

LIFE19 NAT/IT/000264
*Seagrass transplantation for transitional
Ecosystem Recovery*
(LIFE- TRANSFER)

Action D2: Monitoring of C2 action

Second monitoring progress report

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EXECUTIVE SUMMARY

The D.2.1 Sub-action concerns the monitoring of the growth of transplanted angiosperms. The D.2.2 Sub-action concerns 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 the stations monitored at Comacchio (Fattibello) and Goro (Seganda, Bassunsin) lagoons.

This **Second Monitoring Progress Report** is divided in four sections: the first two sections, edited by DAIS-UNIVE, concern angiosperms growth and water and sediment analyses, the other two sections, edited by UNIFE, concerns the benthic and the fish fauna, and the ecological quality.

Regarding transplanted angiosperms rooting and growth, we have not found significant results until now, due to high turbidity (Fattibello) or excessive algal growth episodes (Goro). The transplants will be intensified also considering different lagoon sub-areas.

As regards to water parameters, concentrations of the Reactive Phosphorus in the two lagoons was quite similar, while the concentration of the Total Inorganic Nitrogen was approx. 4 times higher in Goro than in Fattibello. Chlorophyll-*a* and total suspended solids were significantly higher in Fattibello than in Goro. The high values of TSS and Chl-*a* recorded mostly at Fattibello, with a water transparency of approx. 30-40 cm, seem not suitable for the rooting of aquatic phanerogams.

On the basis of the recorded species the ecological conditions obtained by the application of the index MaQI were Poor for Goro and Bad for Fattibello.

Concerning the benthic fauna, 29 macrobenthic taxa were found in the Sacca di Goro, and 20 in Valle Fattibello. The ecological quality status through M-AMBI was Good for one transplant site and Poor and Bad at the other transplant and the control site, respectively, at Goro (High, Moderate, Poor, respectively, through BITS). It resulted moderate at the two sites (transplant and control) at Fattibello. The majority of the macrobenthic taxa present at all the sites belongs to the indifferent (EG-II) and tolerant (EG-III) group at Goro, and tolerant and opportunistic (EG-IV) group at Fattibello.

Concerning the fish fauna, the fish community was richer in species and abundance at Goro respect to Fattibello. The application of the HFBI index resulted in a quality judgement of high at Goro, and sufficient at Fattibello.

SubAction D.2.1 Monitoring angiosperm growth

Aquatic angiosperms at the beginning of the project were absent in both the lagoons. The only species present in the past was *Ruppia cirrhosa*, but it disappeared in the last three decades of the last century with the increasing of eutrophication (Sfriso et al., 2016; Munari et al., 2023).

This project aims to reintroduce this species and/or others aquatic angiosperms present in similar environments to reconstruct, where possible at least in part, the ancient prairies.

Table 1 shows the number of transplants foreseen in each lagoon in the Proposal for a total of 648 sods equivalent approx. to 6480 rhizomes.

Table 1. Number of planned transplants in each lagoon.

Year	2021		2022		2023		2024		2025		Total
Season	---	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	
Stations	---	8	8	8	8	8	8	8	8	8	72
N° of sods	---	72	72	72	72	72	72	72	72	72	648
Equivalent rhizomes	---	720	720	720	720	720	720	720	720	720	6480

Between 2021 and 2022 the number of transplants was higher than anticipated (**Table 1**, **Figure 1**).

Indeed, the total transplant at Goro were 419 sods of *Ruppia cirrhosa* (approx. 4190 equivalent rhizomes) (**Table 2**). The donor sites were Valle Campo and Valle Bertuzzi. In this lagoon, in suitable areas, 100 rhizomes of *Cymodocea nodosa* (approx. 10 equivalent sods), 300 rhizomes of *Zostera marina* (approx. 30 equivalent sods) and 600 rhizomes of *Zostera noltei* (approx. 60 equivalent sods) coming from the Venice Lagoon were also transplanted. At Fattibello the number of transplants was 220 (approx. 2200 equivalent rhizomes). No other species were transplanted because the ecological conditions were not suitable.

Table 2. Number of effective transplants

		Species	Sods (diameter 15 cm)	Equivalent rhizomes	Donor	Rhizomes	Equivalent Sods	Donor
Goro	2021	<i>R. cirrhosa</i>	207	2070	Valle Campo			
	2022	<i>R. cirrhosa</i>	212	2120	Valle Bertuzzi			
	2022	<i>C. nodosa</i>				100	10	Venice Lagoon
	2022	<i>Z. marina</i>				300	30	
	2022	<i>Z. noltei</i>				600	60	
Total			419	4190		1000	100	
Fattibello	2021	<i>R. cirrhosa</i>	80	800	Valle Bertuzzi	-	-	-
	2022	<i>R. cirrhosa</i>	140	1400	Valle Bertuzzi	-	-	-
	Total			220	2200			

The monitoring of the angiosperm transplants occurred in late spring (31 May) and autumn (10 October) 2022 and in late spring 2023 (30 May).

At each angiosperm monitoring we have not recorded significant plant attachment but only some sods which then disappeared and were not recorded in the subsequent surveys because the excessive growth of macroalgae (Goro) or the high water turbidity due to phytoplankton blooms (Fattibello). In the latter lagoon we are currently selecting new areas with more suitable conditions and intensifying the number of transplants.



Figure 1. Transplant activities at Goro and Fattibello

SubAction D.2.2 Monitoring biodiversity and the environmental quality status

SECTION 1 – WATER, SEDIMENTS, MACROALGAE AND EQ

Field sampling

Once the ecologically suitable sites had been selected for the transplantation of aquatic angiosperms, the sampling of the environmental matrices of water and sediments started. In each station some environmental parameters were monitored by means of portable instruments in accordance with what was reported in the sampling forms.

In field the following parameters and macrophyte variables were recorded:

- Date and time of the surveys;
- Air and water temperature;
- Water depth and transparency by Secchi disk;
- Dissolved oxygen;
- pH and Eh in the water column by dedicated probe for water measures;
- pH and Eh in the surface sediments by dedicated probe for sediment measures;
- Water samples for salinity determination;
- Water samples for nutrient determination;
- Sediment samples for physico-chemical and nutrients determination;
- Macroalgal coverage, macroalgal biomass, taxa dominance, samples to determine the complete macroalgal check list.

Water samples (250-500 ml) were manually collected at a depth of approx. 20-30 cm and immediately filtered throughout glass fiber filters GF/F (porosity 0.7 μ m). Filters were retained in filter-holder boxes until the determination of Chlorophyll-a (Chl-a) and Phaeopigments (Pheo-a). Water samples of 250 ml were retained in polyethylene bottles for the determination of nutrients and transported in laboratory by a fridge bag. Both filters and water samples were refrigerated until the determination.

Surface sediment samples (5 cm top layer) were collected by a manual Plexiglas corer ($\varnothing=10\text{cm}$). They were carefully mixed together and pH and Eh were immediately measured on the total homogenized sample. Then two subsamples were retained, one for the analysis of nutrients and the other for the determination of the sediment characteristics. Sediment samples were transported by fridge bag to the laboratory where they were frozen and lyophilized for the determination of fines (fraction $<63\mu\text{m}$), density, moisture, porosity and the total, inorganic, organic phosphorus; total, inorganic, organic carbon and total nitrogen.

Measures in water column and surface sediments

Temperature

The determination of the temperature in the water column at a depth of approx. 30 cm was obtained by means of a thermocouple probe (precision 0.1°C) combined with a portable pH-meter model Delta Ohm HD8705.

Dissolved oxygen

The determination of dissolved oxygen (OD) at about 20-30 cm depth was carried out using an Oximeter (OXI 196) from Wissenschaftlich-Technische Werkstätten GmbH (Germany). The data expressed instrumentally in mg L^{-1} were then converted into saturation percentage (%OD) taking into account the temperature and salinity values. The instrument was calibrated before each series of measurements in its wet container.

pH determination

The determination of the pH (acidity or basicity or neutrality) in the water column was carried out using the Delta Ohm HD8705 portable pH-meter, equipped with a combined electrode (accuracy 0.01 pH units). The instrument was calibrated before each sampling campaign with a pH 7.0 solution.

