SEAGRASS TRANSPLANTATION FOR TRANSITIONAL ECOSYSTEM RECOVERY

Deliverable D.3 Action

Final monitoring progress report in Mar Menor lagoon (Spain)

Project responsible

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Content

EXECUTIVE SUMMARY

This report provides the results obtained from Action D.3 – Monitoring of Action C3, as described in official technical documentation of the LIFE TRANSFER project. It outlines the monitoring methods and results for sub-actions D.3.1 – Monitoring of angiosperm growth, and D.3.2 – Monitoring of biodiversity and environmental quality status, conducted during 2022, 2023, 2024, and 2025 within the framework of the LIFE Transfer project.

Action C.3, the transplantation of submerged aquatic angiosperms in the Mar Menor lagoon, was carried out in the areas selected by the executive project of Action A4 in the spring of 2022 and was repeated three times a year throughout the project. This task (C.3) was carried out in the receiving areas of Los Urrutias from spring 2022 for the species *Cymodocea nodosa* (Ucria) Ascherson 1870 and in the receiving areas of Isla Perdiguera for the species *Ruppia cirrhosa* (Petagna) Grande 1918. *C. nodosa* transplants were carried out in two areas with different depths: deeper (> 1 m) and shallower (< 0.8 m). Furthermore, additional *C. nodosa* transplants were carried out in the locality of Los Nietos, in autumn 2024, only in shallow areas (< 0.8 m). This last was considered additional in case of possible failure of the transplants carried out in Los Urrutias. The transplantation methods were carried out following the operational protocol learned in Action A5.1.

The follow-up sampling campaigns (Action D.3) have been carried out following the official technical specifications of the project. The follow-up action began in May 2022 for the different species of angiosperms, both at the donor and receiving stations, and will end in autumn 2025.

The different parameters measured were: monitoring of plant growth and the expansion rate of newly formed meadows (survival of transplants, expansion rate of transplants and coverage of newly formed meadows); water column parameters (including temperature, salinity, dissolved oxygen, pH, Eh, suspended particles, chlorophyll and nutrients: total ammonium; oxidised nitrogen; dissolved inorganic phosphorus; dissolved silicates); sediment parameters (organic and inorganic carbon, total nitrogen, organic and inorganic phosphorus, and other physical-chemical parameters). The main characteristics of benthic communities (macroinvertebrates and microphytobenthos, and fish fauna were also measured).

Regarding *C. nodosa,* transplants performed in deeper areas (> 1 m depth) maintained survival rates of approximately 100%, while those transplants performed in shallower areas (< 0.8 m depth), they declined to approximately 42 ± 5% in Los Urrutias. The average diameter of sod reached 1 054 ± 40 cm (deep areas) vs 181 ± 28 cm (shallow areas). Coverage exceeded the transplanted area in deep areas (up to 138%), but in shallow areas it remained between 12 – 40%. In Los Nietos, coverage

was <1%. This is similar to what was observed in the first few months of results in transplants carried out in shallow areas in the town of Los Urrutias. A similar follow-up to that carried out in that town is envisaged in order to ensure similar success. In *R. cirrhosa*, the dynamics were unstable: disappearance in summer 2022, reinforcement in 2023, a maximum was reached in spring 2024, and during 2025 coverage was <5%.

Regarding water column parameters, at *C. nodosa* stations, nitrates decreased from 9.1 to 4.2 µg at $N - NO_3^-$/l and nitrites from 0.8 to 0.2 µg at $N - NO_2^-$/l between 2021 and 2025; *at R. cirrhosa* stations, values were lower overall. In contrast, ammonium, silicate and phosphates were higher in *R. cirrhosa*, with NH^{4+} rising to 3.8 ±1 µg at N - NH^{4+}/l. Maximum TSS values were obtained in 2024 (18 mg/L), high salinity in 2024 (46 PSU) and minimum temperatures in the same year. The pH was practically stable. Maximum turbidity in *C. nodosa* was in 2022-23 and in *R. cirrhosa* in 2023-25 (up to 16 NTU). Dissolved O_2 was higher in *C. nodosa* (7.8 – 8 mg/L in 2023-24). Eh was positive in 2021-22 and minimum in 2024 (-76.8 mV *C. nodosa*; -103 mV *R. cirrhosa*). Pigments: minimum Chl *a* in 2024 and maximum in 2025 in *R. cirrhosa* (2.1 µg/L).

In sediments, humidity and porosity increased towards 2025 in all the transplant sites. Densities were higher in *C. nodosa* (up to 1.6 g/cm^3 in 2025). Fine particles and organic matter were much higher in *R. cirrhosa* (10% OM and 42% fine particles). Total carbon was dominated by the inorganic fraction; organic carbon was relatively low but somewhat higher in *R. cirrhosa*. Total nitrogen was higher in donors and in muddy sediments. Total phosphorous was dominated by the inorganic fraction and was higher and more variable in donors areas. Much higher settled particulate matter (SPM) values were obtained in the receiving areas of *C. nodosa* (up to > 6 g $DW \cdot m^{-2} \cdot day^{-1}$), and there were a generalised decline and homogenisation in *R. cirrhosa* towards 2025 (0.2 – 0.3 g $DW \cdot m^{-2} \cdot day^{-1}$).

With regard to macrophytes and the MaQi index, a total of 36 species were observed, with the areas receiving *C. nodosa* having the greatest richness (32 species). The MaQI was normally GOOD-HIGH after 2023 (*C. nodosa).* In *R. cirrhosa*, there was a decline to POOR in 2024 and partial recovery in 2025 (MODERATE). Regarding benthic macroinvertebrates, in *C. nodosa* transplants, the density of individuals was relatively low and similar at depths, up to 2024, but from that year onwards it increased markedly, reaching 4 573 ± 1 476 ind/m^2 in 2025, especially in deep stations. Specific richness was always higher in deep areas: at the end of the monitoring period, 10.6 ± 2 taxa were recorded in deep areas compared to 3.5 ± 1 taxa in shallow areas. *R. cirrhosa* also showed a seasonal increase and an upward trend, but much more intense: the maximum occurred in spring 2025 with 56 977 ind/m^2. Richness went from low values in 2022 to a maximum of 14 taxa in spring

2023 and remained high in 2025 (12 taxa). The BITS and M-AMBI indices improved in both areas. Of the total taxa identified (95 taxa), 39 belong to the group of polychaete annelids, 31 to molluscs, 18 crustacean arthropods. Other smaller groups were Chordata (Class Ascidiacea), Porifera and Cnidaria, with 2, 1 and 1 taxon, respectively. As for fish fauna, more than 20 species were found. There was a clear increase in abundance and a growing appearance of bioindicators (*Syngnathus* spp Linnaeus, 1758, *Hippocampus gutulatus* Cuvier, 1829) in the areas receiving *C. nodosa*. Species of commercial interest and some species listed in the Habitats Directive were also found in the receiving areas across the monitoring period.

In summary, the receiving areas for *C. nodosa* in the Mar Menor show a clear process of consolidation, especially in transplants located at greater depths, which translates into consistent and multi-compartmental ecological improvements. In contrast, *R. cirrhosa* is a species whose restoration is possible, but its natural dynamics are much more fluctuating. This means that transplants also have a high temporal variability and require a more careful approach to ensure their success and to be able to assess their results.

Introduction

This report presents the results obtained from action D.3 - Monitoring of C3 action, as outlined in the official technical report for the project. It presents the monitoring methods and results of sub-actions D.3.1 - Monitoring angiosperm growth and D.3.2 - Monitoring biodiversity and environmental quality status, carried out in 2022, 2023, 2024 and 2025 as part of the LIFE Transfer project. The results presented in this report are in line with those anticipated (particularly with regard to the expected direct results listed on page 38 of the Grant Agreement) and justify any deviations from expectations.

Sub action D.3.1 Monitoring angiosperm growth

Table 1. Mar Menor monitoring action deliverables status.

Name of the Deliverable	Number of action	Deadline	Status	Deliverable date
Final ex-ante monitoring report, Spanish Lagoon	A2	November 2021	Delivered	March 2022
First monitoring progress report	D3	June 2022	Delivered	July 2022
Second monitoring progress report	D3	June 2023	Delivered	July 2023
Third monitoring progress report	D3	June 2024	Delivered	July 2024
Final report	D3	November 2025	Delivered	November 2025

Methods

For *Cymodocea nodosa* (Ucria) Ascherson 1870, Los Urrutias was proposed both for donor and receiving stations (Figure 1). Within this locality, two areas with different depths were selected: deeper (> 1 m) and shallower (<0.8 m), for a total of 8 receiving sites. Each site was composed by 9 sods (sod diameter: 0.16 m^2). Regarding the donor stations: 4 different donors were considered in relation to the different threats considered in *C. nodosa* meadows, in the Mar Menor: Port (LUDP), watercourse affected by heavy metals (BEDW), emersion risk (LUDS) and natural meadows nearby (LUDN).

The locality of Los Nietos (LNR), was considered as additional location, as stated in the executive project (Deliverable from Action A.4). In this locality, transplants would be considered when those of Los Urrutias have not been successful. However, although the transplants carried out in Los Urrutias were successful, it was decided to carry out *C. nodosa* transplants in Los Nietos. Three shallow (<

0.8 m) sites were transplanted in autumn 2024. A total of 27 sods were transplanted. A natural donor site was stablished in the same locality of Los Nietos (LNDN).

For *Ruppia cirrhosa* (Petagna) Grande 1918, 1 transplant location (9 sods) was stablished in the Perdiguera island (IPR), and the donor sites in Las Encañizadas (ENDN), and Santiago de la Ribera (LR), considering these last two as a natural donor meadow. Original *R. cirrhosa* donor site proposed in Ex-Ante monitoring report (Deliverable A.2.2) was refused for use as a donor due to difficulty accessing the site.

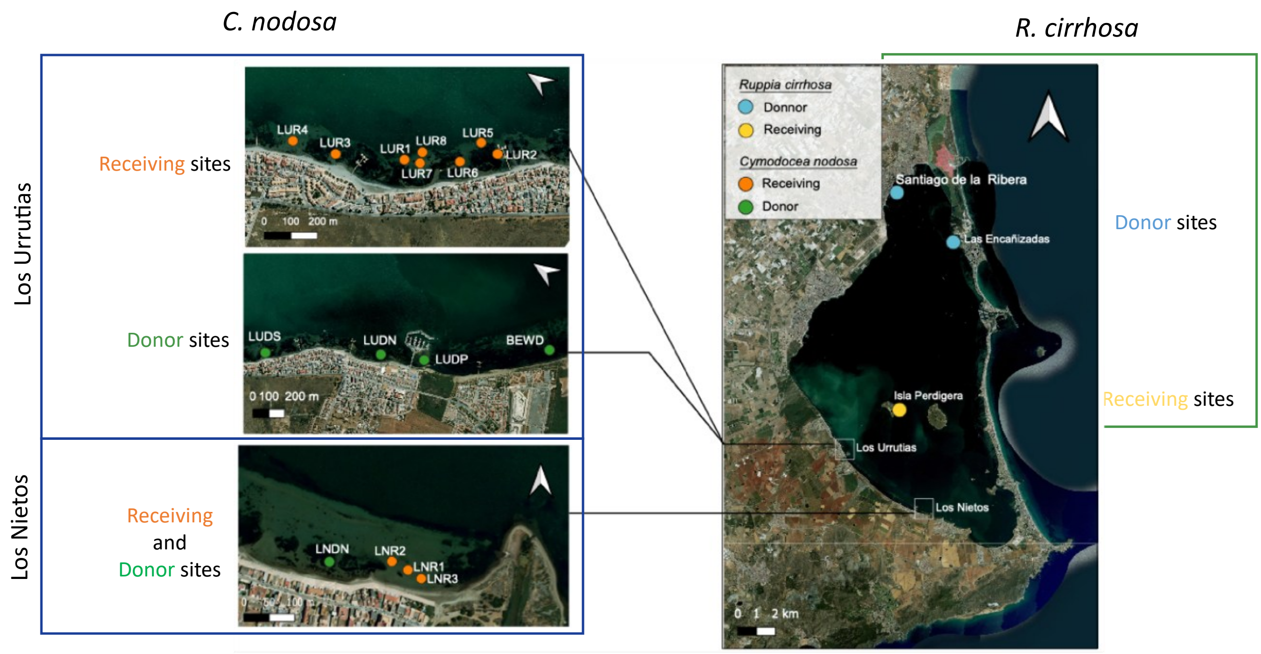


Figure 2. Location of *C. nodosa* and *R. cirrhosa* donor and receiving meadows in the Mar Menor coastal lagoon monitored included in this report.

Table 2. Number of transplanted sods of the different species, in the different locations and periods. Wi: Winter; Sp: Spring; Au: Autumn. Area transplanted estimated on the basis of a mean area of 0.16 m^2 per sod.

Year	**2022**		**2023**				**2024**				**2025**				**TOTAL**
Season	**Sp.**	**Total**	**Wi.**	**Sp.**	**Au.**	**Total**	**Wi.**	**Sp.**	**Au.**	**Total**	**Wi.**	**Sp.**	**Au.**	**Total**	
C. nodosa	72	72	72	72	72	216	72	72	99	243	99	90	99	288	**819**
Los Urrutias	72	72	72	72	72	216	72	72	72	216	72	63	72	207	**711**
Los Nietos									27	27	27	27	27	81	**108**
R. cirrhosa	9	9	9	9	9	27	9	9	9	27	9	9	9	27	**90**
Isla Perdiguera	9	9	9	9	9	27	9	9	9	27	9	9	9	27	**90**
Total	**81**	**81**	**81**	**81**	**81**	**243**	**81**	**81**	**108**	**270**	**108**	**99**	**108**	**315**	**909**

According to the general plan of the project, monitoring of the plant growing and the rate of expansion of the newly formed meadows have been carried out once a month for the first 6 months after first transplantation and, from the 7th month, 1 sampling campaign in early spring, 1 in early summer, and 1 in early autumn of each year. Monitoring have been carried out at all transplant stations for *C. nodosa* and *R. cirrhosa,* for a total of 12 stations (11 for *C. nodosa*, and 1 for *R. cirrhosa*).

