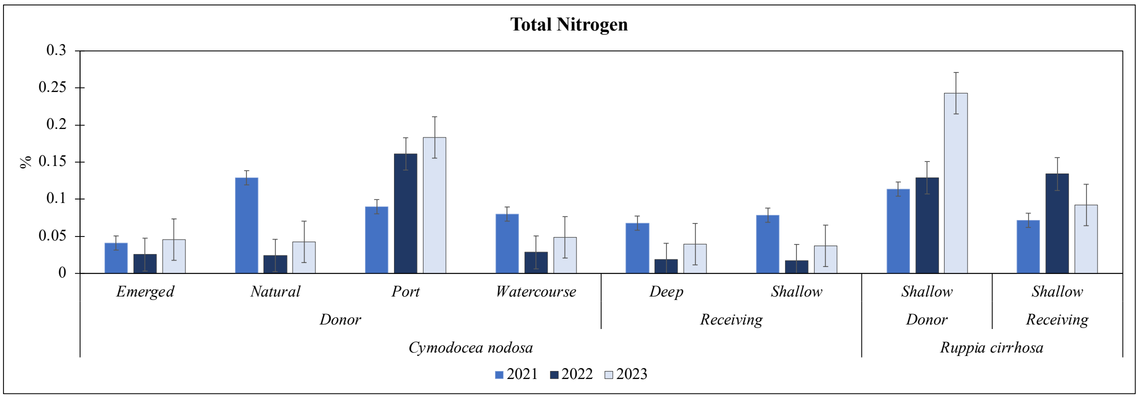

Figure 12. Total nitrogen mean values of parameters measured in surface sediments for the Ex Ante monitoring (2021), first year of monitoring (2022) and second year (June 2023) in both species transplanted stations: receiving and donor.

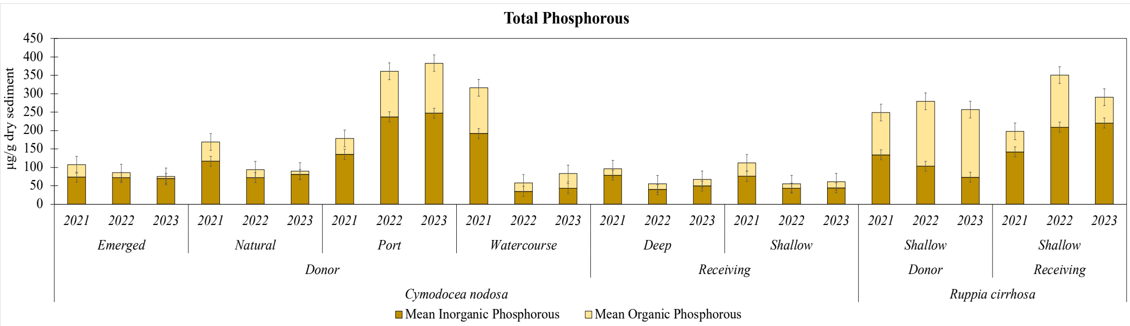

Figure 13. Total phosphorous mean values of parameters measured in surface sediments for the for the Ex Ante monitoring (2021), first year of monitoring (2022) and second year (June 2023) in both species transplanted stations: receiving and donor.

Table 5. Physico-chemical parameters determined in sediments. Mean values correspond to Ex-Ante monitoring (2021), first year of transplant (2022) and second year (June and October 2023) of transplants. < 0.063: fine particle percentage.