Redox potential determination

The determination of the red-ox potential in the water and in the surface sediment was carried out by means of a Delta Ohm HD8705 portable pH-meter equipped with a combined Ag (AgCl) electrode (precision 1 mV). The measurement in water was carried out at a depth of 20-30 cm while in the sediment it was carried out on a sample of 3 sub-samples (5 cm top layer), carefully homogenized, collected by means of a Plexiglas corer ($\varnothing=10\text{cm}$). The measurement on the homogenized sample avoids the enormous variations that occur

depending on very small variations in the insertion of the electrode into the superficial sediment.

Salinity determination

Salinity was determined in laboratory as chlorinity by means of [Oxner \(1962\)](#) argentometric titration. The chlorinity values corrected with a standard solution of sea water of known chlorinity were converted into salinity by means of the relationship: $\text{Salinity} = \text{Cl}^- \times 1.805 + 0.03$.

Suspended solids (filtered particulate matter) determination

Samples of the water column (250-500 ml) were filtered, in duplicate, throughout glass fiber filters GF/F (0.7 μm) pre-dried at 105 ° C for 1 hour and weighed for the measurement of the total suspended solids (TSS). After filtration by a Millipore Swinnex manual apparatus the samples were washed with 2-3 aliquots of distilled water (20 ml) to remove the salts. Filters were placed in filter-holder boxes and refrigerated until the moment of the determination which took place by drying in an oven at 70 ° C for one night. The coefficient of variation as a measure of reproducibility of the analysis was kept below 5%.

Nutrients and chlorophylls in water

The filtered seawater was analyzed for nutrient concentrations following the methods described in [Strickland & Parsons \(1972\)](#) whereas chlorophylls and phaeopigments were determined according to the [Lorenzen \(1967\)](#) method.

Briefly, phytoplankton concentration was determined as Chl-a and Pheo-a by extraction with acetone 90% in ultrasonic bath for 30 min. Reactive phosphorus (RP) was measured as in [Murphy & Riley \(1962\)](#) and reactive silicate (Si) was quantified using the reaction of [Mullin & Riley \(1965\)](#). Ammonium was measured with the phenol-hypochlorite reaction of [Riley \(1953\)](#) modified by [Solarzano \(1969\)](#). The simultaneous determination of nitrite and nitrate concentrations was obtained using the cadmium reduction method as in [Wood et al. \(1967\)](#). The results were expressed as $\mu\text{g L}^{-1}$ for chlorophylls and as μM for nutrients.

Sediment grain-size determination

Sediments were sieved with a 1 mm mesh sieve to remove the coarser part mainly represented by shell residues. Then the fine fraction (Pelite) and the sands were separated with a 63 μm mesh sieve.

Sediment density

The sediment density was determined on wet and dry basis. In particular the values on dry basis (g/cm^3) allow to calculate the concentration of nutrients or pollutants per volume unit.

The density determination was performed in duplicate by using porcelain crucibles of known volume comparing the sediment weight before and after drying at $110\text{ }^\circ\text{C}$ for one night.

Sediment Moisture and Porosity

The same porcelain crucibles of known volume were also used to determine the sediment moisture (ml of water/ weight of wet sediment) and porosity (ml of water/ volume of wet). All data are reported as a percentage.

Elemental Analysis of C, N and P

In the laboratory, sediments were freeze-dried and pulverized using a sediment mill (Fritsch Pulverisette, Germany). The concentration of total nitrogen (TN) and total carbon (TC) were measured in duplicate by a CHNS Analyzer (Vario-MICRO, Elementar CHNS by Elementar Italia S.r.l.) after an accurate sample powdering of ca. 0.3 g of sample. The standard used for nitrogen determination was the “low level N- and S-contents” with $\text{N} = 0.74\%$, art. no. 05 000 959 (Elementar Italia S.r.l.) and the standard used for carbon determination was “C2”, with $\text{C} = 2.00\%$, art. no. S05 005 343 (Elementar Italia S.r.l.). Organic carbon was measured as carbon loss on ignition after ashing at $430\text{ }^\circ\text{C}$ for 2h taking into account the weight loss of the sample following the ashing.

Total phosphorus (TP) was determined following [Aspila et al. \(1976\)](#) after sample combustion in the muffle at $550\text{ }^\circ\text{C}$ for at least 2 h of 0.3–0.4 g of sample. Subsequently, the residue thus obtained was suspended in 50 mL of 1 N HCl and sonicated for ca. 30 min. After allowing the sample to settle for at least 1 h, 0.5 mL of the supernatant were taken with a graduated gaschromatographic syringe and brought to exactly 10 mL using volumetric flasks for a final dilution of 1 L, with the result expressed directly in μM . At this point, the phosphorus concentration was determined spectrophotometrically by the molybdenum blue method adding the mixed reagent and reading the absorbance at 885 nm after ca. 10–15 min according to [Murphy et Riley \(1962\)](#) and [Strickland et Parsons \(1972\)](#). Inorganic phosphorus (IP) was obtained with the same procedure used for TP but without combustion at $550\text{ }^\circ\text{C}$. Organic phosphorus (OP) was determined by difference.

All samples were analysed in duplicate and the analyses were replicated on two different days to obtain an accuracy > 95. Otherwise, the analyses were repeated until the coefficient of variation (standard deviation/mean) between two replicates was <5%. Carbon and nitrogen content were expressed as mg g⁻¹ and phosphorus as µg g⁻¹.

Macrophyte variables

Macrophyte coverage and taxa determination.

Macroalgae samples were collected in accordance with the method for the determination of the Macrophyte Quality Index (MaQI, ISPRA, 2011; Sfriso et al., 2014, **Figure 2**) in order to determine the ecological status as required by Water Framework Directive (2000/60/EC).

Macrophyte Quality Index (MaQI)						
Macroalgae	Macroalgae (score)			Ecological Quality Ratio (EQR)		
	Opportunistic 0	Indifferent 1	Sensitive 2			
Macroalgae	Every coverage ⁽¹⁾		≥25%	0.85		1
			15-25%	0.65	0.75	0.85
			≤15%	0.55	0.55	
	Total coverage ≤5%	2 species	0.45			
	Total coverage >5%	Rhodophyta dominance	≤2 species	0.35		
		Chlorophyta dominance	≤2 species	0.25		
	Total coverage ≤5%		1	0.15	0.65	0.85
Absent/Trace ⁽²⁾		0	0			
aquatic angiosperms	<i>Ruppia cirrhosa</i> , <i>R. maritima</i> , <i>Zostera noltii</i>		missing	<50% ⁽³⁾	50-75%	>75%
	<i>Zostera marina</i>			<25%	25-75%	>75%
	<i>Cymodocea nodosa</i>		missing	<25%	>25%	
	<i>Posidonia oceanica</i>		missing			Present
(1)	Per cent species number.					
(2)	The Xanthophyceae: <i>Vaucheria</i> spp. can be present with a coverage up to 100%. Seasonal growth of Rhodophyta and/or Chlorophyceae which are not able to bloom.					
(3)	Per cent species coverage					

Figure 2. MaQI Scheme

At each station, the relative coverage of macroalgae was assessed by using the Visual Census Technique in clear waters, or touching the bottom 20 times with a rake in turbid waters, in order to discriminate a coverage ≥5%, as required by the application of the index. Subsequently 5-6 macroalgal samples were collected reporting the percentage of

Chlorophyta and Rhodophyta. Representative samples of all the species present in the stations were stored in 4% formaldehyde for the taxonomic determination.

Results

At present we have one year of monthly samples both at Goro and Fattibello. The sampling period was between 29 March 2022 and 07 March 2023.



Figure 3. On the left lagoon of Goro with reeds indicating the transplant points of *Ruppia cirrhosa*. On the right trap for the collection of the particulate matter.



Figure 4. On the left, sampling operation on the lagoon of Fattibello. On the right, equipment and materials for sampling operations.