For the *C. nodosa* transplants in the location of Los Urrutias and those corresponding to *R. cirrhosa*, first angiosperm growth monitoring data were recorded in June 2022, and continued monthly during the same year. Monitoring of angiosperms growth continues in 2023 – 2025 three times a year: 1 campaign in March, 1 campaign in June, and 1 campaign in October. A total of 9 stations (8 for *C. nodosa* in Los Urrutias + 1 for *R. cirrhosa*) were monitored at each campaign. For this report, results are shown as an updated state of the sub-action (up to autumn 2025). Monitoring of angiosperm growth in the location of Los Nietos (3 stations) started in late 2024, with the first transplant period in this extra locality.

Angiosperm growth monitoring was carried out measuring the diameter of each transplanted 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

Results

For the case of *C. nodosa,* in Los Urrutias*,* transplant survival reached 100% in deeper receiving stations. However, those shallower receiving stations showed a decreasing transplant survival tendency. In 2025, the mean percentage of survival in *C. nodosa* shallow transplants was 42 ± 5 % of total sods transplanted. Receiving station for *R. cirrhosa* transplants increased its survival in 2023 (93 ± 7 % of sods transplanted survived) and it was maintained in 2025 (Figure 3).

In the locality of Los Nietos, all transplant stations were located in shallow areas. Their average survival rate observed in 2025 was 30 ± 8%. This survival rate was similar to that observed at the shallower transplant sites in Los Urrutias in 2022. This seems to indicate the viability of carrying out transplants in this other location. Future monitoring actions of the transplants proposed within the framework of the After-Life plan will allow the success of transplants in other locations to be monitored.

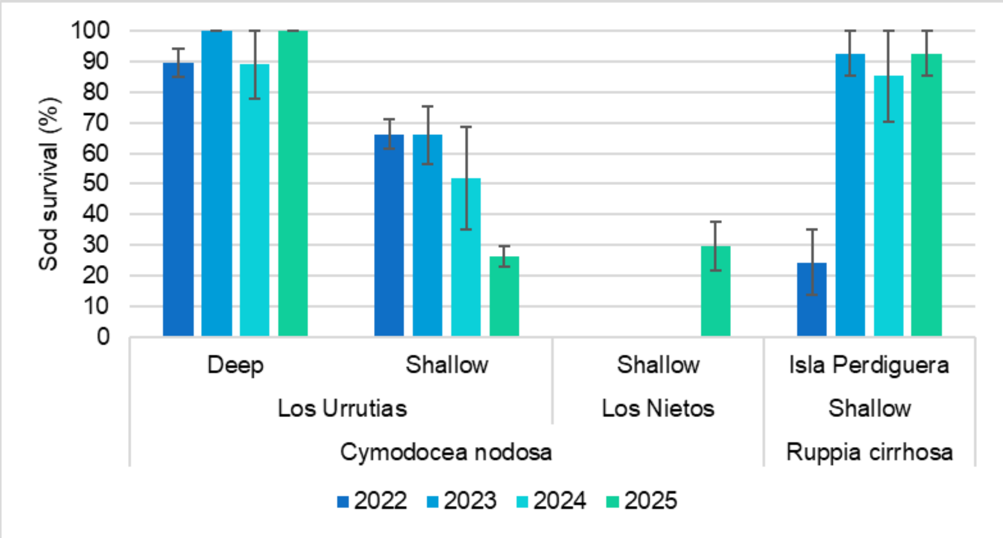


Figure 3. Mean percentage of transplanted sod survival for the different years of monitoring of *C nodosa* and *R. cirrhosa* of Los Urrutias and Perdiguera Island respectively. Survival measured as the percentage of presence or absence of the 9 sods in each transplant within the 10 x 10 m area.

In compliance with the specific objectives of the project (especially regarding the direct expected results as listed on page 38 of the Grant Agreement), the total newly formed surface by angiosperms in the Mar Menor was 935 m^2 (900 m^2 *C. nodosa* + 35 m^2 *R. cirrhosa*).

The rate of expansion was referred in terms of mean sod diameter (Figure 4). In general, higher mean values of *C. nodosa* sod diameters was observed in those deeper transplants. In shallower transplants, despite lower than deeper, sod diameter trended to increase up to the end of the project. Highest mean diameter in both depths was observed in 2024. Whereas shallow transplants showed the greatest mean diameter in spring 2024, in those deeper transplants, highest mean diameter was observed in autumn same year. In the last study period (2025), the mean diameter of deeper area transplants was 1054 ± 40 cm. In shallow area transplants, in the same period, the mean sod diameter measured was 181 ± 28 cm (Figure 4).

The case of *R. cirrhosa* resulted different in terms of rate of expansion (Figure 4). This species transplants disappeared in summer 2022, and was transplanted as a reinforcement in spring 2023, with a decrease in its surface to autumn 2023. However, in 2024 the situation was turned inverse: a great expansion was observed in spring 2024, with highest sod diameter in a single patch (1 210 cm), followed by a decrease in autumn 2024 (21 ± 3 cm), and recovery in spring 2025 (300 cm). However, in autumn last year, a decrease in the diameter of *R. cirrhosa* transplants was observed (11 ± 3 cm).

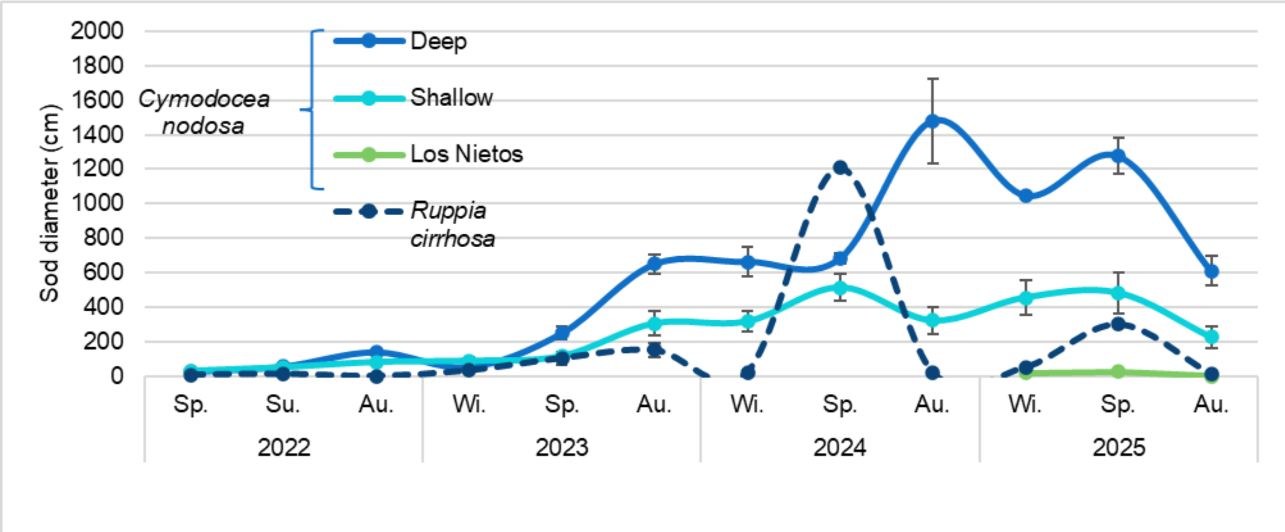


Figure 4. Interannual mean sod diameters (cm) of *C. nodosa* and *R. cirrhosa* transplants. Wi: Winter; Sp: Spring; Su: Summer; Au: Autumn.

Regarding the covering of newly formed meadows (%), in general, all the transplant stations, for both species, increased from spring 2023 to 2025 (Figure 5). The *C. nodosa* transplants reached the maximum coverage in spring 2025, whereas those transplants of *R. cirrhosa* covered the whole transplant site in June 2024 (late spring), followed by a sudden disappearance in autumn same year, and low recovery in last spring (May 2025). In spring 2025, within the *C. nodosa* transplants, deeper receiving stations showed a superior covering to the transplanted area (10 x 10 m) (138 %). This indicates that, some deeper transplants grew above 100 m^2. However, in those shallower transplants, covering reached a mean of 40% of the transplanted surface (for around 40 m^2). In Los Nietos location, due to the low survival, meadows covering represented < 1 %. Again, this last result corresponded only for the firs monitoring periods in these transplants carried out in Los Nietos. Further monitoring must be taken. In *R. cirrhosa* transplant, despite its maximum coverage in spring 2024, in last monitoring period it was < 5% of the total transplanted area (Figure 5). In the last study period, *C. nodosa* deeper station covered 56 % of the total transplanted surface. For the contrast, in those shallower stations, in average, the covering surface in autumn 2025 was 12%.

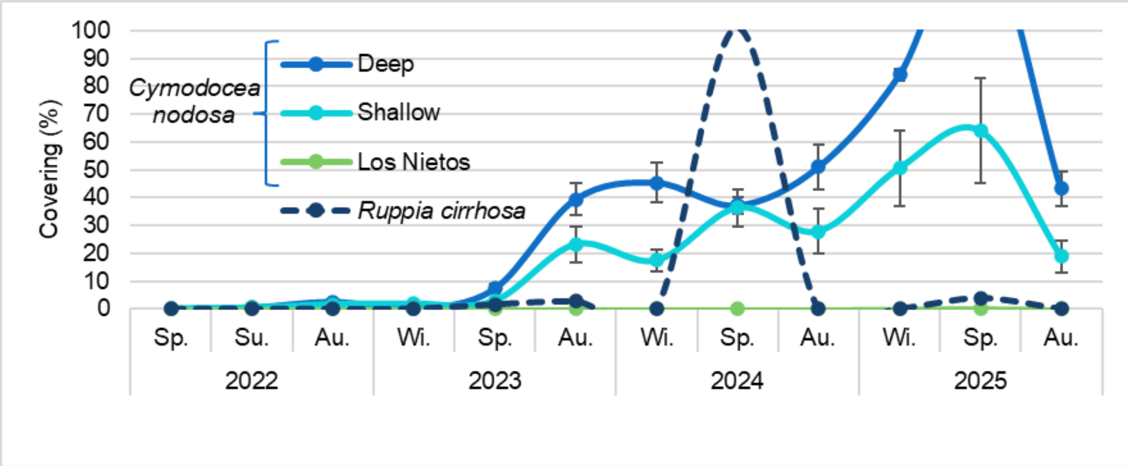


Figure 5. Meadow covering (%) of each transplant area of *C. nodosa* and *R. cirrhosa*, across the study periods. Wi: Winter; Sp: Spring; Su: Summer; Au: Autumn.

Figure 6. *C. nodosa* transplant station LUR4, in Los Urrutias. A particular case of success of transplant evolution, from 2022 (first transplant action) to 2025 (last monitoring period).

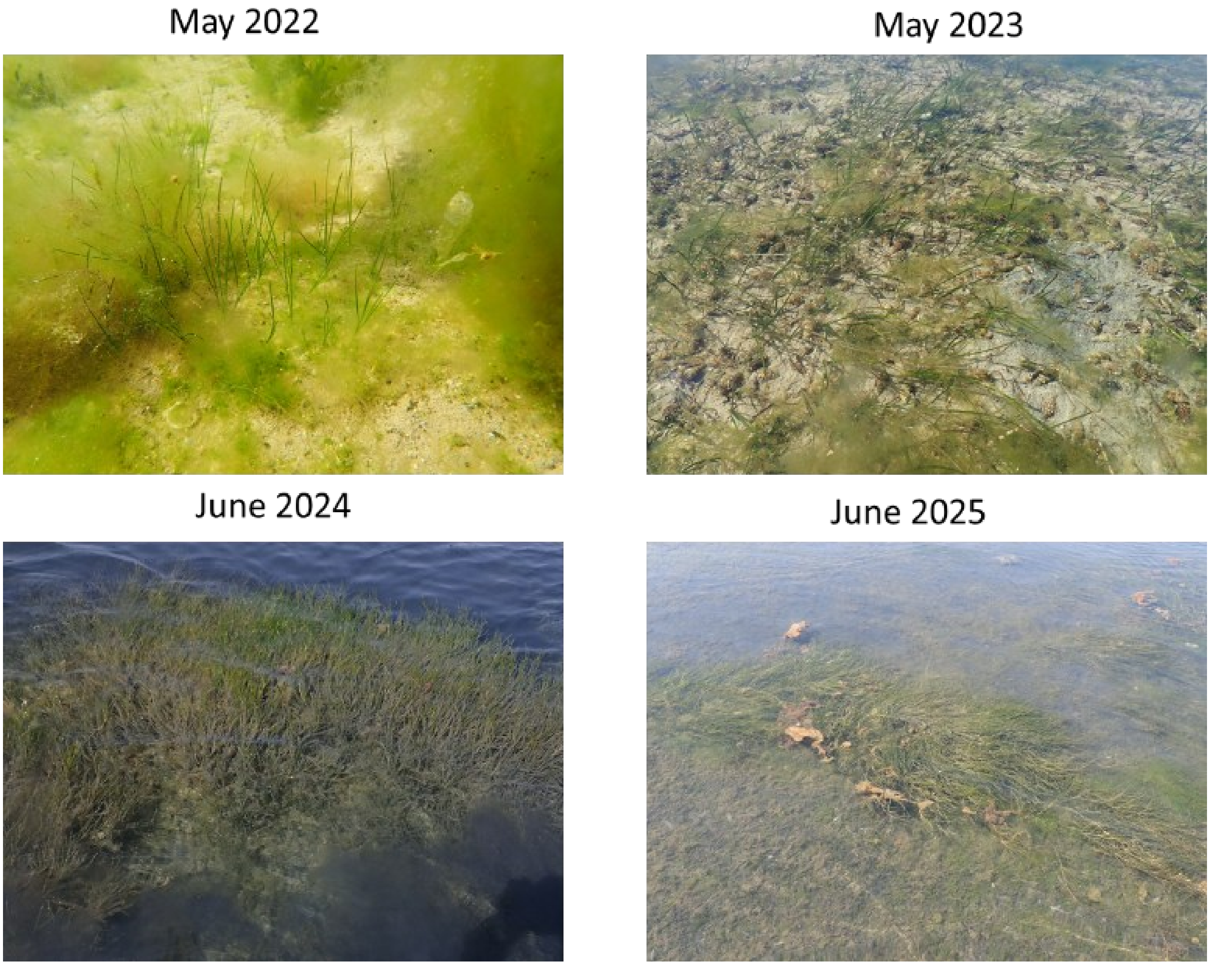


Figure 7. *R. cirrhosa* transplant across the monitoring periods.