Year	Sp.	Receiving Donor	Zone	Humidity	Wet density (g/cm3)	Dry density (g/cm3)	O.M. (%)	<0,063 (%)	Porosity
2021	*Cymodocea nodosa*	D	Emerged	0.30 ± 0.11	1.21 ± 0.08	1.39 ± 0.04	1.79 ± 0.43	4.20 ± 1.94	0.36 ± 0.11
			Natural	0.35 ± 0.05	1.33 ± 0.04	1.31 ± 0.04	1.58 ± 0.07	3.82 ± 0.78	0.47 ± 0.07
			Port	1.27 ± 0.19	0.85 ± 0.03	0.91 ± 0.01	4.84 ± 0.18	67.53 ± 14.15	1.08 ± 0.19
			Watercourse	0.64 ± 0.02	1.68 ± 0.14	1.50 ± 0.05	1.81 ± 0.15	0.50 ± 0.11	1.06 ± 0.06
		R	Deep	0.30 ± 0.01	1.51 ± 0.02	1.52 ± 0.01	0.92 ± 0.07	1.27 ± 0.30	0.46 ± 0.02
			Shallow	0.72 ± 0.27	1.54 ± 0.04	1.50 ± 0.01	1.13 ± 0.11	1.93 ± 0.91	1.11 ± 0.40
	Ruppia cirrhosa	D	Natural	2.58 ± 0.39	0.83 ± 0.08	0.82 ± 0.12	6.69 ± 1.07	7.88 ± 6.82	2.07 ± 0.18
		R	Shallow	1.73 ± 0.21	1.00 ± 0.05	0.97 ± 0.04	3.57 ± 0.35	4.62 ± 1.21	1.73 ± 0.27
2022	*Cymodocea nodosa*	D	Emerged	0.4 ± 0.01	1.55 ± 0.02	1.55 ± 0.02	1.07 ± 0.07	15.68 ± 13.07	0.07 ± 0.02
			Natural	0.65 ± 0.36	1.46 ± 0.03	1.47 ± 0.03	1.07 ± 0.26	6.03 ± 0.39	0.96 ± 0.54
			Port	0.37 ± 0.14	1.04 ± 0.04	1.04 ± 0.04	6.25 ± 0.51	31.37 ± 18.65	0.39 ± 0.15
			Watercourse	0.5 ± 0.06	1.56 ± 0.01	1.57 ± 0.01	2.31 ± 0.10	18.77 ± 16.25	0.14 ± 0.09
		R	Deep	0.3 ± 0.02	1.58 ± 0.01	1.58 ± 0.01	1.50 ± 0.17	2.16 ± 0.23	0.13 ± 0.03
			Shallow	0.84 ± 0.70	1.58 ± 0.02	1.58 ± 0.01	1.49 ± 0.14	2.23 ± 0.23	1.65 ± 1.43
	Ruppia cirrhosa	D	Natural	0.27 ± 0.13	1.25 ± 0.10	1.25 ± 0.10	5.76 ± 2.12	28.63 ± 11.30	0.29 ± 0.14
		R	Shallow	0.44 ± 0.09	1.19 ± 0.02	1.20 ± 0.02	4.37 ± 0.60	26.60 ± 5.30	0.52 ± 0.10
2023	*Cymodocea nodosa*	D	Emerged	0.19 ± 0.09	1.52 ± 0.06	1.53 ± 0.07	0.84 ± 0.10	7.65 ± 4.16	0.28 ± 0.13
			Natural	0.16 ± 0.10	1.31 ± 0.05	1.31 ± 0.05	0.72 ± 0.10	3.58 ± 1.93	0.22 ± 0.13
			Port	1.03 ± 0.30	1.07 ± 0.09	1.11 ± 0.11	2.96 ± 0.95	48.66 ± 5.46	1.10 ± 0.36
			Watercourse	0.77 ± 0.26	1.43 ± 0.09	1.43 ± 0.09	2.57 ± 0.59	47.36 ± 9.83	1.01 ± 0.31

	R	Deep	0.19 ± 0.04	1.49 ± 0.03	1.50 ± 0.03	0.87 ± 0.09	1.98 ± 0.39	0.29 ± 0.06
		Shallow	0.24 ± 0.07	1.52 ± 0.03	1.54 ± 0.03	1.07 ± 0.11	2.61 ± 0.47	0.36 ± 0.10
Ruppia cirrhosa	D	Natural	0.13 ± 0.04	1.10 ± 0.17	1.26 ± 0.24	1.88 ± 0.50	39.89 ± 13.98	0.16 ± 0.07
	R	Shallow	0.49 ± 0.18	1.25 ± 0.07	1.26 ± 0.08	2.69 ± 0.68	30.66 ± 4.97	0.61 ± 0.24

Macrophyte monitoring and MaQI application.

Benthic macroinvertebrates sampling has been carried out in each of the donor and receiving sites proposed for both species under study, taking 3 replicates by site. Monitoring campaigns were carried out in spring and autumn, in each year of monitoring (2022, 2023, and 2024). Samples are under expertise identification. Biomasses are under estimation. Data presented in this report correspond to Ex-Ante Monitoring (Action A.2.2), collected in July 2021.

Despite the results presented in this monitoring report correspond to the ex-ante monitoring program (Action A.2.2), some discussion could be interpreted as in terms of the previous status of both the donor and receiving transplantation stations, for both species (*C. nodosa* and *R. cirrhosa*). For a total of 28 species identified in this campaign, filums Chlorophyta and Rhodophyta dominated in number of taxa found for this period (12, and 11 taxa, respectively) (**Table 6**). For the contrast, the three species corresponding to the Ochrophyta phylum were only found in the natural donor of Las Encañizadas, in connection with the Mediterranean Sea (**Figure 14**), or in the Perdiguera island (receiving station for *R. cirrhosa* transplants).

Regarding to the taxonomic richness to the different sampling stations (both donor and receiving for each species transplanted), the main differences are due to the presence or absence of the phanerogams species under study: *Cymodocea nodosa,* and *Ruppia cirrhosa*. These species were only found in stations corresponding to donor sites. Differences among the donor stations for the case of *C. nodosa* were found, in terms of number of species of the different phyla. However, no dominance of any phylum was observed for any case of study (**Figure 14**).

Table 6. Taxon list of macrophyte species identified in the different donor and receiving station for both transplantation species during July 2021.