Currently, all the physico-chemical analyses of the water column and surface sediments and the macrophyte determination collected during the first year (**Figures 3, 4**) have been carried out.

Water Column

Figure 5 shows the variations of Temperature, Salinity, Oxygen Saturation, pH, Eh, Total Suspended Solids, Settled Particulate Matter, Silicates, Reactive Phosphorus and Total Dissolved Nitrogen (sum of ammonium, nitrite, nitrate) in the water column.

The mean water temperature at Fattibello (20.4 ± 8.6 °C) was slightly higher than at Goro (18.5 ± 8.5 °C) with the minimum value recorded at Goro (6.6 °C) and the highest (31.0 °C) at Fattibello. Salinity on average was higher at Goro (26.7 ± 3.6 psu) than at Fattibello (20.6 ± 7.4 psu) where it fluctuated in a higher range (5.0 - 29.5 psu) with the lowest value in November. The oxygen saturation was slightly higher at Fattibello ($210 \pm 69\%$) than at Goro (199 ± 63) with the minimum values in November and December. At Goro the values of pH (8.30 ± 0.37), on average, were higher than at Fattibello (8.10 ± 0.37), with the highest values in winter. Similarly, the redox potential (Eh) was higher at Goro (291 ± 56 mV) than at Fattibello (264 ± 100 mV) where in July values close to 0 mV were recorded.

At Goro the mean amount of Total Suspended Solids (TSS) was quite twice higher (40.2 ± 11.4 mg L⁻¹) than at Fattibello (20.6 ± 7.4 mg L⁻¹) whereas the particulate collected by sedimentation traps at Goro was slightly lower (199 ± 63 mg L⁻¹ at Goro; 210 ± 69 mg L⁻¹ at Fattibello).

Silicates at Fattibello (33.8 ± 27.7 μM) were significantly higher than at Goro (23.8 ± 12.4 μM). The mean concentrations of the Reactive Phosphorus (RP) in the two lagoons was a bit lower at Goro (0.36 ± 0.22 μM) than at Fattibello (0.43 ± 0.23) with the highest values recorded in June and July. Conversely, the Dissolved Inorganic Nitrogen (DIN) monitored at Fattibello (35.8 ± 52.7 μM) was on average almost 4 times higher than at Goro (9.45 ± 8.36 μM). In addition, at Fattibello in winter values up to 154.5 μM were found.

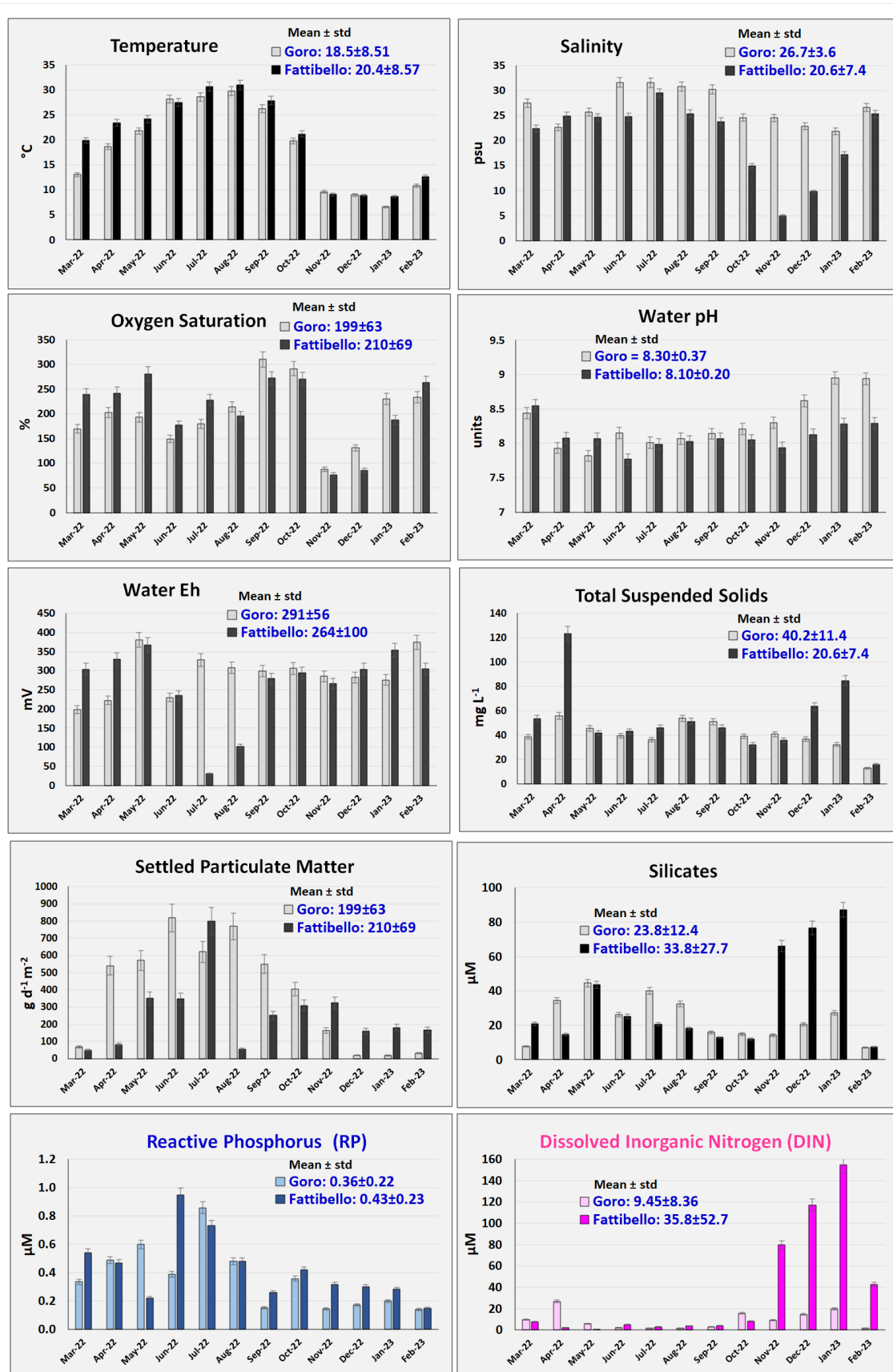


Figure 5. Variation of some environmental parameters in the Water Column in the Goro and Fattibello stations.

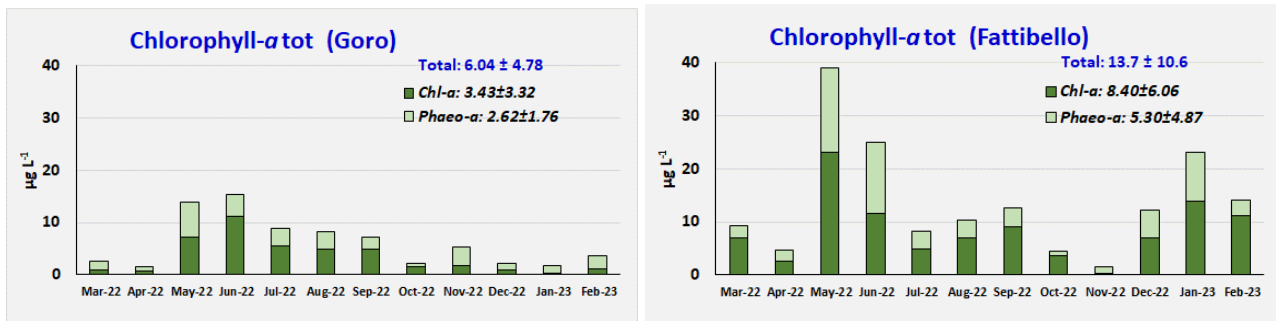


Figure 6. Variation of the total Chlorophyll-a at Goro and Fattibello.