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

SECTION 1 – Environmental parameters: water and sediments

Methods

Water Column

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 ($N\text{-}NH_4^+$); oxidized nitrogen ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$); dissolved inorganic phosphorous (SRP); dissolved silicates ($Si\text{-}SiO_4^{2-}$); 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 ($N\text{-}NH_4^+$); oxidized nitrogen ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$); dissolved inorganic phosphorous (SRP) and dissolved silicates ($Si\text{-}SiO_4^{2-}$) 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).

Surface sediments

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 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, total organic, and total carbon (TIC, TOC, TC).
- Total nitrogen (TN).
- Total inorganic, total organic, and total phosphorous (TIP, TOP, TP).

Total carbon, total inorganic carbon, and total nitrogen were analysed by Elemental Analyzer LECO CN 828. Total organic carbon (TOC) was analysed 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, total organic, and total 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 of the relation: g wet sediment – g dry sediment) / g wet sediment x 100); Porosity (ml water/ml wet sediment x 100); 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.

Settle particulate matter (SPM)

During 2022 and 2025, sedimentation traps were placed at the transplant sites. A total of 12 sedimentation traps were placed for the *C. nodosa* sites (8 receiving + 4 donor) and 2 for the *R. cirrhos*a study (1 receiving + 1 donor). The sedimentation rate is estimated as the dry weight of sediment deposited over a month, and the daily average is then calculated (g $DW \cdot m^{-2} \cdot day^{-1}$).

Results

Water Column

Differences in water matrix nutrients were observed between those two phanerogam species transplanted: whereas, in average, in those *C. nodosa* receiving sites, nitrate (NO_3^-) concentration was 3.8 ± 0.4 µg at N-NO_3^-/ l. For the contrast, in receiving station corresponding to *R. cirrhosa* transplants, the concentration was lower (0.2 ± 0.2 µg at N-NO_3^- / l). Similar, nitrite (NO_2^-) concentration in *C. nodosa* receiving sites resulted higher (0.3 ± 0.02 µg at N-NO_2^-/ l), than in *R. cirrhosa* ones (0.1 ± 0.01 µg at N-NO_2^-/ l). In both transplanted species receiving sites, concentration of nitrate (NO_3^-) and nitritie (NO_2^-) were lower in the last monitoring period (2025) than in the Ex-ante monitoring period (2021; Action A.2) (Figure 1). However, differences across monitoring period resulted greater in those *C. nodosa* receiving sites, than in *R. cirrhosa* receiving sites. Nitrate concentration, in *C. nodosa* receiving sites decreased from 9.1 ± 2 µg at N-NO_3^- / l in 2021, to 4.2 ± 1 µg at N-NO_3^- / l in 2025. Similarly, nitrite concentration, in *C. nodosa* receiving sites, decreased from 0.8 ± 0.2 to 0.2 ± 0.02 µg at N-NO_3^- / l.

Contrary, ammonium (NH_4^+), silicate (SiO_4^{2-}), and phosphate (PO_4^{3-}) concentrations, resulted higher in *R. cirrhosa* receiving sites than in *C. nodosa* ones. Concentration of ammonium in *R. cirrhosa* increased from 2021 (0.8 ± 0.3 µg at N- NH_4^+ / l) to 2025 (3.8 ± 1 µg at N-NH_4^+/ l). However, in *C. nodosa* receiving sites, ammonium concentration remained similar across the monitoring periods. In both transplant species sites, silicate concentration trended to increase from 2021 (9.1 ± 3 µg at Si-SiO_4^{2-} / l *C. nodosa*; 15 ± 6.6 µg at Si - SiO_4^{2-} / l *R. cirrhosa*), to 2025 (26.1 ± 4 µg at Si - SiO_4^{2-} / l *C. nodosa*; 28.6 ± 12 µg at Si - SiO_4^{2-}/ l *R. cirrhosa*). For the case of phosphorous, concentration in *R. cirrhosa* transplant sites resulted similar in the first and last monitoring period (0.3 ± 0.1 µg at P - PO_4^{3-} / l). However, in those *C. nodosa* receiving sites, that concentration decreased from 2021 (0.2 ± 0.01µg at P - PO_4^{3-} / l) to 2025 (0.1 ± 0.02 µg at P - PO_4^{3-} / l).

Regarding to total suspended solids (TSS), greater values in both transplanted species were observed in 2024, reaching mean values of 18 ± 2 mg / l in *C. nodosa*, and 18.3 ± 3 mg / l in *R. cirrhosa*. In the last monitoring period (2025), in *R. cirrhosa* transplant sites, mean value of TSS was greater (14.9 ± 4 mg / l) than in *C. nodosa* ones (9.6 ± 1 mg / l) (Figure 7).

Salinity reached a mean maximum in 2024 in both species transplantation sites (46.2 ± 0.1 PSU in *C. nodosa*, and 46 ± 1 PSU in *R. cirrhosa* sites). However, in the same period (2024), water temperature resulted minimum (compared to the rest of periods). In 2024, in *R. cirrhosa* transplanted sites, temperatures reached a mean of 19.5 ± 3 ºC. In those *C. nodosa* transplant sites, temperatures ranged from 21 ± 1 º C (2024), to 24.5 ± 1 ºC (2022). The case of Ex-ante monitoring period (2021), sampling was carried out only in summer period so, temperature and Eh resulted higher than the rest of monitoring periods. For the case of pH, it remained more or less constant across the monitoring program, in both species transplant sites. Slight differences were observed among those of *R. cirrhosa*, and *C. nodosa*, being higher in this last.

Regarding turbidity (NTU), its value resulted highest in *C. nodosa* transplant sites in the periods 2022 and 2023, but not in 2023 and 2025, when *R. cirrhosa* transplant sites showed the mean highest value of turbidity. This last case, in 2023, showed an average of 16.4 ± 11 NTU. In the last period, the same site showed higher water matrix turbidity (7.4 NTU) than those of *C. nodosa* (5.1 ± 2 NTU). In almost all the monitoring periods, dissolved oxygen (DO) resulted higher in *C. nodosa* transplant sites, than in *R. cirrhosa* ones. In average, in 2023 and 2024, the mean DO concentration resulted highest in *C. nodosa* (7.8 ± 0.2 and 8 ± 0.3 mg / l, respectively). Contrary, *R. cirrhosa* transplant site, in 2023, showed the minimum mean dissolved oxygen concentration (6.4 ± 2 mg / l).

Redox potential (Eh, mV) showed a positive mean value in both species transplant sites in 2021 (Ex-Ante monitoring) and 2022 (first monitoring period), correlated to nitrate reduction. However, in the rest of monitoring periods, redox potential reached minimums values of -76.8 ± 10 mV in *C. nodosa* and -103.0 ± 49 mV in *R. cirrhosa* transplant sites, in 2024.

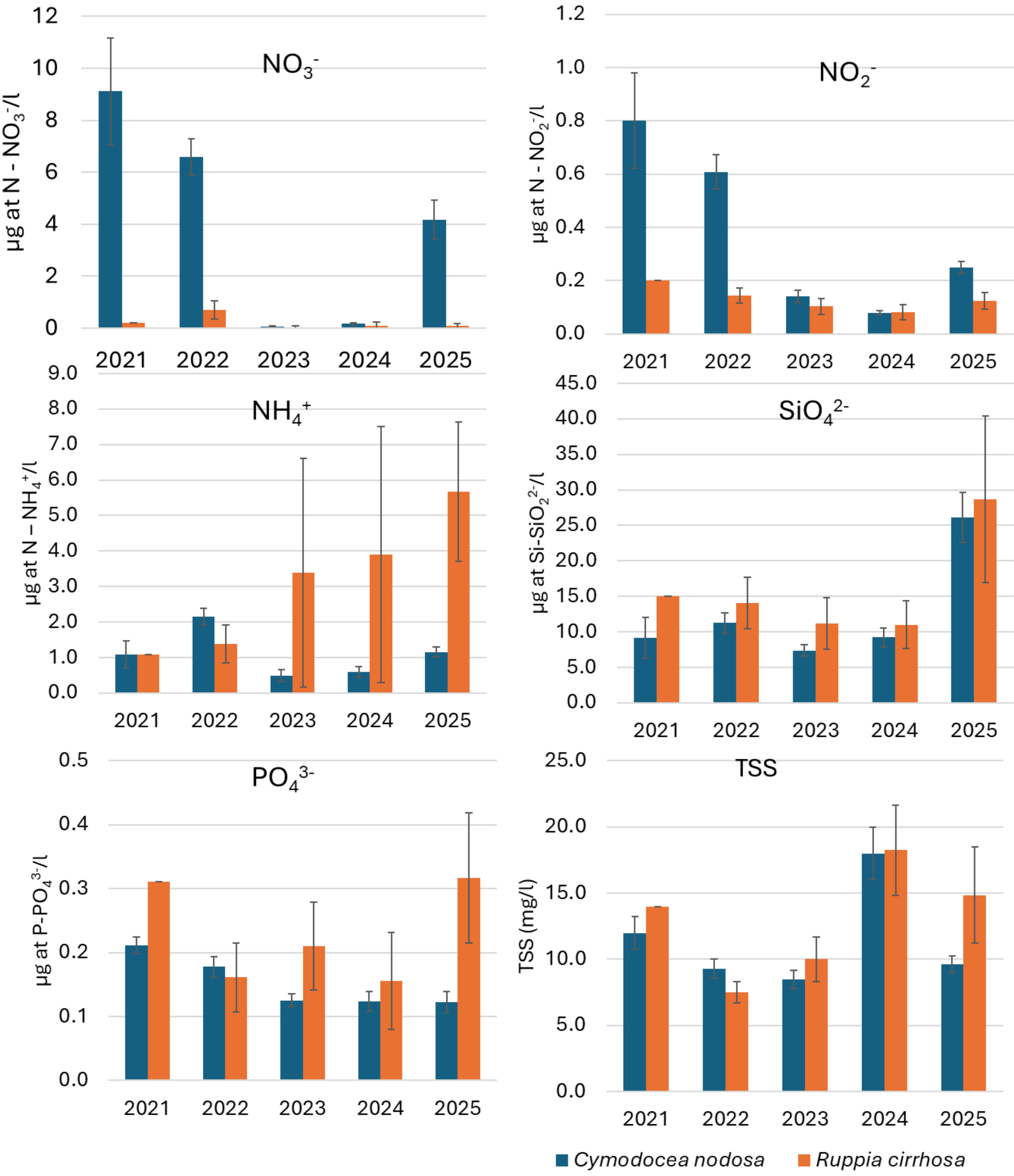


Figure 7. *C. nodosa* (dark blue) and *R. cirrhosa* (orange) transplant stations nutrients content in water matrix, across periods.

Figure 8. Physicochemical parameters water matrix in *C. nodosa* (dark blue) and *R. cirrhosa* (orange) transplant stations across the periods.

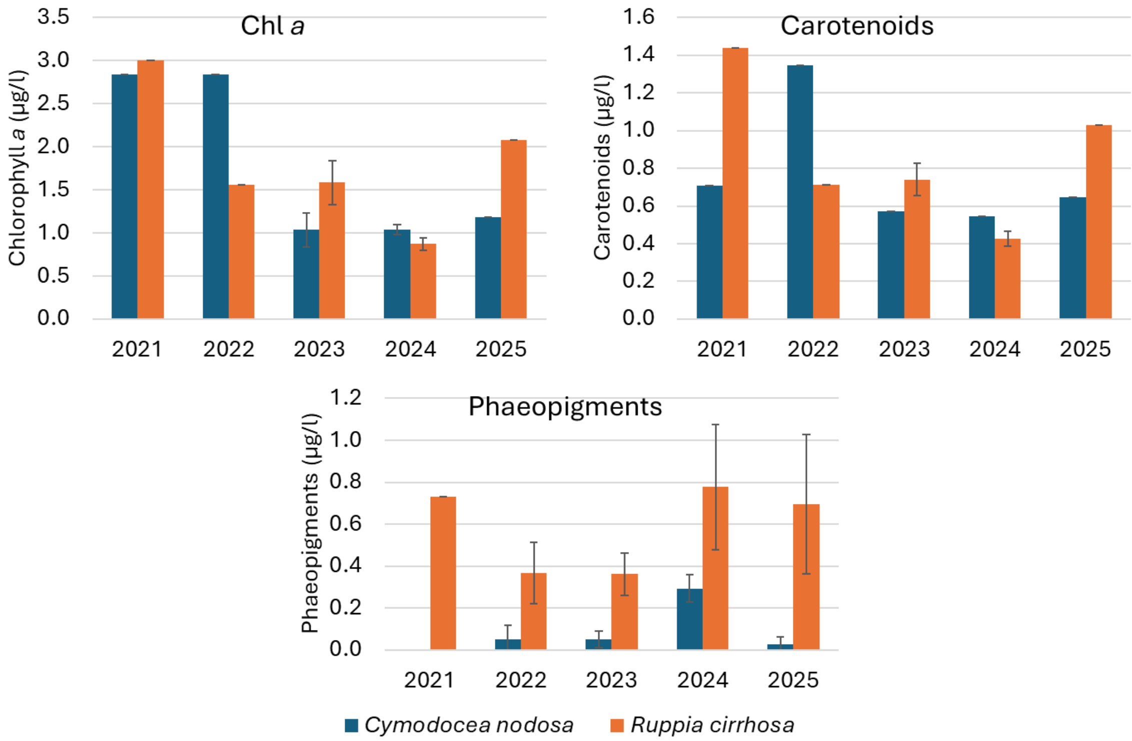


Figure 9. Pigments in water matrix in *C. nodosa* (dark blue) and *R. cirrhosa* (orange) transplant stations across the periods.