Chlorophyta
Acetabularia acetabulum (Linnaeus) P.C.Silva, 1952
Acetabularia calcyculus J.V.Lamouroux, 1824
Batophora occidentalis (Harvey) S.Berger & Kaever ex M.J.Wynne, 1998
Caulerpa prolifera (Forsskål) J.V.Lamouroux, 1809
Chaetomorpha ligustica (Kützing) Kützing, 1849
Chaetomorpha linum (O.F.Müller) Kützing, 1845
Cladophora echinus (Biasoletto) M.J.Wynne, 2017
Cladophora spp Kützing, 1843
Cladophora vagabunda (Linnaeus) Hoek, 1963
Ulothrix flacca (Dillwyn) Thuret, 1863
Ulva flexuosa Wulfen, 1803
Ulva prolifera O.F.Müller, 1778
Ochrophyta
Dictyopteris lucida M.A.Ribera Siguán, A.Gómez Garreta, Pérez Ruzafa, Barceló Martí & Rull Lluch, 2005
Dyctiota implexa (Desfontaines) J.V.Lamouroux, 1809
Feldmannia irregularis (Kützing) Hamel, 1939
Rhodophyta
Alsidium corallinum C.Agardh, 1827
Ceramium codii (H.Richards) Mazoyer, 1938
Ceramium diaphanum (Lightfoot) Roth, 1806
Chondria capillaris (Hudson) M.J.Wynne, 1991
Erythrotrichia carnea (Dillwyn) J.Agardh, 1883

Herposiphonia tenella (C.Agardh) Ambronn, 1880
Hypnea musciformis (Wulfen) J.V.Lamouroux, 1813
Jania rubens (Linnaeus) J.V.Lamouroux, 1816
Lophosiphonia obscura (C.Agardh) Falkenberg, 1897
Palisada tenerrima (Cremades) D.Serio, M.Cormaci, G.Furnari & F.Boisset, 2010
Spyridia filamentosa (Wulfen) Harvey, 1833
Tracheophyta
Cymodocea nodosa (Ucria) Asch.
Ruppia cirrhosa (Petagna) Grande, 1918

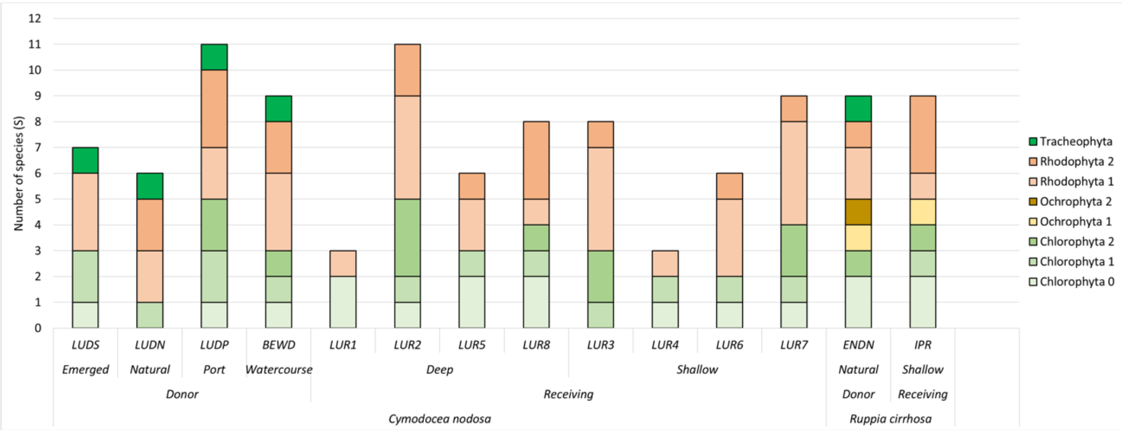

Figure 14. Number of macrophyte species found in the different donor and receiving station for both transplantation species during July 2021.

<u>Benthic macroinvertebrates</u>

Results of benthic macroinvertebrates and the application of ecological index (BITS and M-AMBI) were referred in this report as an updated data to year 2023 (second monitoring year).

A total of 87 taxa corresponding to the Annelida, Mollusca, Arthropoda, Cnidaria, and Porifera, were identified during the ex-ante, and monitoring program carried out up to the date referred in this report (**Table 7**). The dominant Phylum was Annelida (48 taxa identified for this periods), followed by Mollusca and Arthropoda (18 and 19, respectively). For the case of Cnidaria and Porifera, only one taxon was identified. Across the different periods, the richness trended to increase for the case of deeper *Cymodocea nodosa* transplanting stations, and in the receiving stations of *Ruppia cirrhosa* (**Figure 15**). The highest number of species corresponds to the second monitoring period (2023) in the receiving station of *R. cirrhosa* transplants (24 taxa). The lowest mean number of taxa was found in the same station for the year 2021 (4 taxa). Regarding to *C. nodosa* receiving stations, deeper ones increased both the number of species and diversity index up to year 2023. For the contrast, in the shallower receiving stations, despite in 2022 both species richness and diversity index were greater than those from deeper stations, in 2023 these variables were lower than the previous years.