If we consider the water transparency this mainly depends on the amount of TSS (**Figure 5**) and the concentration of Chlorophyll-a (Chl-a) (**Figure 6**). Both these parameters were twice higher at Fattibello than at Goro. As above reported, the mean TSS ranged from 20.6 mg L⁻¹ at Goro to 40.2 mg L⁻¹ at Fattibello where in April a peak of 123 mg L⁻¹ was recorded. Similarly, the mean total Chl-a was 6.04±4.78 µg L⁻¹ at Goro and 13.7±10.6 µg L⁻¹ at Fattibello where a first peak was recorded in May-June (up to 38.9 µg L⁻¹) and a second peak in January-February. In both stations the concentration of the active pigment (Chl-a) was greater than the degraded one (Phaeo-a). The high values of TSS and Chl-a recorded mostly at Fattibello, with a water transparency of approx. 30-40 cm, are prohibitive both for the presence of macroalgae and the rooting of aquatic phanerogams.

Surface sediments

Some physico-chemical characteristics of the 5 cm sediment top layer in the two stations are reported in **Figure 7**.

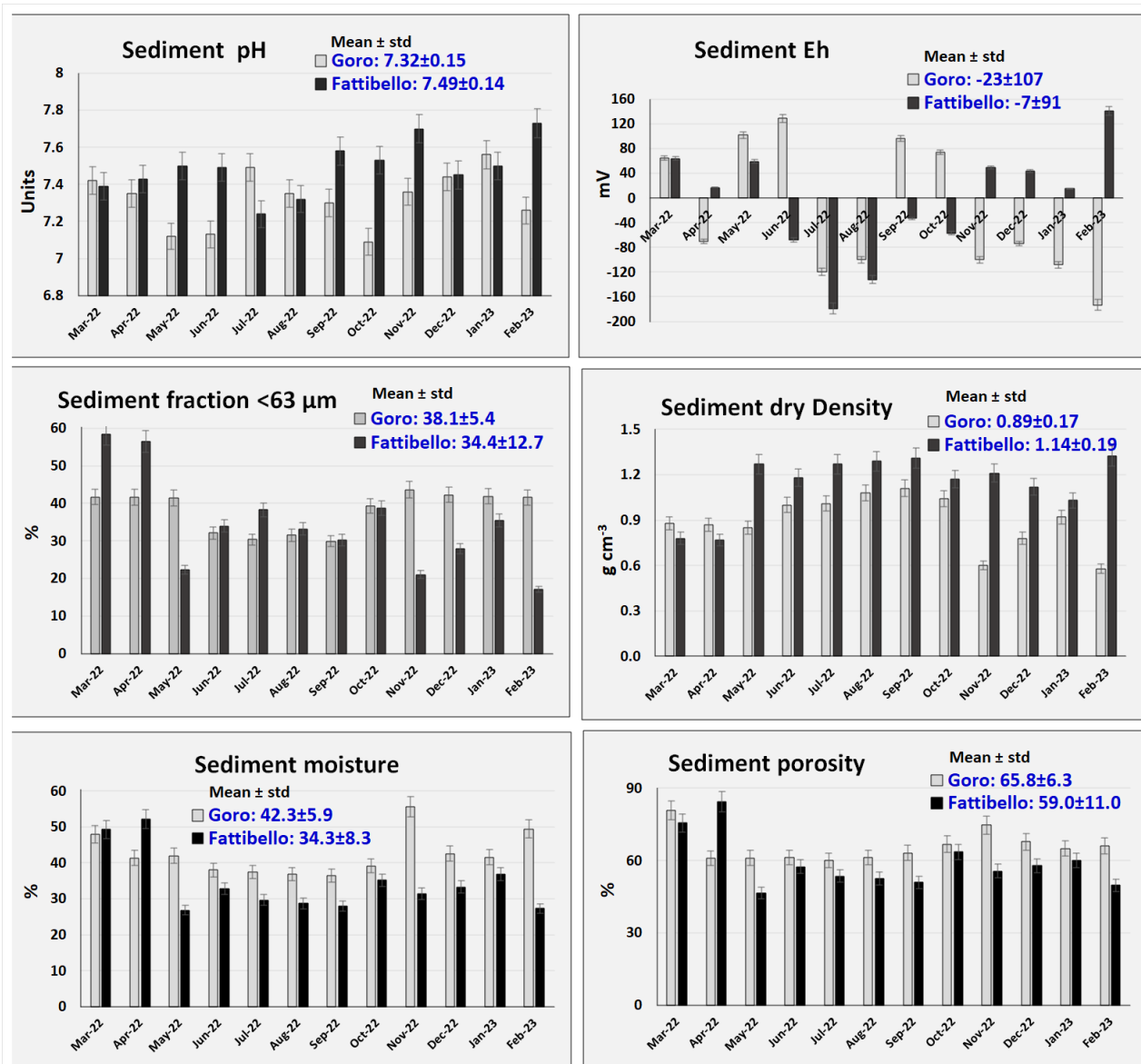


Figure 7. Variation of some physico-chemical characteristics of surface sediments

The pH of surface sediments in the two lagoons was slightly lower at Goro (7.32 ± 0.15 units) than at Fattibello (7.49 ± 0.14 units). Similarly, the value of Eh was lower at Goro (-23 ± 107 mV) than at Fattibello (-7 ± 91 mV). The amount of Fines (fraction $<63 \mu\text{m}$) in the two stations was quite similar ranging from $34.4 \pm 12.7\%$ at Fattibello to $38.1 \pm 5.4\%$ at Goro. The highest amount of Fines was coupled with the lower dry density recorded at Goro (0.89 ± 0.17 g DW cm^{-3}) in comparison to Fattibello (1.14 ± 0.19 g DW cm^{-3}). Instead, higher values of moisture and porosity were recorded at Goro ($42.3 \pm 5.9\%$ and $65.8 \pm 8.3\%$, respectively).