Chlorophyll *a*, and carotenoids varied across the periods in terms of concentration either in *C. nodosa* and *R. cirrhosa*. In ex-ante monitoring period, Chl *a* reached a maximum of 2.8 ± 0.3 and 3 ± 0.7 µg / l, in each species transplant sites. These highest values were due to it was only sampled in summer 2021. However, in the rest of monitoring periods (2022 – 2025), Chl *a* concentration decreased from 2022 to 2024. In this last, both species transplant sites showed similar Chl *a* concentration: 1 ± 0.1 and 0.9 ± 0.3 µg / l, respectively. In the last monitoring period (2025), Chl a concentration in *R. cirrhosa* transplant sites increased up to 2.1 ± 1 µg / l, whereas, for the case of *C. nodosa* transplant sites, its concentration did not result such high (1.2 ± 0.1 µg / l) (Figure 9).

Carotenoids showed a similar dynamic across the monitoring periods. In the first year, 2022, *C. nodosa* transplant sites showed higher mean values (1.3 ± 0.2 µg / l) than that of *R. cirrhosa* (0.7 ± 0.4 µg / l). Minimum values were observed in 2024 (0.5 ± 0.04 µg / l in *C. nodosa*, and 0.4 ± 0.2 µg / l in *R. cirrhosa* transplant sites) and, in the last monitoring period (2025), *R. cirrhosa* transplant sites showed higher mean concentrations (1 ± 0.3 µg / l) than *C. nodosa* ones (0.6 ± 0.1 µg / l (Figure 9).

Respect to phaeopigments, they are almost absent in *C. nodosa* transplant sites, except in 2024 (0.3 ± 0.1 µg / l). In the case of *R. cirrhosa* transplant sites, higher mean concentrations of phaeopigments were also observed in 2024 (0.8 ± 0.3 µg / l) and 2025 (0.7 ± 0.3 µg / l) (Figure 9).

Surface sediments

Changes in the humidity (or moisture content) in sediments can impact nutrient cycling. In both transplanted species, sediments humidity increased across the monitoring periods, mostly due to the increase of transplanted covering (Figure 10). In the case of *C. nodosa*, in the middle terms of monitoring, moisture content was lower than in the final period (2025: 0.4 ± 0.1 %). For the case of *R. cirrhosa* transplant sites sediments, humidity resulted higher than the *C. nodosa* sites. Similarly, in the last monitoring period, it was higher than the previous ones (0.6 ± 0.1 %).

Porosity is a key parameter in sediment water surface diffusion. Differences in sediment porosity were observed among the species transplanted sites (Figure 10). In the case of *C. nodosa*, transplant sites sediment porosity varied across the monitoring period, increasing up to year 2025 (0.7 ± 0.0 %), similar to that observed in ex-ante monitoring (2021: 0.9 ± 0.3%). For the case of *R. cirrhosa* transplant sites, sediment porosity remained similar across the monitoring periods, being higher than in *C. nodosa* transplant sites (2021: 1.73 ± 0.27%; 2025: 0.65 ± 0.12%).

The difference between dry density and wet density in marine sediments is that dry density measures the weight of sediment without water per unit volume, while wet density includes the weight of the water contained in the pores of the sediment. The wet density is always higher than the dry density due to the presence of moisture. In the case of study of the Mar Menor, no differences were observed between dry and wet density, due mainly to the lower moisture in sediments (Figure 10). However, differences in sediment densities were detected between the species transplanted sites: in the case of *C. nodosa*, in average, transplant sites sediment showed higher mean density than in the case of *R. cirrhosa* ones. In the first case, highest mean dry density values were observed in 2025 (*C. nodosa* sites: 1.6 ± 0.02 g / cm^3; *R. cirrhosa*: 1 ± 0.05 g / cm^3). For the contrast, in *R. cirrhosa* transplant sites, highest mean dry sediment density was observed in 2023 (1.3 ± 0.1 g / cm^3).

Regarding the organic content (O.M.) and fine fraction (< 0.063 mm particles), differences were observed among the two species transplanted sites (Figure 10). In the case of *R. cirrhosa* transplant sites, either organic content or fine fraction resulted higher than in the case of *C. nodosa* sediments. In the first, up to the end of monitoring period (2025), highest mean values were recorded both in organic matter content (10.4 ± 1.1 %), or fine fraction (41.5 ± 8.2 % < 0.063 mm particles). Despite lower, similar dynamic was observed in the case of *C. nodosa* transplant site sediments: an increase in organic matter content (1.6 ± 0.1 %) and fine fraction (7.3 ± 3.1 % < 0.063 mm particles), in 2025.

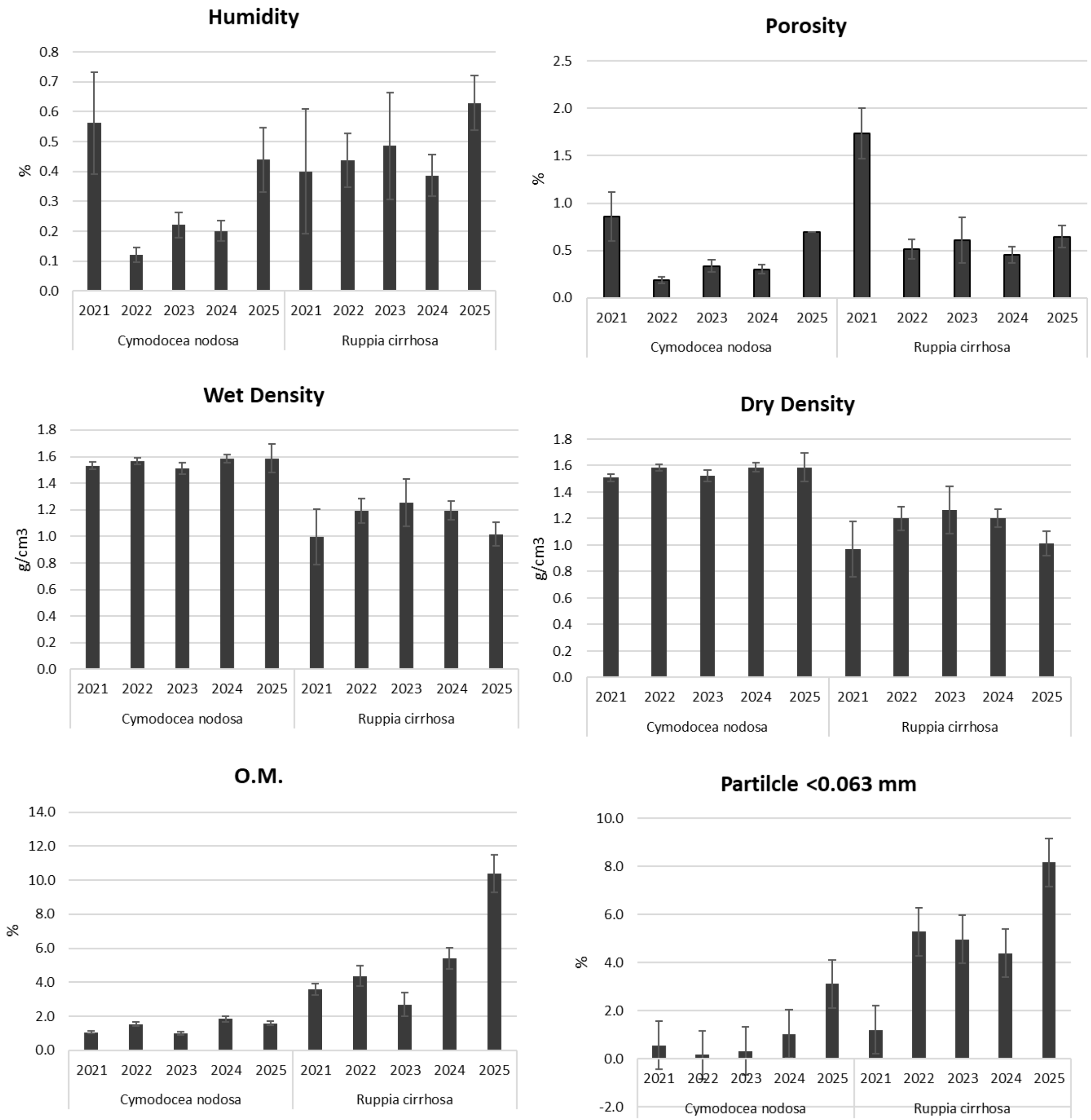


Figure 10. Mean values of surface sediment humidity (%), porosity, wet and dry densities (g / cm^3), organic matter content (O.M., %), and fine fraction content (< 0.063 mm; %) of the different species transplant sites, across the monitoring periods (2021 – 2025).

In the case of *C. nodosa* receiving sites, the results obtained showed that in all sites and years monitored, the total inorganic carbon fraction represented the majority of total carbon, while total organic carbon constituted a much smaller proportion (Figure 11). This pattern was consistent at both the Los Urrutias and Los Nietos localities, on where sediments were composed, mainly, of mollusc shells, foraminifera, polychaete annelid tubes, with a high carbonate content.

From a spatial point of view, moderate variations were observed between transplanted sites, although the predominance of inorganic carbon remained in all of them. At some sites, total carbon values were slightly lower, which could be due to local differences in sedimentation rate, substrate composition, or hydrodynamics. In this sense, Los Nietos, with greater hydrodynamics, could be more carbonated, while Los Urrutias could show slightly higher organic carbon contents associated with lower water renewal and greater accumulation of organic matter.

In terms of temporal evolution, the values of both fractions (inorganic and organic carbon) showed slight fluctuations between years, with no clear trend of increase or decrease. This stability suggests that the processes of mineralization and carbon accumulation in the system remain in equilibrium throughout the period studied. The low proportion of organic carbon indicates efficient degradation of organic matter, probably favoured by oxidizing conditions and a high rate of mineralization.

Overall, these results reflected that the sediments of Los Urrutias and Los Nietos were characterized by a predominance of inorganic carbon and low organic carbon accumulation, which is an indicative of stable and highly mineralized environments.

In *R. cirrhosa* transplant site, the dynamic was similar as for *C. nodosa*, although in this case, the sediment was muddier and had higher concentrations of organic carbon due to the higher contribution of organic matter by the system. With the exception of the Las Encañizadas donor area in 2025, where the concentration of organic carbon was much higher, the temporal trend in the rest of the stations and years remained relatively stable.

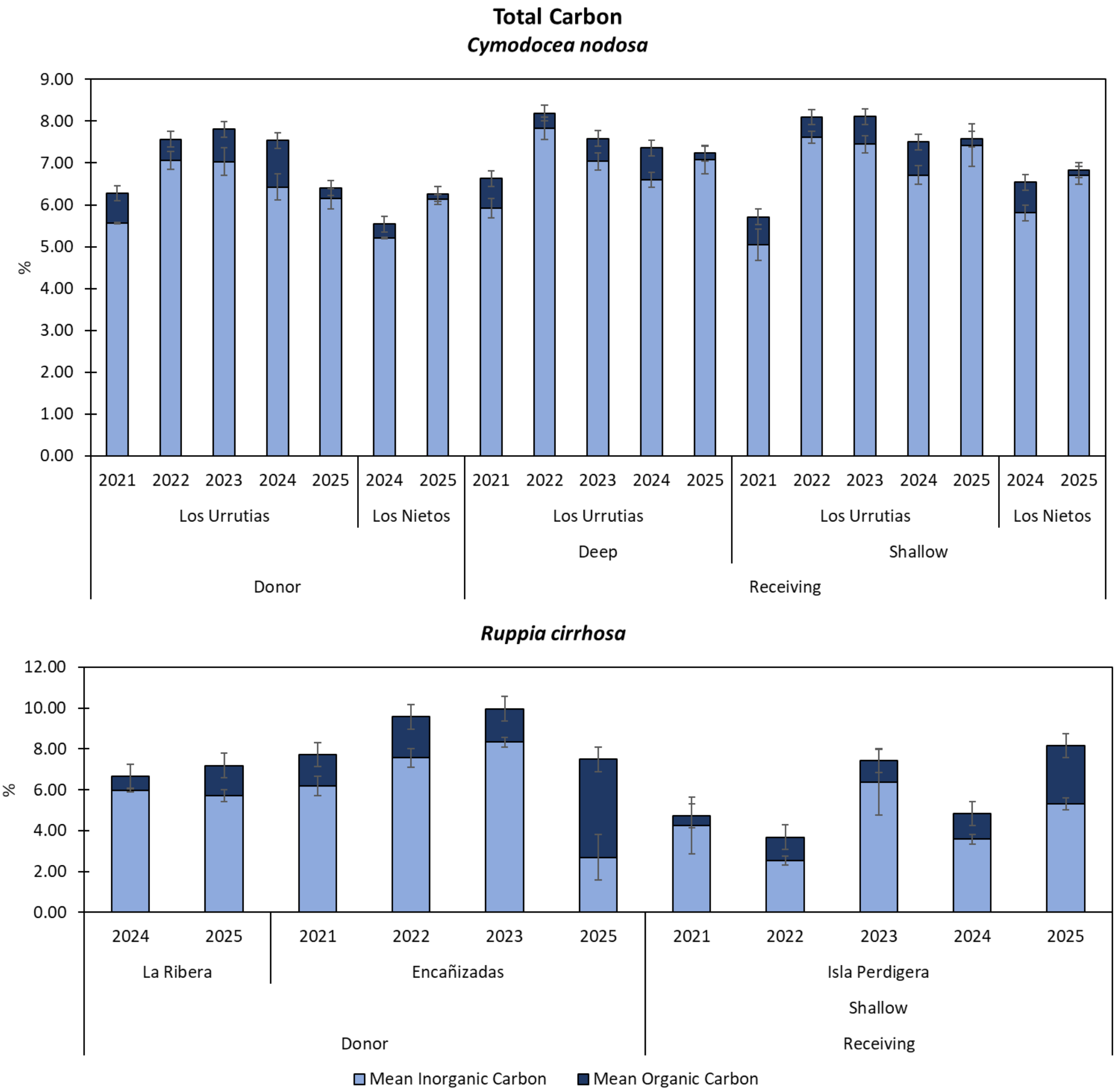


Figure 11. Total carbon, total inorganic and total organic carbon (%) content in sediments, across the monitoring periods, of the donor and receiving sites for the *C. nodosa* and *R. cirrhosa* transplants.