These results also provided the values of the index estimated for this report in the receiving stations for both species. For the case of the BITS index (**Figure 16**), in the case of study of *C. nodosa*, the dynamic followed is similar to those corresponding to the diversity and richness. In the case of deeper receiving stations, BITS index reached a mean value of 1.52 (± 0.1), in 2023. However, in the shallowest receiving stations, this index decreased from the year 2021 (1.31 ±

0.14) up to 2023 (1.05 ± 0.13). Despite these changes, the mean ecological quality status of all receiving stations remained "Moderate" for all years. For the case of *R. cirrhosa* receiving stations, despite its increase in the number of taxa, and diversity index, the BITS index mean value showed a decrease from 2021 (1.31 ± 0.29) to 2023 (1.05 ± 0.05). Similar to those from shallow receiving stations of *C. nodosa* transplants.

Focusing on the estimation of the M-AMBI index, the relative dominance of the different ecological groups was analysed (**Figure 17**). For *C. nodosa* deeper receiving stations, an increase in the mean number of individuals of species corresponding to the group I (sensitive species), increased from 2021 (21.43%), to 2023 (71, 13%). Pollution indication species (group V) appeared in the years 2022 and 2023, for the *C. nodosa* deeper receiving stations. Mean number of individuals of species classified as opportunistic (group IV) decreased from 2021 to 2023. The case of *C. nodosa* shallower receiving stations showed a different dynamic in terms of relative dominance of ecological groups. Whereas in 2021 the indifferent species group (II) decreased its mean relative importance from 2021 (36.34 %) to 2023 (18 %), those corresponding to tolerant species group (III) remained constant their dominance during the monitoring (~ 45 %, in all years). Moreover, for the same receiving stations, both opportunistic and pollution indicators groups increased their mean percentage of relative dominance in 2023 (4 % in both cases). The case of *R. cirrhosa* transplantation stations were different. Despite in 2021 (Ex-ante monitoring), the receiving station was dominated by the tolerant species group (III) (97.8 %), in 2022 (first monitoring period), a greater equitability was found in terms of relative importance of the different groups for this receiving station. Species referring to the groups V and I increased their mean relative importance in 2022, in the same *R. cirrhosa* receiving station (10.95%, and 15.9%, respectively). In the case of the same receiving station, for the year 2023, the number of individuals from the group III increased, and those from group V, decreased.

When application of the M-AMBI index to establish the ecological quality status of the different receiving stations for both species transplanted, differences were found when comparing those receiving stations from the different depth for the case of *C. nodosa*, and across year in *R. cirrhosa* receiving stations (**Figure 18**). Whereas for the case of deep receiving stations of *C. nodosa* was classified as good status, the shallower stations were classified as a moderate quality status for all the monitoring years. For the contrast, in the case of receiving *R. cirrhosa* transplant stations, the quality status improved from poor (2021), to good (2022, and 2023).

Table 7. Taxa list found in the different donor and receiving stations for both transplantation species for the two first years of monitoring.

ANNELIDA
Polychaeta
Capitellidae
Capitella capitata (Fabricius, 1780)
Capitella giardi (Mesnil, 1897)
Heteromastus filiformis (Claparède, 1864)
Mediomastus fragilis Rasmussen, 1973
Notomastus latericeus Sars, 1851
Cirratulidae
Caulleriella sp Chamberlin, 1919
Cirratulidae Ryckholt, 1851
Cirriformia tentaculata (Montagu, 1808)
Dorvilleidae
Dorvillea sp Parfitt, 1866

<u>Eunicidae</u>
Lysidice unicornis (Grube, 1840)
<u>Glyceridae</u>
Glycera tridactyla Schmarda, 1861
<u>Hesionidae</u>
Syllidia armata Quatrefages, 1866
<u>Microphthalmidae</u>
Microphthalmus pseudoaberrans Campoy & Vieitez, 1982
<u>Nereididae</u>
Nereididae Blainville, 1818
Perinereis cultrifera (Grube, 1840)
<u>Opheliidae</u>
Armandia cirrhosa Filippi, 1861
Opheliidae Malmgren, 1867
<u>Orbiniidae</u>
Naineris laevigata (Grube, 1855)
Orbiniidae Hartman, 1942
Phylo foetida (Claparède, 1868)
Scoloplos haasi (Monro, 1937)
<u>Paraonidae</u>
Cirrophorus furcatus (Hartman, 1957)
Paradoneis lyra (Southern, 1914)
Paraonidae Cerruti, 1909
<u>Phyllodocidae</u>
Nereiphylla rubiginosa (Saint-Joseph, 1888)
Phyllodocidae Örsted, 1843
<u>Sabellidae</u>
Branchiomma boholense (Grube, 1878)
Sabellidae Latreille, 1825
<u>Serpulidae</u>
Hydroides dianthus (Verrill, 1873)
Hydroides elegans (Haswell, 1883)
Serpulidae Rafinesque, 1815
<u>Spionidae</u>
Boccardia sp Carazzi, 1893
Dipolydora sp Verrill, 1881
Dispio sp Hartman, 1951
Malacoceros sp Quatrefages, 1843
Microspio sp Mesnil, 1896
Prionospio fallax Söderström, 1920
Prionospio sp Malmgren, 1867
Pseudopolydora sp Czerniavsky, 1881
Scolelepis sp Blainville, 1828
Spio filicornis (Müller, 1776)
Spio sp Fabricius, 1785
Spionidae Grube, 1850
<u>Syllidae</u>
Syllidae Grube, 1850