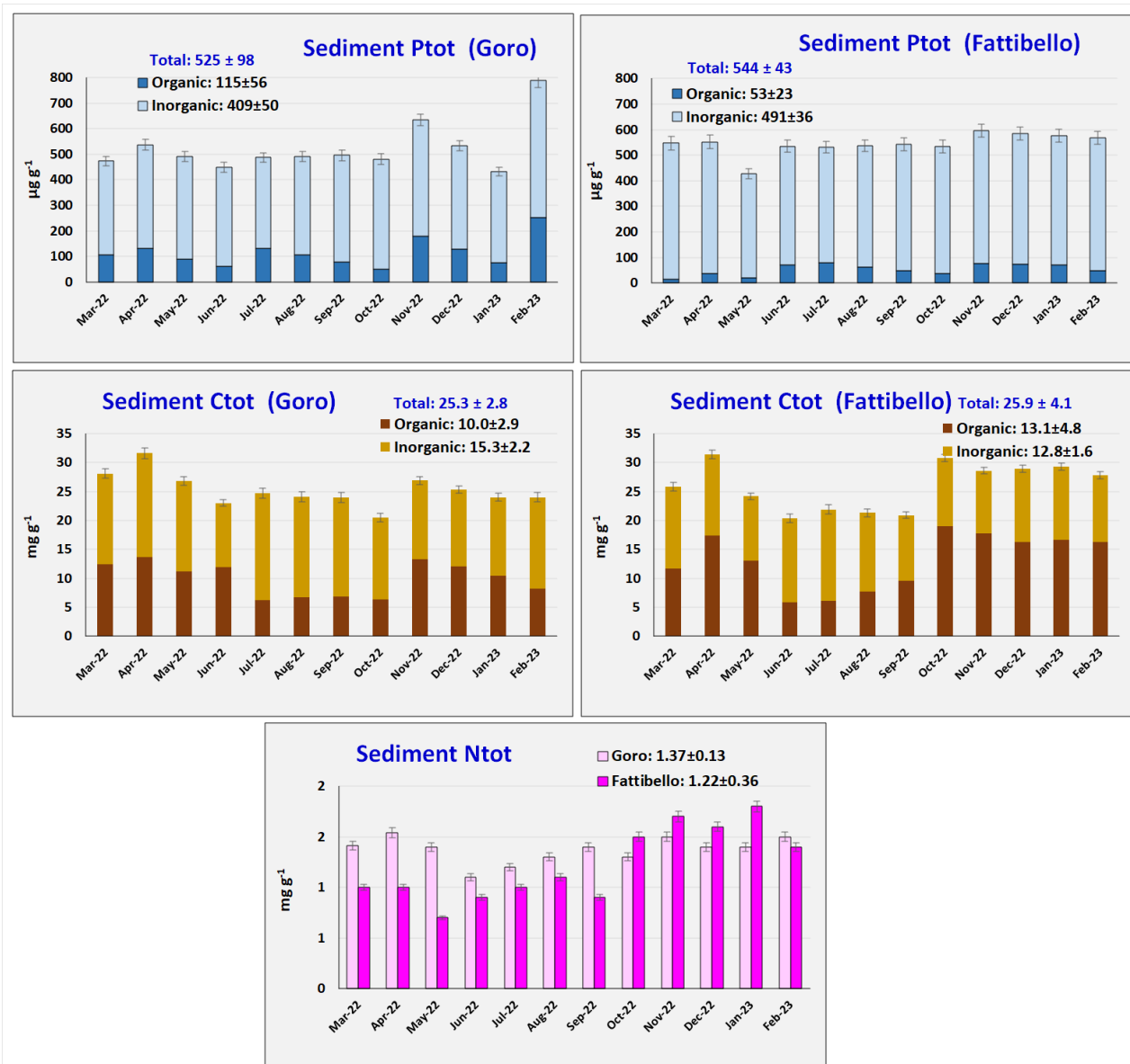


Figure 8. Variation of nutrient concentrations in surface sediments.

Regarding the trophic status, the nutrient and carbon concentrations were quite similar (**Figure 8**). The Total Phosphorus ranged from $525 \pm 98 \mu\text{g g}^{-1}$ at Goro to $544 \pm 43 \mu\text{g g}^{-1}$ at Fattibello. However, the concentration of the organic fraction (Porg) at Goro was twice higher ($115 \pm 56 \mu\text{g g}^{-1}$) than at Fattibello ($53 \pm 23 \mu\text{g g}^{-1}$). Total Nitrogen (Ntot) ranged from 1.37 ± 0.13 to $1.22 \pm 0.36 \text{ mg g}^{-1}$ at Goro and Fattibello, respectively. The concentrations of Total Carbon (Ctot) was basically the same (25.3 and 25.9) with minor differences between the organic and inorganic fractions.

Macrophytes

Macroalgae and MaQI determination

The quantitative and qualitative consistency of macroalgae present in the two stations is reported in **Figure 9**.

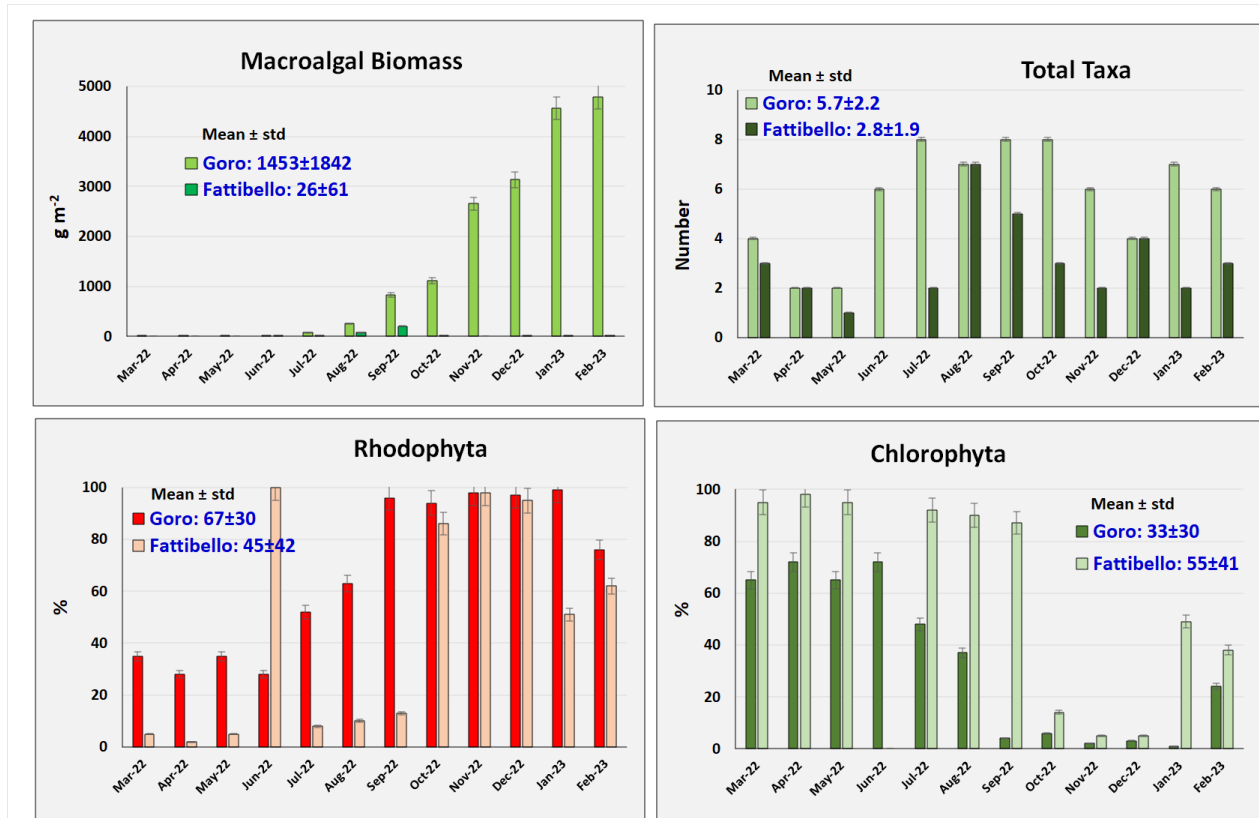


Figure 9. Variation of macroalgae in the two stations.

The mean biomass on fresh weight basis was very high at Goro ($1453 \pm 1842 \text{ g m}^{-2}$) and negligible at Fattibello where the main producer was phytoplankton (**Figure 6**). At Goro the highest values (up to 4787 g m^{-2}) were recorded in the cold seasons whereas at Fattibello it was zero or close to zero most to the year with a maximum in July (79 g m^{-2}). In both the lagoons the dominant species were the Rhodophyceae: *Gracilariopsis vermiculophylla* and *Gracilariopsis longissima* and the Chlorophyceae *Ulva australis* species that characterize degraded environments. No taxa of high ecological value were recorded.

On the basis of the recorded species the ecological conditions obtained by the application of the index MaQI were Poor for Goro (**Table 3**) and Bad for Fattibello (**Table 4**).

Table 3. Macrophytes sampled at Goro.