In the receiving sites of *C. nodosa*, total nitrogen contents were mostly low and relatively homogeneous, although with some specific exceptions where higher values were observed, possibly associated with local accumulation processes or external inputs of organic matter (Figure 12). These reduced concentrations may be related to the lower maturity or density of the transplanted *C. nodosa* meadows, which could have lower nutrient retention and recycling compared to established donor meadows. In contrast, at donor sites, total nitrogen values tended to be slightly higher, reflecting higher organic matter content and biological activity. Consolidated *C. nodosa* meadows favoured the accumulation of fine sediments and organic detritus, which could increase total nitrogen reserves.

Overall, the pattern observed indicates that sediments from donor sites had higher concentrations of total nitrogen, associated with greater ecological stability and maturity of *C. nodosa* meadows. In contrast, receiving sites showed lower and more dispersed nitrogen content, reflecting initial colonization conditions or lower nutrient retention capacity. These results highlight the influence of the structure and state of development of seagrass beds on nitrogen storage and dynamics in the sediment at both locations.

The general pattern indicated that the muddy sediments associated with *R. cirrhosa* had high concentrations of total nitrogen, reflecting their high nutrient storage capacity. The donor areas (Encañizadas and Santiago de la Ribera) showed slightly higher concentrations, associated with more mature and stable meadows, while in the receiving area (Isla Perdiguera) the total nitrogen in sediment tended to be lower, which may reflect a lower capacity for nitrogen retention and recycling in the sediments after transplantation. However, given that the sediments at Isla Perdiguera were highly muddy, the differences were not very marked, suggesting that the fine texture and low oxygenation of the sediment favour total nitrogen retention even in areas where the meadows are still incipient.

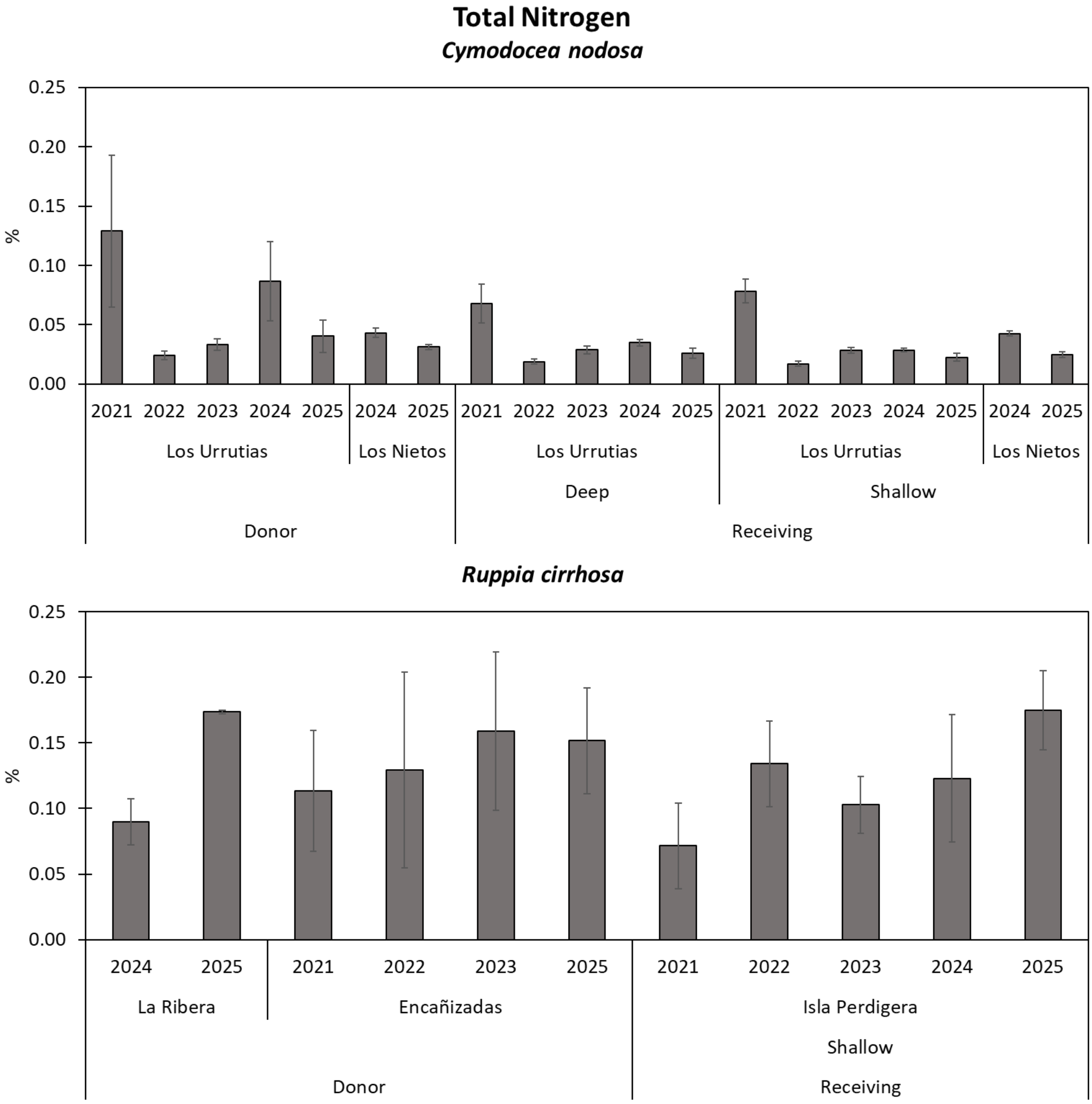


Figure 12. Total nitrogen content (%), across the monitoring periods, in sediments of donor and receiving sites for the transplanted species *C. nodosa* and *R. cirrhosa*.

In general terms, inorganic phosphorus constituted the predominant fraction of total phosphorus at all sites, while organic phosphorus represented a smaller proportion (Figure 13).

At donor sites, total phosphorus values were generally higher than those recorded at recipient sites, especially with regard to the inorganic fraction. This trend could be related to greater accumulation and mineralization of organic matter in sediments associated with established *C. nodosa* meadows, which would favour the release and retention of phosphate compounds. In contrast, the receiving sites, which were in the process of colonization after transplantation, had lower concentrations, which could reflect a lower organic matter content or a lower nutrient retention capacity in the sediment.

When comparing the two locations, it can be seen that Los Urrutias showed greater variability between sites, including some high concentration peaks, suggesting greater heterogeneity in sedimentary conditions or in the degree of vegetation cover. In contrast, Los Nietos showed more homogeneous and generally lower values, possibly because the transplants were carried out in the fall of 2024 and are therefore more recent than those in Los Urrutias so, comparisons must be taken carefully.

In the case of *R. cirrhosa*, the results indicated that sediments associated with this species had moderate total phosphorus values, with a predominance of the inorganic fraction, and that the differences between donor and recipient sites were less pronounced than in the case of *C. nodosa*. This could be due to ecological and functional differences between the two species, as *R. cirrhosa*, in the Mar Menor, tends to occupy shallower and more variable areas, with muddier sediments and higher organic matter content, with more active sediment dynamics that could limit the differential accumulation of phosphorus between sites.

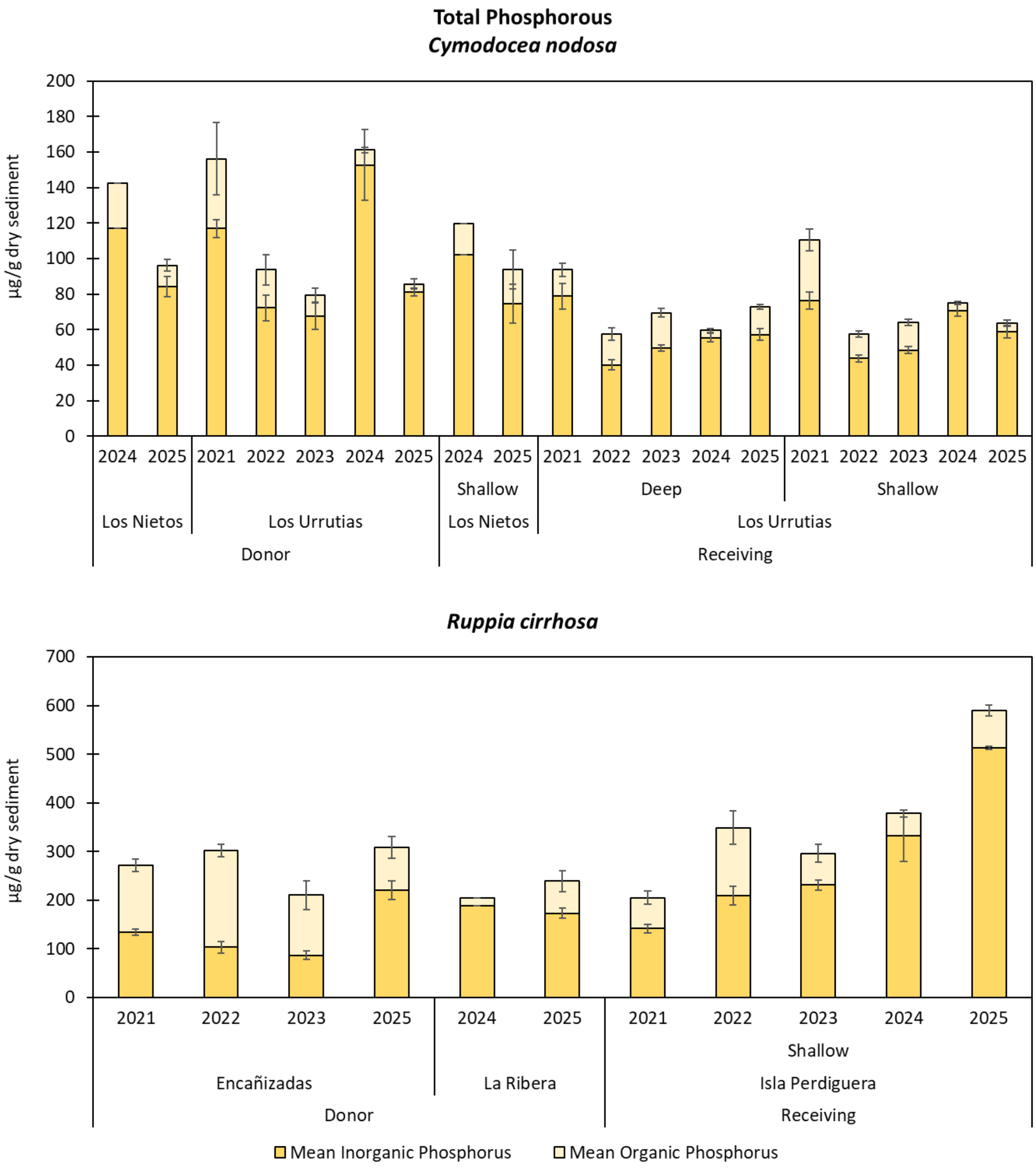


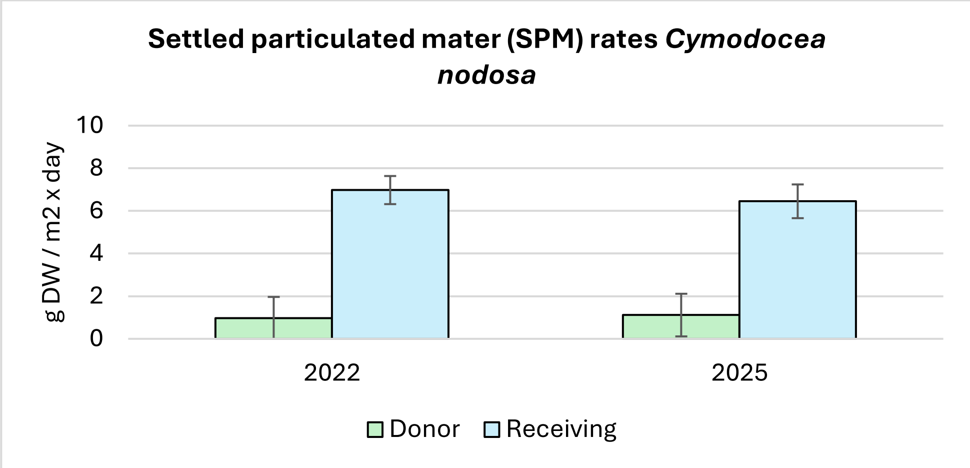
Figure 13. Total phosphorus, total inorganic phosphorous, total organic phosphorous (µg P / g dry sediment), across the monitoring periods, in sediments of donor and receiving sites for the transplanted species *C. nodosa* and *R. cirrhosa*.

Settled particulated mater (SPM)

Sedimentation rates of suspended particulate matter (SPM) showed marked differences between species and associated bottom types. On sandy bottoms dominated by *C. nodosa*, receiving areas had significantly higher sedimentation values compared to donor areas in both 2022 and 2025. In both years, rates in recipient areas exceeded 6 g DW·m^{-2}·day^{-1}, while donor areas remained below 2 g DW·m^{-2}·day^{-1}. This trend suggested that, after transplantation, the recipient areas of *C. nodosa* experienced greater deposition of particulate matter, possibly associated with less consolidation of the sandy substrate and still incomplete vegetation cover, which would favour the resuspension and subsequent sedimentation of suspended material. The consistency of this pattern across years indicated a persistence in the hydrodynamic or structural differences between the two types of areas.