Syllis benelihaue (Campoy, 1982)
Syllis parapari San Martín & López, 2000
Syllis sp Lamarck, 1818
Terebellidae
Terebellidae Johnston, 1846

ARTHROPODA
Malacostraca
Aoridae
Microdeutopus algicola Della Valle, 1893
Microdeutopus gryllotalpa A. Costa, 1853
Microdeutopus sp A. Costa, 1853
Corophiidae
Corophium insidiosum (Crawford, 1937)
Corophium sp Latreille, 1806
Monocorophium insidiosum (Crawford, 1937)
Gammaridae
Gammarus insensibilis Stock, 1966
Idoteidae
Idoteidae Samouelle, 1819
Ischyroceridae
Ericthonius difformis H. Milne Edwards, 1830
Ericthonius punctatus (Spence Bate, 1857)
Ericthonius sp H. Milne Edwards, 1830
Siphonoecetes sabatieri Rouville, 1894
Leptocheliidae
Chondrochelia savignyi (Kroyer, 1842)
Maeridae
Elasmopus rapax A. Costa, 1853
Palaemonidae
Palaemon sp Weber, 1795
Sphaeromatidae
Cymodoce truncata Leach, 1814
Tanaididae
Tanais dulongii (Audouin, 1826)
Varunidae
Brachynotus sexdentatus (Risso, 1827)
CNIDARIA
Hexacorallia
Aiptasiidae
Aiptasia diaphana (Rapp, 1829)
MOLLUSCA
Bivalvia
Cardiidae
Cerastoderma glaucum (Bruguière, 1789)
Parvicardium exiguum (Gmelin, 1791)
Lucinidae
Loripes orbiculatus Poli, 1795
Mytilidae

Modiolus barbatus (Linnaeus, 1758)
Mytilaster minimus (Poli, 1795)
Semelidae
Abra alba (W. Wood, 1802)
Tellinidae
Gastrana fragilis (Linnaeus, 1758)
Veneridae
Dosinia lupinus (Linnaeus, 1758)
Polititapes aureus (Gmelin, 1791)
Gastropoda
Caecidae
Caecum trachea (Montagu, 1803)
Cerithiidae
Bittium reticulatum (da Costa, 1778)
Cerithium scabridum R. A. Philippi, 1848
Hydrobiidae
Hydrobia acuta (Draparnaud, 1805)
Nassariidae
Tritia cuvierii (Brocchi, 1814)
Tritia neritea (Linnaeus, 1758)
Rissoidae
Pussillina inconspicua (Alder, 1844)
Pussillina lineolata (Michaud, 1830)
Polyplacophora
Chitonidae
Rhyssoplax olivacea (Spengler, 1797)
PORIFERA
Sycon raphanus Schmidt, 1862