Taxonomic list first year														
Goro 2022-2023														
N°	N°	Chlorophyceae	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dic	Gen	Feb
1	1	<i>Blidingia dowsonii</i> (Hollenberg & I.A. Abbott) S.C. Lindstrom, L.A. Hanic & L. Golden	X			X	X	X	X	X			X	X
2	2	<i>Cladophora glomerata</i> (Linnaeus) Kützing					X			X				
3	3	<i>Cladophora lehmanniana</i> (Lindenberg) Kützing						X						
4	4	<i>Ulva australis</i> Areschoug	X	X	X	X	X	X	X	X	X			X
5	5	<i>Ulva rigida</i> C. Agardh										X	X	
6	6	<i>Ulva intestinalis</i> Linnaeus								X				
7	7	<i>Ulva polyclada</i> Kraft					X		X					
8	8	<i>Uronema marinum</i> Womersley				X	X		X	X	X		X	
Rhodophyceae														
9	1	<i>Agardhiella subulata</i> (C. Agardh) Kraft et M. J. Wynne					X	X	X	X	X		X	X
10	2	<i>Hypnea cervicornis</i> J Agardh								X	X	X		
11	3	<i>Gracilaria longissima</i> (S. G. Gmelin) Steentoft et al.				X	X	X	X		X	X	X	X
12	4	<i>Gracilaria vermiculophylla</i> Ohmi	X	X	X	X	X	X	X		X	X	X	X
13	5	<i>Melanthamnus harveyi</i> (Bailey) Diaz-Tapia & Maggs				X								
14	6	<i>Polysiphonia morrowii</i> Harvey	X											
15	7	<i>Sahlbergia subintegra</i> (Rosenvinge) Kornmann								X				
16	8	<i>Solieria filiformis</i> (Kützing) P. W. Gabrielson						X	X				X	X
Phaeophyceae														
Total Taxa			4	2	2	6	8	7	8	8	6	4	7	6
Chlorophyceae N°			2	1	1	3	5	3	4	5	2	1	3	2
Rhodophyceae N°			2	1	1	3	3	4	4	3	4	3	4	4
Phaeophyceae N°			0	0	0	0	0	0	0	0	0	0	0	0
Cover %			5	10	0.01	5	25	30	75	60	60	70	80	100
Biomassa g/m ²			1.6	3.4	0.4	1.8	83	254	831	1114	2653	3138	4565	4787
Rhodophyceae%			65	72	65	72	48	24	37	6	2	3	1	24
Chlorophyceae%			35	28	35	28	52	63	4	94	98	97	99	76
Chlorophyceae abundance%			3.3	7.2	0.007	3.6	12	7.2	27.8	3.6	1.2	2.1	0.8	24.0
Rhodophyceae abundance%			1.8	2.8	0.004	1.4	13	18.9	3.0	56.4	58.8	67.9	79.2	76.0
MaQI			0.25	0.25	0.25	0.35	0.25	0.25	0.25	0.35	0.35	0.35	0.35	0.35
Poor														

Table 4. Macrophytes sampled at Fattibello.

Taxonomic list first year														
Fattibello 2022-23														
N°	N°	Chlorophyceae	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
1	1	<i>Blidingia dowsonii</i> (Hollenberg & I.A. Abbott) S.C.Lindstrom, L.A.Hanic & L.Golden	X					X	X			X		
2	2	<i>Blidingia ramifera</i> (Bliding) Garbary & L.B. Barkhouse												X
3	3	<i>Cladophora albida</i> (Nees) Kützing						X						
4	4	<i>Cladophora glomerata</i> (Linnaeus) Kützing						X	X					
5	5	<i>Cladophora sericea</i> (Hudson) Kützing								X		X	X	
6	6	<i>Rhizoclonium</i> sp.						X						
7	7	<i>Ulva australis</i> Areschoug	X	X	X		X	X	X	X	X	X		X
8	8	<i>Ulva compressa</i> Linnaeus	X	X										
9	9	<i>Ulva flexuosa</i> Wulfen							X	X				
10	10	<i>Ulva linza</i> Linnaeus						X						
11	11	<i>Ulva polyclada</i> Kraft					X		X					
12	12	<i>Ulva prolifera</i> O. F. Müller						X			X	X	X	
13	13	<i>Uronema marinum</i> Womersley												X
Rhodophyceae														
14	14	<i>Agardhiella subulata</i> (C. Agardh) Kraft et M. J. Wynne						X	X	X				
15	15	<i>Bostrychia scorpioides</i> (Hudson) Montagne	X											
16	16	<i>Catenella caespitosa</i> (Withering) L. M. Irvine	X											
17	17	<i>Dasya baillouviana</i> (S. G. Gmelin) Montagne	X			X			X					
18	18	<i>Gracilaria longissima</i> (S. G. Gmelin) Steentoft et al.					X	X						
19	19	<i>Gracilaria vermiculophylla</i> Ohmi		X			X	X	X	X	X	X	X	X
20	20	<i>Melanothamnus harveyi</i> (Bailey) Diaz-Tapia & Maggs					X			X				X
21	21	<i>Polysiphonia morrowii</i> Harvey						X			X		X	
22	22	<i>Solieria filiformis</i> (Kützing) P. W. Gabrielson						X	X			X	X	
Phaeophyceae														
23	1	<i>Kuckuckia spinosa</i> (Kützing) Kornmann	X	X										
24	1	<i>Myrionema</i> sp.	X	X										
Total Taxa			8	5	1	1	5	12	9	6	4	6	5	5
Chlorophyceae N°			3	2	1	0	2	7	5	3	2	4	2	3
Rhodophyceae N°			3	1	0	1	3	5	4	3	2	2	3	2
Phaeophyceae N°			2	2	0	0	0	0	0	0	0	0	0	0
Cover %			0.05	0.05	0.05	0.05	30	0.05	0.05	70	0.05	0.1	0.1	0.05
Biomassa g/m ²			0	0	0	0	19.2	0	1.2	207	17	4	13	1.3
Chlorophyceae %			0	98	100	0	92	14	7	87	5	16	49	38
Rhodophyceae %			0	2	0	100	8	86	93	13	95	84	51	62
Chlorophyceae abundance %			0	0.049	0.05	0	27.6	0.007	0.004	61	0.003	0.02	0.05	0.02
Rhodophyceae abundance %			0	0.001	0	0.05	2.4	0.043	0.047	9	0.05	0.08	0.05	0.03
MaQI			0	0	0	0	0.25	0	0	0.25	0	0	0	0
Bad														

Statistical analyses

The relationship between environmental parameters of the water column and surface sediments and macrophyte variables was determined by the application of the Principal Component Analysis (PCA) which showed a variance of 74.5% with 5 components. The combination of the first two components is plotted in **Figure 10**.

Two main groups of parameters/variables were detected. One with Chl-a, Phaeo-a and TSS responsible of water turbidity which were associated with the nitrogen species (ammonium, nitrite, nitrate), silicate, Ehs, Corg and Pinorg. The other with the algal biomass and cover which were associated with Salinity, some sediment characteristics (Fines, Moisture, Porosity), pH of the water column (pHw), Porg and Cinorg. Among the macroalgae Chlorophyceae were associated with total nitrogen (Ntot) and the redox potential of the water column (Ehw), whereas Rhodophyceae with reactive phosphorus (RP), the sediment collected by sedimentation traps (SPM) and water temperature (Temp)Temperature.