In contrast, considerably lower sedimentation rates were observed in the muddy bottoms occupied by *R. cirrhosa*. In 2022, this species receiving areas had slightly higher values than the donor areas (2.04 g DW·m^{-2}·day^{-1} versus 0.55 g DW·m^{-2}·day^{-1}), but in 2025 both decreased dramatically to values close to 0.24 and 0.25 g DW·m^{-2}·day^{-1}, respectively, with no appreciable differences between sites. This overall decline and convergence between areas suggests a gradual stabilisation of sediment in the recipient areas, probably as a result of the recovery of vegetation cover and the restoration of habitat structure after transplantation.

Overall, the results showed that the sedimentary response after transplantation depends on both the species and the type of substrate. The sandy bottoms of *C. nodosa* appeared to be more dynamic and sensitive to initial disturbance, while the muddy bottoms of *R. cirrhosa* showed faster recovery and a tendency towards homogenisation of sedimentation rates over time (Figure 14).



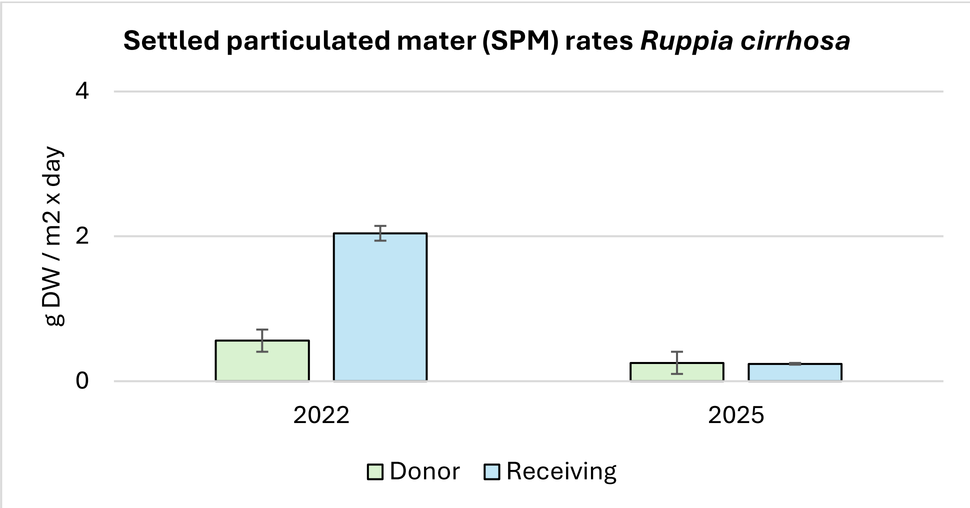


Figure 14. Sedimentation rates of suspended particulate matter (SPM; g $DW \cdot m^{-2} \cdot day^{-1}$) in donor and recipient areas of *Cymodocea nodosa* (sandy bottoms) and *Ruppia cirrhosa* (muddy bottoms) transplants in 2022 and 2025. The bars indicate the mean ± standard error.

SECTION 2 – Ecological Quality Status (EQS)

Macrophytes and MaQI determination

Methods

Macrophyte monitoring sampling campaigns were carried out twice a year (spring and autumn) for all the project periods. In each donor and receiving sites proposed for both species under study it was taken 3 replicates.

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 solution for its posterior taxonomic identification in the laboratory of Universidad Complutense of Madrid (Spain), with the collaboration of experts (Dra. 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 Sfriso (2010): species with punctuation 2 (high ecological value) and species with punctuation 0-1, which were subdivided in Chlorophyta and Rhodophyta. Wet weigh (g) of all the classes will be measured using an electronic balance. With all the parameters mentioned above, the Macrophyte Quality Index (MaQI; Sfriso & Facca, 2011; Sfriso *et al*., 2014) were determined in order to assess the ecological status as required by Water Framework Directive (2000/60/EC).

Results

A total of 36 macrophyte species were identified in all the transplant sites (donor and receiving) for both study species (*C. nodosa* and *R. cirrhosa*): 17 Rhodophyta,16 Chlorophyta, 1 Ochrophyta, and the two phanerogams. Among these two transplanted species, *C. nodosa* (donor and receiving) showed highest macrophyte species richness (32 species), compared to *R. cirrhosa* (22 species). In the first, Rhodopyta was the richest group (17 species), followed by Chlorophyta (14 species). For the contrast, in the case of *R. cirrhosa* stations, the number of species of Chlorophyta resulted higher (10 species) than Rhodophyta (9 species). Ectocarpaceae taxa (Ochrophyta) was only identified in one donor station of *R. cirrhosa* transplants (Table 3).

Differences in species composition were observed in each study species, among donor and receiving sites, and within these last, among deep and shallow receiving areas. Difference across the monitoring period was also perceived in both transplanted species (Figure 15). For the case of *C. nodosa*, in average, in spring 2023, highest number of species was identified either in donor or receiving sites. At the end of the monitoring program, it was identified higher number of species than in the first year (2022). In average, donor sites showed lower number of macrophyte species than in receiving ones. Among these lasts, some differences were detected in relation to the depth, showing changes in the richest across the periods.

Table 3. Species list of macrophyte identified in the different species transplanted sites. E-MaQI score is specified for each species.

Phylum	Specie	Score	*C. nodosa*	*R. cirrhosa*
Chlorophyta	*Acetabularia acetabulum* (Linnaeus) P.C. Silva, 1952	2	X	X
	Acetabularia calcyculus J.V.Lamouroux, 1824	2	X	
	Batophora occidentalis (Harvey) S. Berger & Kaever ex M.J.Wynne, 1998	2		X
	Caulerpa prolifera (Forsskål) J.V.Lamouroux, 1809	1	X	X
	Chaetomorpha ligustica (Kützing) Kützing, 1849	0	X	X
	Chaetomorpha linum (O.F.Müller) Kützing, 1845	1	X	X
	Cladophora albida (Nees) Kutzing, 1843	1	X	X
	Cladophora coelothrix Kützing, 1843	2	X	
	Cladophora fracta f. nigrescens-cassior Rabenhorst	1	X	
	Cladophora pellucida (Hudson) M.J.Wynne, 2017	2	X	
	Cladophora spp Kützing, 1843	0	X	X
	Cladophora vagabunda (Linnaeus) Hoek, 1963	0	X	X
	Phaeophila dendroides (P.Crouan & H.Crouan) Batters, 1902	0	X	
	Ulva clathrata (Roth) C.Agardh, 1811	0	X	X
	Ulva compressa Linnaeus, 1753	0		X
	Ulva spp Linnaeus, 1753	0	X	
Ochrophyta	Ectocarpaceae C. Agardh, 1828	1		X
Rhodophyta	*Alsidium corallinum* C.Agardh, 1827	2	X	
	Anotrichium tenue (C.Agardh) Nägeli, 1862	2	X	
	Carradoriella denudata (Dillwyn) Savoie & G.W.Saunders, 2019	1	X	X
	Ceramium codii (H.Richards) Barros-Barreto & Maggs, 2023	2	X	X
	Ceramium diaphanum (Lightfoot) Roth, 1806	1	X	X
	Ceramium spp Roth, 1797	1	X	
	Chondria capillaris (Hudson) M.J.Wynne, 1991	1	X	X
	Erythrotrichia carnea (Dillwyn) J.Agardh, 1883	1	X	X
	Herposiphonia secunda f. tenella (C.Agardh) Ambronn, 1880	2	X	
	Hydrolithon spp (Foslie) Foslie, 1909	2	X	X
	Hypnea musciformis (Wulfen) J.V.Lamouroux, 1813	2	X	
	Hypnea spinella (C.Agardh) Kützing, 1847	2	X	X
	Jania rubens (Linnaeus) J.V.Lamouroux, 1816	2	X	X
	Lophosiphonia obscura (C.Agardh) Falkenberg, 1897	2	X	
	Palisada tenerrima (Cremades) D.Serio, M.Cormaci, G.Furnari & F.Boisset, 2010	2	X	X
	Polysiphonia sertularioides (Grateloup) J.Agardh, 1863	1	X	
	Spyridia filamentosa (Wulfen) Harvey, 1833	1	X	
Tracheophyta	*Cymodocea nodosa* (Ucria) Ascherson, 1870	2	X	X
	Ruppia cirrhosa (Petanga) Grande, 1918	2		X

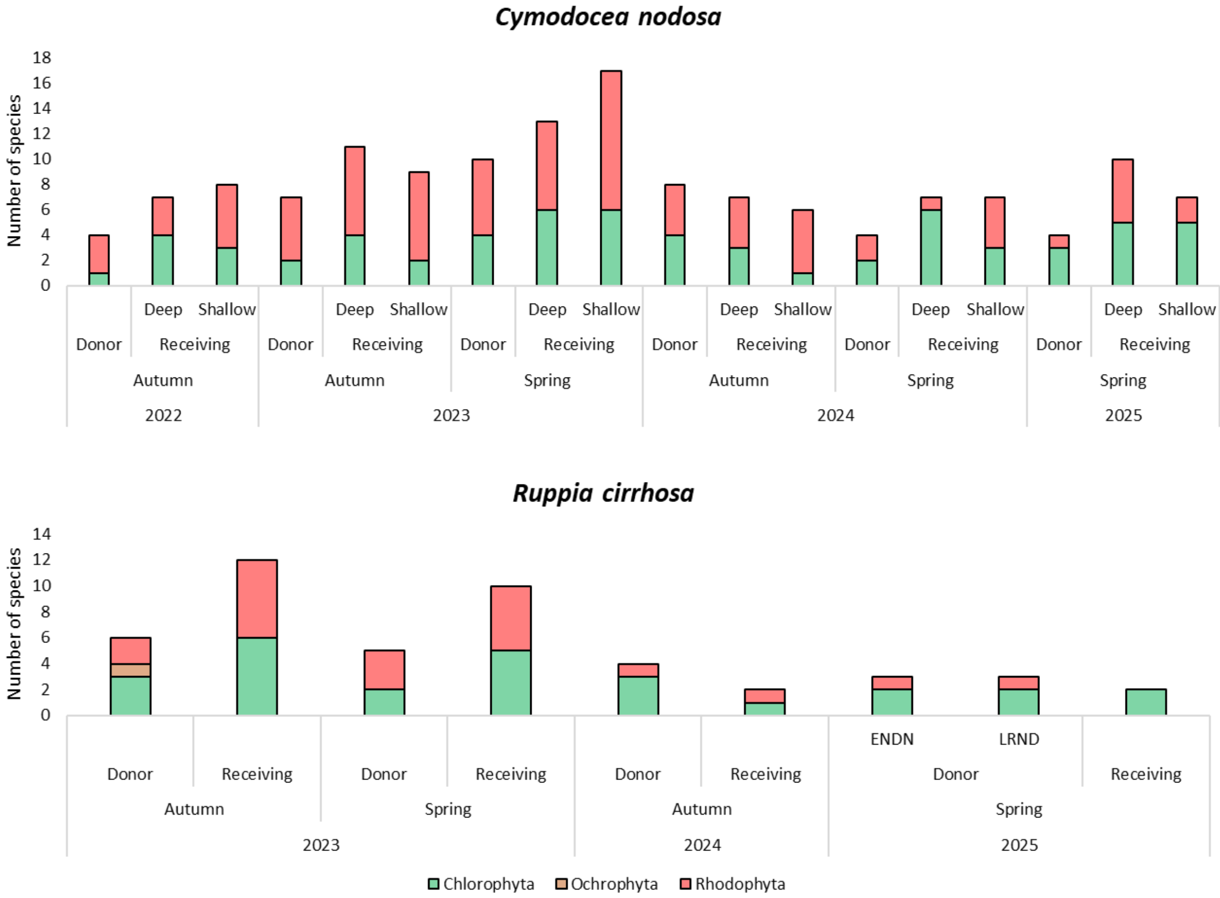


Figure 15. Number of macrophyte species identified in donor and receiving sites for each transplanted species case of study, across the monitoring periods.

Across the monitoring periods, macroalgae covering increased in *C. nodosa* donor sites, but in receiving ones (Table 4). However, in these last, despite total covering remained similar to the first monitoring year, the macrophyte and macroalgae coverage resulted in similar values. Due to this, ecological status at the end of the project, in both donor and receiving sites, was HIGH (MaQI = 0.85). This status was already reached in 2023, with the success of phanerogam transplant, and the presence of it in the macrophyte samples collected. The sensitive taxa values ranged between 0 and 7 species, with greater sensitivity in autumns (Au.) from 2022 and 2024. In spring (Sp.) from 2024 and 2025 sensitive taxa generally dropped or was close to zero species, indicating less presence of sensitive taxa. Total covering varied significantly across seasons and years, highlighting highest values in donor sites in 2023 (spring: 425.8%, autumn: 246.1%). However, receiving sites showed highest total coverage in autumn 2024 (42.3 %). *C. nodosa* coverage in receiving sites peaked in 2023 (12.6 % in autumn) and remained almost constant across the autumn 2024 (12.2%), and spring 2025 (12.4%). However, in spring 2023 and 2024, this species coverage did not suppose higher than

3%. Most years and seasons have MaQi values between 0.65 and 1, which corresponds to ecological statuses from "GOOD" to "HIGH" (indicating good ecological status). In spring 2022, a "POOR" status appears, showing a period of lower environmental quality.

Table 4. MaQI value estimation for donor (D) and receiving (R) sites for *C. nodosa* transplants, seasonally (Au, Autumn; Sp, Spring), across the monitoring periods.