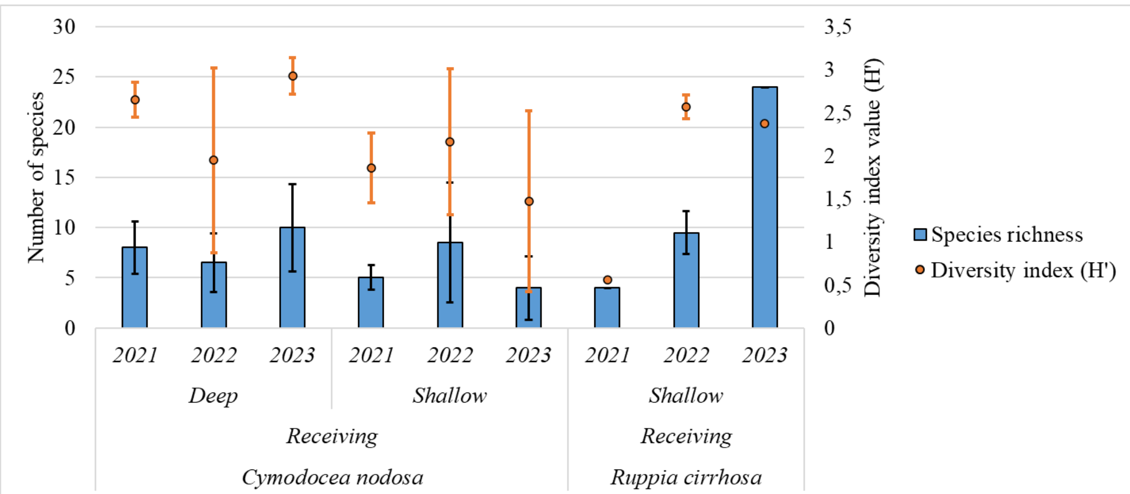

Figure 15. Mean Shannon diversity index (orange dots) and number of macroinvertebrate species (blue bars) estimated for each station for both transplantation species, in Ex-Ante Monitoring (Summer 2021), and Monitoring (2022, and 2023).

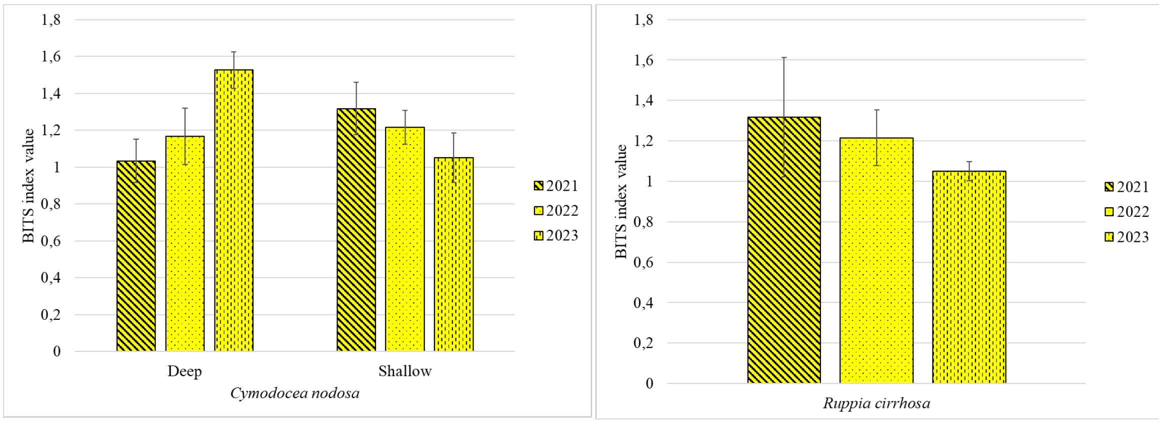

Figure 16. Mean BITS values estimated for both transplantation species receiving stations in Ex-Ante Monitoring (Summer 2021), and Monitoring (2022, and 2023). Color yellow corresponds to a moderate quality status stated in Michele and Munari (2008).

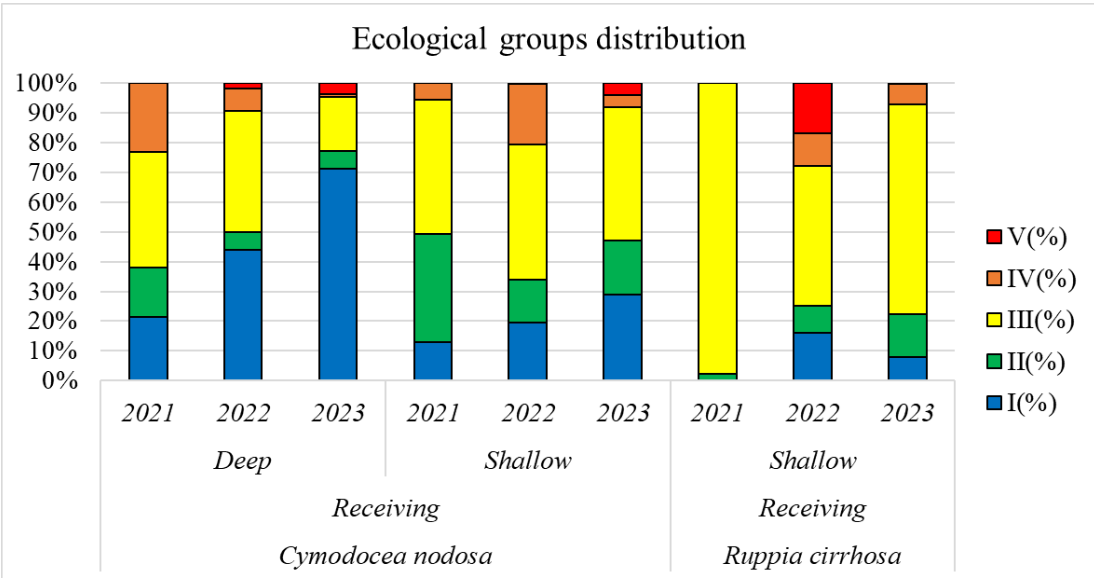

Figure 17. Mean M-AMBI ecological groups distribution for both species transplantation receiving stations, in the different monitoring periods (2022, and 2023), including Ex-Ante Monitoring (2021). Ecological groups: (I) Sensitive species; (II) Indifferent species; (III) Tolerant species; (IV) Opportunistic species; (V) Pollution indicating species. Muxika et al. (2007).