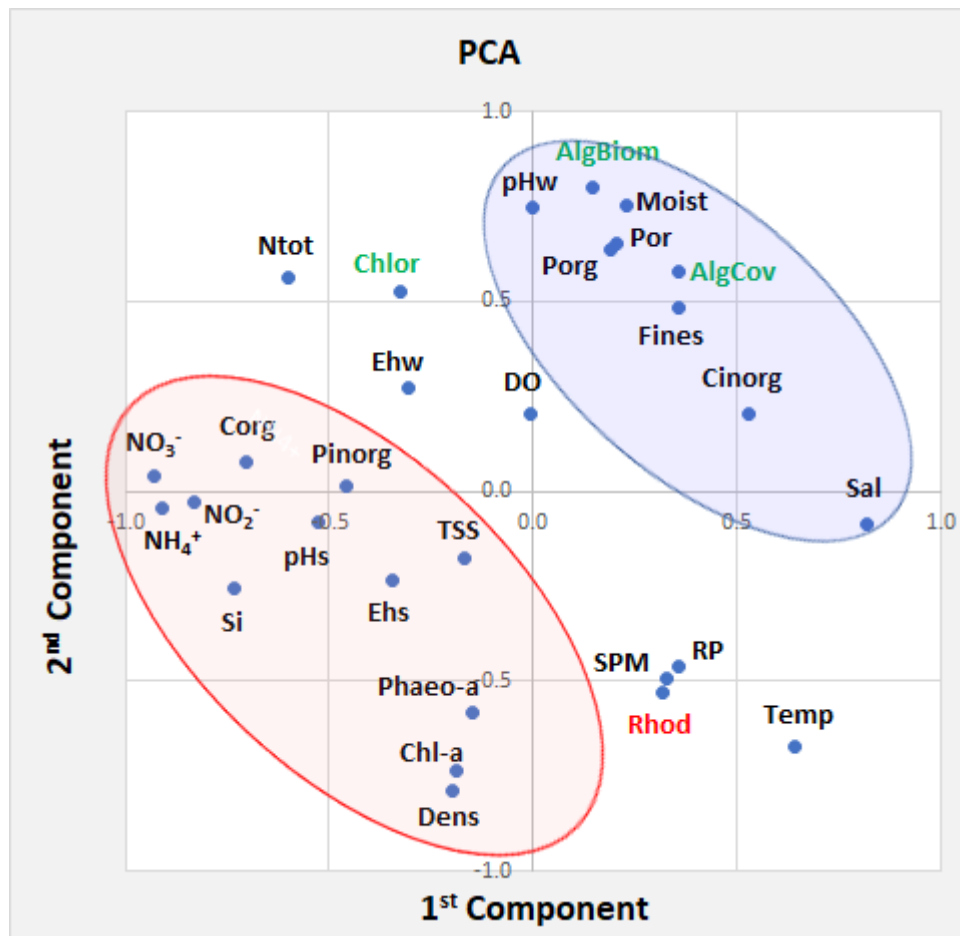


Figure 10. PCA analysis between environmental parameters and macrophyte variables recorded at Goro and Fattibello.

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SECTION 2 – MACROBENTHOS AND EQ

The macrobenthic community analysis (composition, structure and dynamics) is the best approach for assessing the ecological status for a given water body. The DL 260 / 10 explicitly requests the analysis of the "macrobenthos" as biological quality element for the definition of the ecological status of transition and marine-coastal waters. The monitoring of the macrobenthic community on a spatio-temporal scale enables the evaluation of the effectiveness of activities carried out for environmental improvement and it provides an adequate tool for a rigorous assessment of the quality of the environmental conditions.

The macrobenthos monitoring actions are carried out at 2 sites of the Natura 2000 Network, characterized by the presence of the priority habitat 1150 - coastal lagoons, and affected by the conservation activity of this habitat by transplanting marine phanerogams; at each of the 4 sites (Sacca di Goro, Valle Fattibello), the monitoring action is carried out at 2 stations plus the new station in Bassunsin (Goro lagoon) (with 5 replicates per station to infer the intra-site variability).

At each station, macrobenthos is sampled following a BACI (Before-After, Control-Impact) design, that is sampling in a control site and in a site subjected to impact (specifically the transplant operation), before and after impact (intervention) (**Figure 1**).

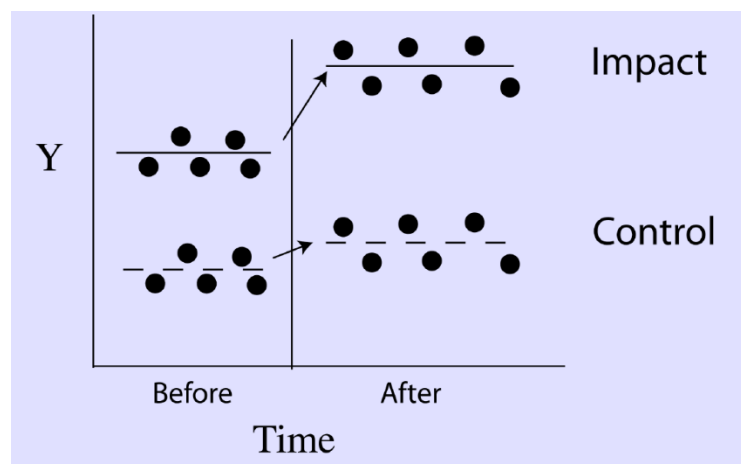


Fig. 1. Before-after-control-impact (BACI) design

At each station 5 replicates of samples are collected in order to achieve for each site (i.e. lagoon) 5 Control replicates, i.e. in areas not directly interested by the phanerogam

transplant; 5 Impact replicates, i.e. in areas directly interested by the phanerogam transplant.

For the assessment of environmental quality, an indicator specifically required by Legislative Decree 260 / 2010 for transitional waters is used, the M-AMBI index (Muxika et al., 2007).

Sampling of macrobenthos was carried out in October 2022 and May 2023. Samples from May 2023 are under analyses, so this report shows data for October 2022.

Structural indices describing the macrobenthic community were calculated on species/abundance data at each replicate of each station.

The ecological quality at each station has been assessed by applying the macrobenthic index M-AMBI (Muxika et al., 2007) on the species/abundance data set. The M-AMBI index is based on a multivariate analysis in which factor analysis combines the values of AMBI, with those of Shannon-Wiener diversity (H') and number of species (S). The M-AMBI is calculated by means a user-friendly software (www.azti.es) to be applied with the latest update of the species list already available.

The index is based on the classification of macrobenthic species into 5 ecological groups (EG) which correspond to different levels of disturbance-sensitivity (Borja et al., 2000). The EGI group includes the most sensitive species; following a tolerance gradient we arrive at the EGV group, which includes strongly opportunistic species, characteristic of heavily polluted environments. The AMBI index is calculated as:

$$AMBI = [(0*\%EGI)+(1,5*\%EGII)+(3*\%EGIII)+(4,5*\%EGIV)+(6*\%EGV)]/100$$

The ecological value (EG) of benthic taxa is reported in the AMBI library. If some species are not assigned an ecological value, as such species are not present in the AMBI library, the accuracy of the result may be compromised if: a) the percentage of unassigned taxa is > 20%, b) the taxa not belonging to some groups have a large number of individuals. Thanks to the calculation method, the M-AMBI Index is able to summarize the complexity of soft bottom communities, enabling the ecological reading of the ecosystem in question. M-AMBI corrects the quality values provided by AMBI through the integration of diversity and specific richness. M-AMBI is an extremely flexible tool for the derivation of the EQR, as it requires the operator to enter the limit values (equivalent to the reference values) for H', S and AMBI. If this step is omitted, the reference values for the "High" quality class are taken as the

highest values of S and H '(and lowest of AMBI) present within the numeric matrix for which the operator is running the calculation. This omission leads to extreme errors in the evaluation of the EQ (ecological quality).

The value of the M-AMBI varies between 0 and 1. Below (**Figure 2**) are reported (i) the type-specific reference values for each metric that makes up the M-AMBI, the M-AMBI class limits, expressed in terms of the ecological quality ratio (RQE), between the High status and the Good status, and between the Good status and the Moderate status as required by current legislation. The values of the reference conditions and the relative Good / Moderate and High / Good limits considered for the calculation are those relating to macrotypes 1 and 2 (M-AT-1, M-AT-2), to which Fattibello and the Sacca di Goro respectively belong.

Valori di riferimento e limiti di classe

Tab. 4.4.1/c – Limiti di classe in termini di RQE per l'M-AMBI

<i>Rapporto di Qualità Ecologica</i>			
<i>Elevato/Buono</i>	<i>Buono/Sufficiente</i>	<i>Sufficiente/Scarso</i>	<i>Scarso/Cattivo</i>
0,96	0,71	0,57	0,46

Le condizioni di riferimento sono state definite sulla base di un criterio misto statistico/geografico. L'indice M-AMBI è un indice multivariato, pertanto le condizioni di riferimento vanno indicate per i tre indici che lo compongono: AMBI, Indice di Diversità di Shannon-Wiener e numero di specie (S).

Tab. 4.4.1/d - Valori di riferimento tipo-specifiche per l'applicazione dell'M-AMBI

Macrotipo	Geomorfologia	Escursione marea	Salinità	AMBI	Diversità di Shannon-Wiener	Numero di Specie (S)
M-AT-1	Laguna costiera	Non tidale	-	1,85	3,3	25
M-AT-2	Laguna costiera	microtidale	Oligo/meso/poli	2,14	3,40	28
M-AT-3	Laguna costiera	microtidale	Eu/iper	0,63	4,23	46

Fig. 2. Reference values for the M-AMBI calculation (from the DL260/10).