	2022		**2023**				**2024**				**2025**	
	Au.		**Sp.**		**Au.**		**Sp.**		**Au.**		**Sp.**	
	D	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**	**D**	**R**
Nº of sensitive taxa (score 2)	1	6	3	6	7	4	0	0	7	1	0	0
Sensitive taxa % (score 2)	25	40	30	20	100	20	0	0	88	8	0	0
Total coverage (%)	56.19	28.4	425.8	33.4	246.1	32.3	145.2	8.7	140.3	42.3	184	25.7
***Cymodocea nodosa* coverage %**	10.50	0	325	2.3	200	12.6	100	2.3	52.5	12.2	125	12.4
Macroalgal coverage %	45.69	28.4	100.8	31.1	46.1	19.7	45.2	6.3	87.8	30.1	59	13.3
Relative abundance Rhodophyta % (score 0 - 1)	26.68	1.8	15.5	34.3	62.5	31.8	0.1	3.8	0.3	1.2	0	0.2
Relative abundance Chlorophyta % (score 0 - 1)	7.66	93.3	51.1	49.6	32.5	18.8	99.6	41.9	87.2	76.2	0.9	0.7
MaQi value	0.65	0.25	1	0.85	0.85	0.85	0.85	0.85	1	0.65	0.85	0.85
Ecological Status (EQR)	GOOD	POOR	HIGH	HIGH	HIGH	HIGH	HIGH	HIGH	HIGH	GOOD	HIGH	HIGH

For the case of *R. cirrhosa* transplants, number of sensitive taxa varied between 0 and 2 species. These species resulted in a percentage of total species identified of 0% (as in the case of receiving sites in autumn 2024) to 67% (receiving sites in spring 2025). Total macrophyte coverage resulted highest in receiving site in spring 2023, mostly due to the greatest coverage of macroalgae in this site (747 %). In the rest of seasons and periods, total coverage resulted lower than in 2023, with differences across the monitoring periods. Despite this data correspond to *R. cirrhosa* transplant sites, *C. nodosa* was also identified. This species was present mainly in donor sites, in autumn 2023, and in the new donor location (Santiago de la Ribera, LR) in spring 2025. Regarding to *R. cirrhosa*, it showed highest coverage values in donor sites, mainly in spring 2023 (173.7%). In the receiving site, *R. cirrhosa* covering increased in spring 2023 (50%), disappeared in samples collected in 2024, and supposed a 7.5% of coverage in 2025. Macroalgae, for its case, showed a coverage peak in 2023 (747 %) and always remained higher than that from *R. cirrhosa*. Regarding to MaQI and Ecological status estimated, in *R. cirrhosa* transplant receiving sites, its ecological status decreased from HIGH (2023) to POOR (2024). This decreasing was mainly due to the disappearance of *R. cirrhosa* in receiving sites, and the dominance of species with MaQI scores 0 – 1. In the last period, despite the number of sensitive taxa was higher, and the presence of *R. cirrhosa* was notorious, the highest relative abundance of Chlorophyta species with scores 0 – 1 (100%), maintained receiving sites in a MODERATE (MOD) status.

Table 5. MaQI value estimation for donor (D) and receiving (R) sites for *R. cirrhosa* transplants, seasonally (Au, Autumn; Sp, Spring), across the monitoring periods. Donor locations: EN, Encañizadas; LR, Santiago de la Ribera.

	2023				**2024**		**2025**		
	Au.		**Sp.**		**Au.**		**Sp.**		
	D	**R**	**D**	**R**	**D**	**R**	**D**		**R**
	EN		**EN**		**LR**		**EN**	**LR**	
Nº of sensitive taxa (score 2)	2	2	2	1	1	0	1	2	2
Sensitive taxa % (score 2)	29	17	33	9	20	0	25	67	67
Total coverage (%)	77.8	45.4	185.5	797.1	23.1	23.8	19.8	119.8	63.8
***Cymodocea nodosa* coverage %**	70.2	0	0	0	0	0	0	35	0
***Ruppia cirrhosa* coverage %**	0	12	173.7	50	8.7	0	17.5	25	7.5
Macroalgal coverage %	7.6	33.4	11.8	747.1	14.4	23.7	2.2	59.7	56.2
Relative abundance Rhodophyta % (score 0 - 1)	38	11.2	95.5	0.1	2.6	0	44.4	1.7	0
Relative abundance Chlorophyta % (score 0 - 1)	15.5	26.2	0.2	92.1	27.8	21.1	0	73.2	100
MaQI value	0.85	0.85	0.85	0.85	0.55	0.25	0.55	0.85	0.55
Ecological Status (EQR)	HIGH	HIGH	HIGH	HIGH	MOD.	POOR	MOD.	HIGH	MOD.

Macrobenthos and M-AMBI and BITS determination

Methods

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 autumn. 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 (Ethanol 70% solution) and identification in the laboratory. Abundance and specific richness were 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.
- BITS index value
- M-AMBI index value

Results

Of the total taxa identified (95 taxa), 39 taxa belong to the group of polychaete annelids, 31 taxa to molluscs, 18 taxa crustacean arthropods. Other smaller groups were Chordata (Class Ascidiacea), Porifera and Cnidaria, with 2, 1 and 1 taxon, respectively (Table 6).

Table 6. Taxa list found in the different donor and receiving stations for both transplantation species in the monitoring program.

Annelida
Capitellidae
Heteromastus filiformis (Claparède, 1864)
Mediomastus fragilis Rasmussen, 1973
Notomastus latericeus Sars, 1851
Cirratulidae
Caulleriella sp Chamberlin, 1919
Cirriformia tentaculata (Montagu, 1808)
Scoloplos haasi (Monro, 1937)
Dorvilleidae
Dorvillea sp Parfitt, 1866
Eunicidae
Lysidice unicornis (Grube, 1840)
Glyceridae
Glycera tridactyla Schmarda, 1861
Nereididae
Nereididae Blainville, 1818
Perinereis cultrifera (Grube, 1840)
Orbiniidae
Naineris laevigata Grube, 1855)
Scoloplos haasi (Monro, 1937)
Paraonidae
Cirrophorus furcatus (Hartman, 1957)
Paradoneis sp Hartman, 1965
Sabellidae
Branchiomma boholense (Grube, 1878)
Serpulidae
Spionidae
Dispio sp Hartman, 1951
Malacoceros sp Quatrefages, 1843
Microspio sp Mesnil, 1896
Pseudopolydora sp Czerniavsky, 1881
Spio sp Fabricius, 1785
Syllidae
Syllis sp Lamarck, 1818
Arthropoda
Aoridae
Microdeutopus algicola Della Valle, 1893
Gammaridae
Gammarus insensibilis Stock, 1966
Ischyroceridae
Corophium insidiosum (Crawford, 1937)
Ericthonius difformis H. Milne Edwards, 1830
Siphonoecetes sabatieri Rouville, 1894
Leptochelidae
Chondrochelia savignyi (Kroyer, 1842)
Maeridae
Elasmopus rapax A. Costa, 1853
Sphaeromatidae
Cymodoce truncata Leach, 1814
Varunidae
Brachynotus sexdentatus (Risso, 1827)
Mollusca
Cardiidae
Cerastoderma glaucum (Bruguière, 1789)
Parvicardium exiguum (Gmelin, 1791)
Ceritiidae
Bittium reticulatum (da Costa, 1778)
Cerithium vulgatum Bruguière, 1792
Hydrobidae
Hydrobia acuta (Draparnaud, 1805)
Lucinidae
Dosinia lupinus (Linnaeus, 1758)
Loripes orbiculatus Poli, 1795
Mytilidae
Mytilaster minimus (Poli, 1795)
Rissoidae
Pussillina lineolata (Michaud, 1830)
Semelidae

Abra alba (W. Wood, 1802)	
Tellinidae	
Gastrana fragilis (Linnaeus, 1758)	
Veneridae	
Dosinia lupinus (Linnaeus, 1758)	
Polititapes aureus (Gmelin, 1791)	
Phoronida	
Phoronidae	
Phoronis sp Wright, 1856	
Porifera	
Sycettidae	
Sycon raphanus Schmidt, 1862	

In relation to the density of individuals, in *C. nodosa* receiving sites, differences among deep and shallow transplant sites showed slights variations up to 2024 (Figure 16). From this period, there was a highlighted increase in the density of individuals, reaching maximum up to 2025 (4 572.6 ± 1 476 ind. / m^2), mainly in those deep sites. Species richness varied between 2 and 12 taxa, with greater values of deep transplant sites, compared to shallow ones. Up to the end of the monitoring period (2025), the mean number of taxa identified in *C nodosa* receiving sites was 10.6 ± 2 taxa for deep sites, and 3.5 ± 1 taxa for shallow ones. In average, greater than the first monitoring year.

For the case of *R. cirrhosa* transplant site, density of individuals also showed seasonally increase and a peak in spring 2025 (56 977.3 ± 4 8091 ind. / m^2). Number of taxa increased gradually from 2022 to 2025, with a maximum in spring 2023 (14 ± 3.6 taxa), and 12 ± 1.6 taxa in 2025.

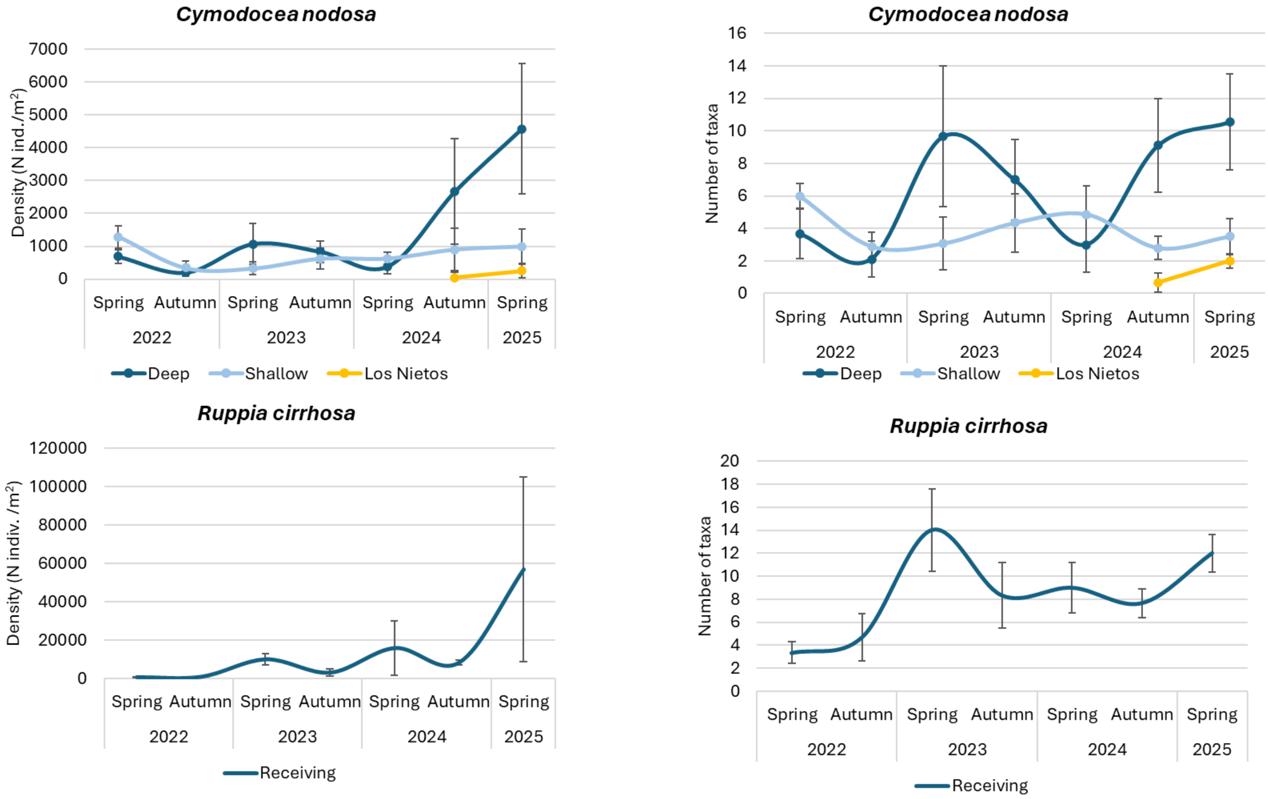


Figure 16. Density of individuals (N indiv. / m^2) and species richness (N taxa) identified in receiving sites of the different transplanted species: *C. nodosa* and *R. cirrhosa*, across the monitoring period (seasonally).

The BITS index showed a positive temporal evolution in the transplanted meadows of *C. nodosa* and *R. cirrhosa*, indicating a progressive improvement in ecological quality at the recipient sites. In *C. nodosa*, values increased gradually from 2023 to 2025, reaching levels similar to or higher than those in the donor areas, suggesting structural and functional recovery of the meadows on sandy bottoms. In *R. cirrhosa*, established on muddy bottoms, the index generally showed higher values and a faster recovery, with evident stabilisation from 2024 onwards. Overall, the results reflect a trend towards ecological consolidation of the transplanted habitats, with differences in response speed associated with the type of substrate and the characteristics of each species (Figure 17). The M-AMBI index showed a general improvement in the ecological status of areas hosting *C. nodosa* and *R. cirrhosa* throughout the monitoring period. In *C. nodosa*, values increased gradually until 2025, indicating a sustained recovery of meadows on sandy bottoms. Deep receiving sites showed higher ecological quality (good), while in shallow areas it was slightly lower (moderate). In *R. cirrhosa*, the increase was faster and values stabilised from 2024 onwards. Overall, the results indicate a positive trend towards the ecological consolidation of the transplanted habitats, with differences in the speed and magnitude of recovery associated with the type of substrate and the biology of each species (Figure 18).