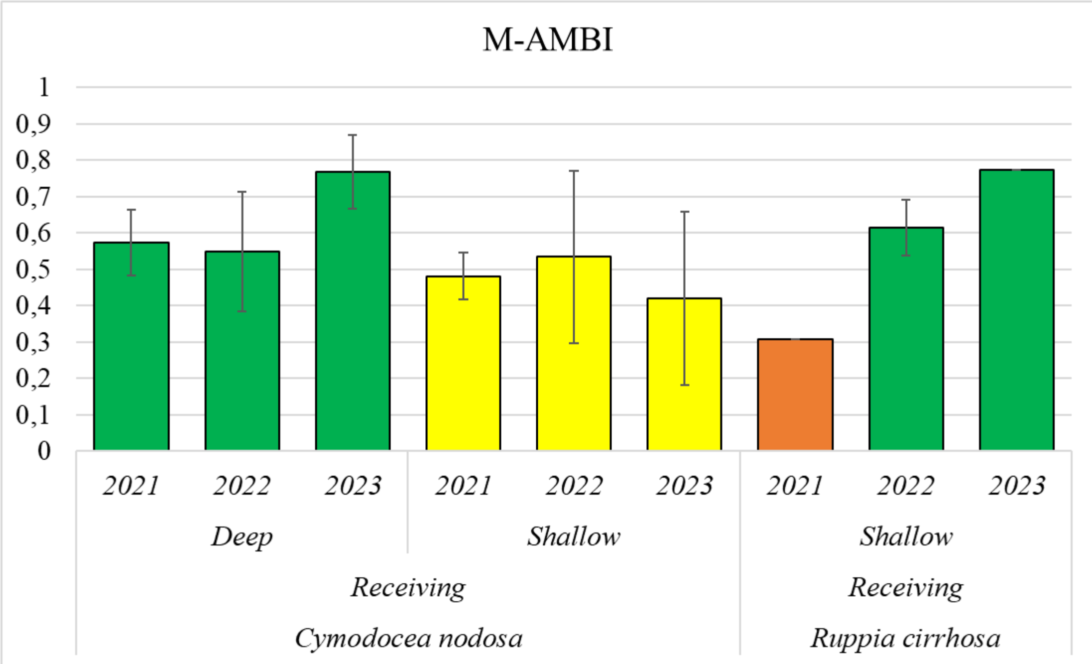

Figure 18. M-AMBI values for the receiving stations for both transplantation species in Ex-Ante Monitoring (Summer 2021), and Monitoring (2022, 2023). Ecological Quality Ratio colour scale: Red = Bad; Orange = Poor; Yellow = Moderate; Green = Good; Blue = High. Muxika et al. (2007).

<u>Fish fauna</u>

Fish fauna monitoring and HBFI application has been studied twice a year (spring and autumn) at each station during the monitoring, with a total of 8 samples. The campaign consisted mean a beach net trawl survey to assess the state of the fish community through the HFI index, as an indicator of the ecological quality of the area.

The campaign has been carried out in June and October 2023 by manual trawling in an area with a maximum depth of 1.5 m, within the transplant area but properly separated from the transplants.

The results of the HBFI index are in the process of being analysed. For this report, the abundance data of the different species obtained are presented.

The taxon *Mugilidae* Jarocki, 1822 has shown the highest abundance at both donor and receiving sites, although at donor sites its abundance is higher. *Atherina boyierii* (Riso, 1820) has also shown high abundances at both sites. The abundance of the seagrass indicator species *Syngnathus abaster* Risso, 1827 has been similar in both donor and receiving areas.

Aphanius iberus (Valenciennes in Cuvier & Valenciennes, 1846) is one of the most endangered Iberian vertebrate species. In recent decades, it has suffered a drastic regression that has led to the species being listed as endangered, both nationally and internationally (Oliva, 2008).

This specie is included in Royal Decree 139/2011, of 4 February, for the development of the List of wild species under special protection and the Spanish Catalogue of endangered species, in the category "In danger of extinction" and in Annex II of the Habitat Directive of 21 May 1992.

Table 8. Abundance of fish found in the transplantation areas for the years 2022 and 2023. Species are allocated to ecological and feeding guilds (Estuarine Use Functional Group; EUFG) and Feeding Mode Functional Guild; FMFG, respectively) according to Franco et al. (2009), and their status as either indicator species for the habitat or allochthonous taxa is shown.