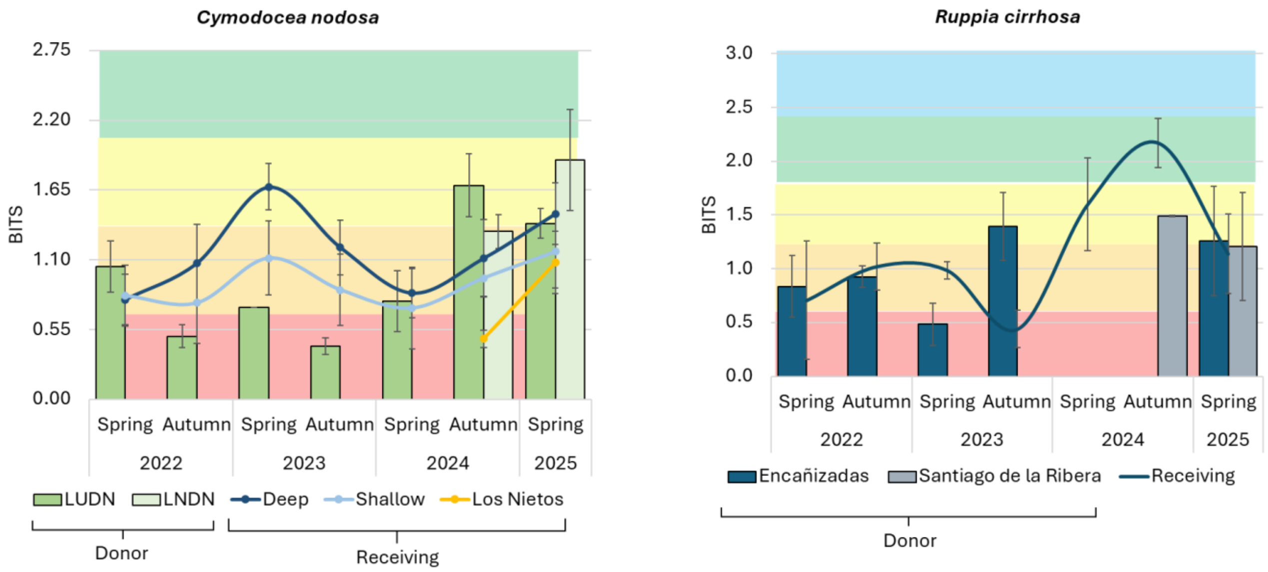


Figure 17. BITS index value estimated for each transplanted species sites, and control sites (donor), across the monitoring periods.

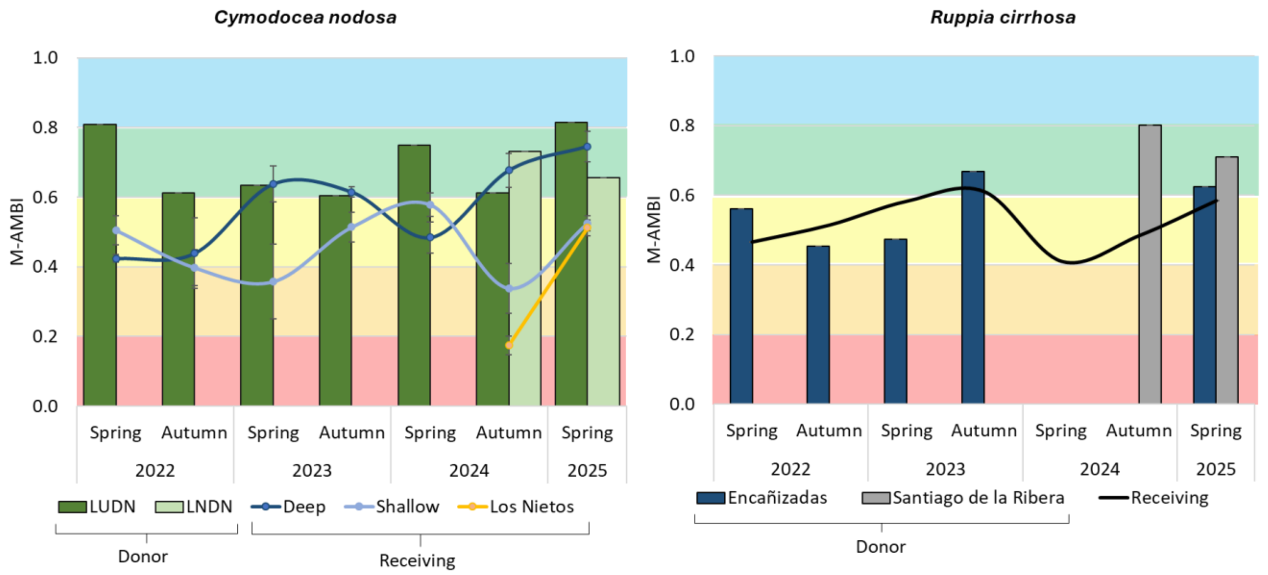


Figure 18. M-AMBI index value estimated for each transplanted species sites, and control sites (donor), across the monitoring periods.

Fish fauna

Methods

Fish fauna monitoring and were 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 20 m beach net trawl survey and 50 m transects were made to assess the state of the fish community by analysing species diversity. In each location, two transects were carried out in places where the transplant sites were located. In addition, in the case of *C. nodosa*, two transects were also carried out in natural seagrass donor locations (control). Given the difficulty of accessing and carrying out transects in the *R. cirrhosa* seagrass donor area, it was not possible to obtain reference data for the natural area (control) for this species. Only receiving sites data was presented for this last case.

The parameters to be determined are those necessary 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, the total length (in mm) of each individual were measured.

Results

Analysis of the average fish abundances recorded between 2022 and 2025 in the three locations studied in the Mar Menor (Los Urrutias, Los Nietos, and Isla Perdiguera/La Ribera) shows a gradual recovery of fish communities in areas receiving transplants of *C. nodosa* and *R. cirrhosa* (Table 8).

In general terms, more than twenty species were identified, representative of families typical of seagrass beds, including *Atherina boyeri* Risso, 1810, *Diplodus* genus species, *Sparus aurata Linnaeus, 1758*, Mugilidae species, and *Syngnathus* Linnaeus, 1758 species. The highest abundances were recorded in Los Urrutias, followed by Isla Perdiguera and Los Nietos, suggesting local differences in vegetation structure and coverage.

The donor areas showed higher and more stable abundances throughout the study period, reflecting consolidated fish communities and mature habitats. In contrast, the receiving areas showed low initial values in 2022 but showed a sustained increase in abundance and species richness from 2023 onwards, especially of euryhaline species such as *A. boyeri,* Mugilidae, and *Sardina pilchardus* (Walbaum, 1792).

The progressive increase in indicator species associated with seagrass habitats—such as *Syngnathus abaster* Risso, 1827, *Syngnathus typhle* Linnaeus, 1758, and *Syngnathus acus* Linnaeus, 1758 —is a relevant ecological indicator of the success of transplantation and functional habitat recovery. These species, together with the sporadic appearance of *Hippocampus guttulatus* Cuvier, 1829, suggest an improvement in the structural complexity and shelter provided by the vegetation.

In *R. cirrhosa* meadows (Perdiguera Island), recolonization was slower than in *C. nodosa*, with low abundances in 2022–2023 but an upward trend towards 2025. This difference could be related to the lower initial coverage and more marked seasonality of *R. cirrhosa*.

Overall, the results show a positive trend of ecological recovery in the recipient areas, reflected in the increase in total abundance and specific diversity of fish, as well as in the appearance of bioindicator species typical of well-developed meadows. The progressive convergence between the communities of donor and recipient areas suggests that, in the medium term, seagrass transplants are effectively contributing to habitat restoration and the recovery of the ecological functionality of the Mar Menor ecosystem.

Table 7. 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.

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

Table 8. Abundance of fish found in the transplantation areas. 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.

Cymodocea nodosa	**EUFG**	**FMFG**	**Status**	**Donor**				**Receiving**			
LOS URRUTIAS				**2022**	**2023**	**2024**	**2025**	**2022**	**2023**	**2024**	**2025**
Anguilla anguilla (Linnaeus, 1758)	Di	HP		1	1						
Apricaphanius iberus (Valenciennes, 1846)	ES	Bmi	Annex II HD	1		1		1	1		
Atherina boyeri Risso, 1810	ES	HZ, HP	Commercial interest	78	101 ± 85.5	86.5 ± 23.5	8.5 ± 3.5	128 ± 26	127 ± 3	110 ± 34	47.5 ± 4.5
Belone belone (Linnaeus, 1760)	MM	HP		1	3			1	1		
Callionymus pusillus Delaroche, 1809	MM/MS	Bmi						1			
Dicentrarchus punctatus (Bloch, 1792)							1				1
Diplodus annularis (Linnaeus, 1758)	MS	Bmi, Bma, HZ									1
Diplodus puntazzo (Walbaum, 1792)	MS	Bmi, Bma, HZ							3		
Diplodus sargus (Linnaeus, 1758)	MS	Bmi, Bma, HZ		1	1		1		1		
Engraulis encrasicolus (Linnaeus, 1758)	MM	PL	Commercial interest	101	4				2		
Gobius cobitis Pallas, 1814	MS	Bmi, Bma, HP					2				2.5 ± 1.5
Gobius niger Linnaeus, 1758	ES	Bmi, Bma, HP		3	1	15 ± 2	2	9.5 ± 5.5	2	7.5 ± 6.5	2
Microlipophrys dalmatinus (Steindachner & Kolombatovic, 1883)	MS	OV, Bmi					1				
Mugilidae sp Jarocki, 1822	D/MM	DV	Commercial interest	50	305.5 ± 37.5	261.5 ± 47.5	45.5 ± 9.5	350 ± 88	278 ± 29	339 ± 58	101 ± 30
Pomatoschistus marmoratus (Risso, 1810)	ES	Bmi, HZ		4	6	48 ± 5	3	4.5 ± 1.5	9 ± 3	20.5 ± 15.5	6
Salaria pavo (Risso, 1810)	ES	OV, Bmi		2	3	7	7.5 ± 5.5	7 ± 4	6 ± 4	9	13 ± 6
Sardina pilchardus (Walbaum, 1792)	MM	PL				10				62 ± 48	
Sarpa salpa (Linnaeus, 1758)	MM/MS	HV		1				2	1		
Serranus cabrilla (Linnaeus, 1758)	MS							1			
Solea solea (Linnaeus, 1758)	MM	Bmi, BMa	Commercial interest								1
Sparidae Rafinesque, 1818	D/MM	DV			7				5 ± 2		
Symphodus sp Rafinesque, 1810	MS	Bmi, BMa		21	15 ± 13	8.5 ± 3.5		39	5.5 ± 2.5	15	
Syngnathus abaster Risso, 1827	ES	Bmi	Indicator (seagrass)	6	2	12 ± 4	8.5 ± 2.5	7.5 ± 5.5	13.5 ± 7.5	19.5 ± 12.5	6
Syngnathus acus Linnaeus, 1758	ES	Bmi	Indicator (seagrass)					1			
Syngnathus typhle Linnaeus, 1758	ES	Bmi, HP, HZ	Indicator (seagrass)	2	1	1	1	6	1	1	
LOS NIETOS											
Atherina boyeri Risso, 1810	ES	HZ, HP	Commercial interest			153 ± 3	754 ± 751			32 ± 9	545 ± 11
Diplodus annularis (Linnaeus, 1758)	MS	Bmi, Bma, HZ					1				
Diplodus puntazzo (Walbaum, 1792)	MS	Bmi, Bma, HZ									0.5
Diplodus sargus (Linnaeus, 1758)	MS	Bmi, Bma, HZ				3					
Gobius niger Linnaeus, 1758	ES	Bmi, Bma, HP				4.5 ± 1.5	1			1.5	0.5
Hippocampus guttulatus Cuvier, 1829	ES	Bmi, HZ								0.5	
Mugilidae Jarocki, 1822	D/MM	DV	Commercial interest			171.5 ± 32.5	13 ± 9			1	28 ± 5
Pomatoschistus marmoratus (Risso, 1810)	ES	Bmi, HZ				16 ± 14				63.5 ± 34.5	2.5
Salaria pavo (Risso, 1810)	ES/MS	OV, Bmi				2.5 ± 1.5	2.5			14.5 ± 4.5	0.5
Sardina pilchardus (Walbaum, 1792)	MM	PL				6.5					
Solea solea (Linnaeus, 1758)	MM	Bmi, BMa	Commercial interest			1					
Sparus aurata Linnaeus, 1758	MM	Bmi, Bma, HZ									0.5
Symphodus tinca (Linnaeus, 1758)	MS	Bmi, BMa					1				
Syngnathus abaster Risso, 1827	ES	Bmi, HZ	Indicator (seagrass)			5.5 ± 2.5					
Ruppia cirrhosa											
LA RIBERA/PERDIGUERA ISLAND											
Apricaphanius iberus (Valenciennes, 1846)	ES	Bmi	Annex II HD						0.5	1	
Atherina boyeri Risso, 1810	ES	HZ, HP	Commercial interest				25 ± 14	1.5		43.5	1.5
Belone belone (Linnaeus, 1760)	MM	HZ, HP						0.5			
Gobius niger Linnaeus, 1758	ES	Bmi, Bma, HP					2.5	0.5		21	
Lithognathus mormyrus (Linnaeus, 1758)	MS	Bmi, BMa							4		
Mugilidae Jarocki, 1822	D/MM	DV	Commercial interest				47 ± 40	42.5 ± 41.5	6.5	123	21.5 ± 5.5
Pomatoschistus marmoratus (Risso, 1810)	ES	Bmi, HZ					5.5				2
Salaria pavo (Risso, 1810)	ES/MS	OV, Bmi					5.5 ± 3.5	3.5		28.5	8.5 ± 2.5
Symphodus sp Rafinesque, 1810	MS	Bmi, BMa						8.5		2	
Syngnathus abaster Risso, 1827	ES	Bmi, HZ	Indicator (seagrass)				1.5	1		4	
Syngnathus acus Linnaeus, 1758	MS	Bmi, HZ	Indicator (seagrass)					1			
Syngnathus typhle Linnaeus, 1758	ES	Bmi, HP, HZ	Indicator (seagrass)						0.5	0.5	

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