Taxon	EUFG	FMFG	Status	*Cymodocea nodosa* Donor 2022	*Cymodocea nodosa* Donor 2023	*Cymodocea nodosa* Receiving 2022	*Cymodocea nodosa* Receiving 2023	*Ruppia cirrhosa* Receiving 2022	*Ruppia cirrhosa* Receiving 2023
Anguilla Anguilla (Linnaeus, 1758)	C	-	-	1	1				
Aphanius iberus (Valenciennes in Cuvier & Valenciennes, 1846)	ES	Bmi	In danger of extinction	1		1	2		1
Atherina boyierii Riso, 1810	ES	HZ	-	155	21	256	254	3	
Belone belone (Linnaeus, 1760)	MM	HP	-	2	5	2	1	1	
Callionymus pusillus Delaroche, 1809	MM/MS	Bmi	-			1			
Diplodus puntazzo (Walbaum, 1792)	MS	-	-				5		
Diplodus sargus (Linnaeus, 1758)	MS	-	-	1	1		1		
Engraulis encrasicolus (Linnaeus, 1758)	MM	PL	-	22	8		4		
Gobius niger (Linnaeus, 1758)	ES	Bmi,HP	-	5	1	19	4	1	
Lithognathus mormyrus (Linnaeus, 1758)	MM	Bmi,BMa	-						8
Mugilidae Jarocki, 1822	D/MM	DV	-	99	611	7	556	85	13
Pomatoschistus marmoratus (Risso, 1810)	ES	Bmi	-	7	12	9	18		
Salaria pavo (Risso, 1810)	ES/MS	OV	-	3	6	14	12	7	

Sarpa salpa (Linnaeus, 1758)	MM/MS	HV	-	1		3	1		
Serranus cabrilla (Linnaeus, 1758)	MS	-	-			1			
Sparidae Rafinesque, 1818	D/MM	DV	-		14		1		
Syngnathus Linnaeus, 1758	D/MM	DV	-				16		
Syngnathus abaster Risso, 1827	ES	Bmi	Indicator(seagrass)	11	4	15	11	1	
Syngnathus acus Linnaeus, 1758	ES	Bmi	Indicator(seagrass)			1		1	
Syngnathus typhle Linnaeus, 1758	ES	HZ	-	3	2	11	1		1
Symphodus sp Rafinesque, 1810	ES	Bmi,BMa	-	42	29	78	12	17	

EUFG	FMFG
ES = estuarine species	Bmi = microbenthivores
MM = marine migrants	BMa = macrobenthivores
MS = marine stragglers	PL = planktivores
F = freshwater species	HZ = hyperbenthivores–zooplanktivores
D = diadromous species	HP = hyperbenthivores–piscivores
	DV = detritivores
	OV = homnivores

REFERENCES

- Aspila K., Agemian H., Chau A. (1976). A Semi-automated Method for the Determination of Inorganic, Organic and Total Phosphate in Sediments. Awalyst. Vol. 101, pp. 287-197.

- Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

- Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal L 327, 22/12/2000 P. 0001 – 0073.

- Franco, A., Torricelli, P., & Franzoi, P. (2009). A habitat-specific fish-based approach to assess the ecological status of Mediterranean coastal lagoons. Marine Pollution Bulletin, 58(11), 1704-1717.

- Mistri, M., Munari, C. (2008). BITS: a SMART indicator for soft-bottom, non-tidal lagoons. Marine Pollution Bulletin, 56: 587-599.

- Murphy, J., Riley, J.P. (1962). A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27: 31-36.

- Muxika I., Borja A., Bald, J. (2007). Using historical data, expert judgement, and multivariate analysis in assessing reference conditions and benthic ecological status, according to the

- Oliva Paterna, F. J. (2008). Biología y conservación de Aphanius iberus (Valenciennes, 1846) en la Región de Murcia.

- Parsons, T., Maita, Y., Lalli, C.M. (1984). A Manual of Chemical and Biological Methods for Seawater Analysis. 1sted. Pergamon Press. England.

- Royal Decree 139/2011, of 4 February, for the development of the List of Wildlife Species under Special Protection Regime and the Spanish Catalogue of Threatened Species.

- Sfriso, A. (2010). Macrophyte Quality Index (MaQI) per la valutazione dello stato ecologico dei sistemi di transizione dell'ecoregione-Mediterranea. In: Bonometto, A., Genaro, P., Boscolo Brusà, R. (Eds). Linee Guida per l'applicazione del Macrophyte Quality Index (MaQI). Implementazione della Direttiva (2000/60/CE). ISPRA, pp. 34.

- Sfriso, A., & Facca, C. (2011). Ecological Indices based on macroalgae and angiosperms in the Mediterranean eco-region: an overview. *Life in the Mediterranean Sea: A Look at Habitat Changes, Nova Science Publishers*, 521-541.

- Sfriso, A., Facca, C., Bonometto, A., & Boscolo, R. (2014). Compliance of the macrophyte quality index (MaQI) with the WFD (2000/60/EC) and ecological status assessment in transitional areas: The Venice lagoon as study case. Ecological indicators, 46, 536-547.