The BITS index (Mistri & Munari, 2008) was also applied. BITS is written:

$$\text{BITS} = \log[(6fl + fll) / (fll + 1) + 1] + \log[nll / (nll + 1) + nll / (nll + 1) + 0,5nll / (nll + 1) + 1]$$

where *fl* is the sensitive families frequency (ratio of the total number of individuals belonging to sensitive families to the total number of individuals in the sample), *fll* is the tolerant families

frequency (ratio of the total number of individuals belonging to tolerant families to the total number of individuals in the sample), and f_{III} is the opportunistic families frequency (ratio of the total number of individuals belonging to opportunistic families to the total number of individuals in the sample). The +1 terms in the equation are needed in order to allow the division operation to be completed even when f_{III} is null, and to prevent the eventuality of a log of zero if f_I and f_{II} are null. The second term of the BITS model allows to weight the number of sensitive families respect the tolerant and the opportunistic ones: n_I is the number of sensitive families, n_{II} is the number of tolerant and n_{III} is the number of opportunistic families. Again, the +1 terms in the equation are needed in order to allow the division operation to be completed. The BITS index is null when there are no sensitive and tolerant families, indicating a very high amount of organic matter in the sediments, and, in lagoonal ecosystems, a very poor water exchange. BITS is high when the environment is good, with few opportunistic families, and it decreases as the environment degrades.

The following scheme (from DL260/10) reports the BITS class limits, expressed in terms of the ecological quality ratio (RQE), between the High status and the Good status, and between the Good status and the Moderate status as required by current legislation, and the type-specific reference values for BITS.

Limiti di classe in termini di RQE per il BITS			
Elevato/Buono	Buono/Sufficiente	Sufficiente/Scarso	Scarso/Cattivo
0,87	0,68	0,44	0,25

Valori di riferimento tipo-specifiche per l'applicazione del BITS	
M-AT-1	2,8
M-AT-2	3,4
M-AT-3	3,4

Sacca di Goro

A total of 29 macrobenthic taxa were found in the Sacca di Goro. **Table 1** shows the faunal list of the macrobenthic taxa collected during the ex-ante monitoring campaign.

<i>Polydora cornuta</i>	<i>Caprella scaura</i>
<i>Streblospio shrubsolii</i>	<i>Monocorophium acherusicum</i>
<i>Streblospio eridani</i>	<i>Cyathura carinata</i>
<i>Hediste diversicolor</i>	<i>Idotea balthica</i>
<i>Caulleriella alata</i>	<i>Gammarus aequicauda</i>
<i>Serpula concharum</i>	<i>Melita palmata</i>
<i>Ficopomatus enigmaticus</i>	<i>Grandidierella japonica</i>
<i>Hydroides nigra</i>	<i>Microdeutopus gryllotalpa</i>
<i>Pileolaria militaris</i>	<i>Monocorophium insidiosum</i>
<i>Hydroides dianthus</i>	<i>Actiniaria ind.</i>
<i>Hydroides elegans</i>	<i>Cerastoderma glaucum</i>
<i>Alitta succinea</i>	<i>Lentidium mediterraneum</i>
<i>Capitella minima</i>	<i>Arcuatula senhousia</i>
<i>Prionospio cirrifera</i>	<i>Platyhelminthes ind.</i>
<i>Corophium orientale</i>	

Tab. 1. Taxonomic list of the macrobenthic taxa identified in the Sacca di Goro

In **Table 2** the values of community descriptors (diversity and species richness), together with AMBI/M-AMBI values and ES are reported. In the Table also the reference parameters are shown (in grey).

Stations	AMBI	H'	S	M-AMBI	Status
Bad	6	0	0	0	Bad
High	2,14	3,40	28	1,00	High
CTR	4,24	1,41	14	0,46	Bad
SEG	3,13	1,47	7	0,48	Poor
BAS	2,63	3,11	21	0,85	Good

Tab. 2. Community parameters and AMBI/M-AMBI values in the Sacca di Goro (CTR. Control; SEG: Seganda; BAS: Bassunsin)

The ecological quality at Goro is also summarized in **Figure 3**. It is evident how the ecological quality is much better at transplant site BAS respect to SEG and also to the control site.

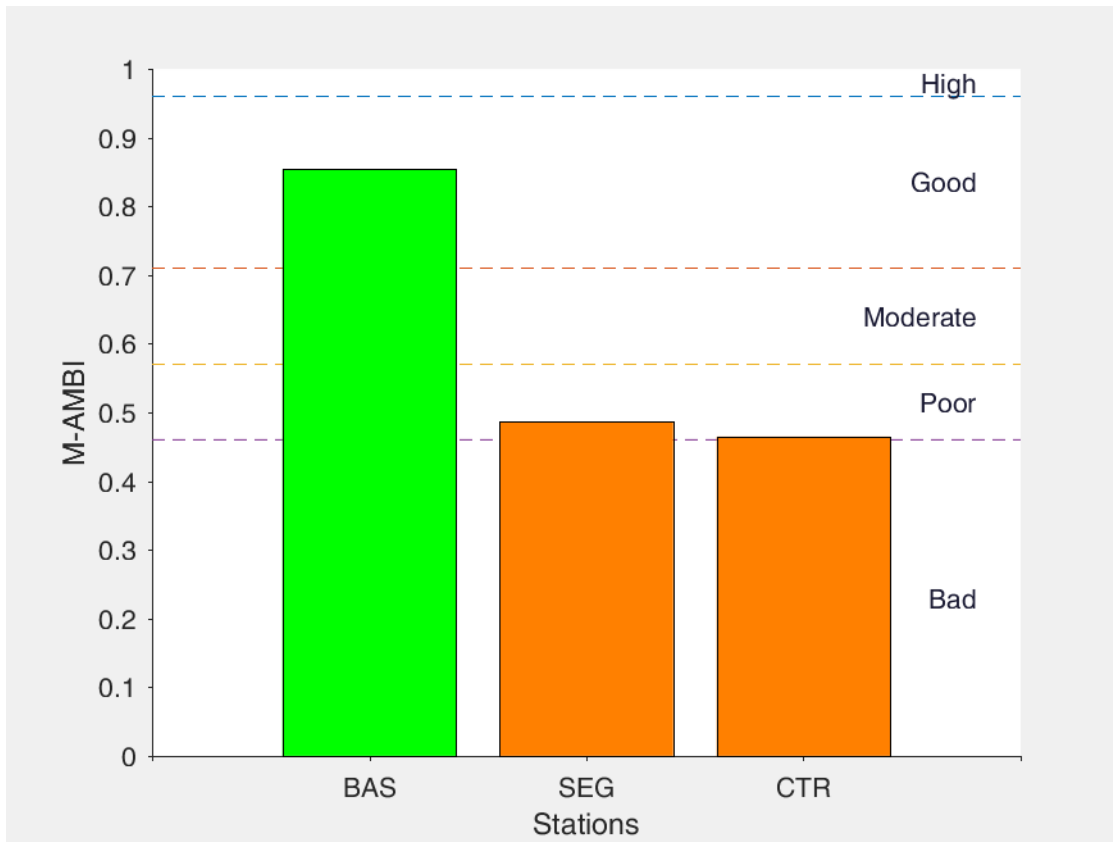


Fig. 3. Ecological quality through M-AMBI at Goro.

Table 3 shows the percentage of invertebrates belonging to the five sensitivity-tolerance groups: EG-I sensitive, EG-II indifferent, EG-III tolerant, EG-IV and EG-V opportunistic of second and first order respectively; the higher the percentage of EG-I (or the lower the percentage of EG-IV and EG-V), the better the ecological quality.

Stations	I(%)	II(%)	III(%)	IV(%)	V(%)
CTR	0	0,9	14,6	84,3	0,1
BAS	4,6	16,2	74,9	4	0,2
SEG	0	0	91,7	8	0,4

Tab. 3. Sensitivity-tolerance distribution of macrobenthos in the Sacca di Goro (CTR. Control; BAS: Bassunsin; SEG: Seganda).

Figure 4 shows the distribution of EGs at the two transplant sites and control site.

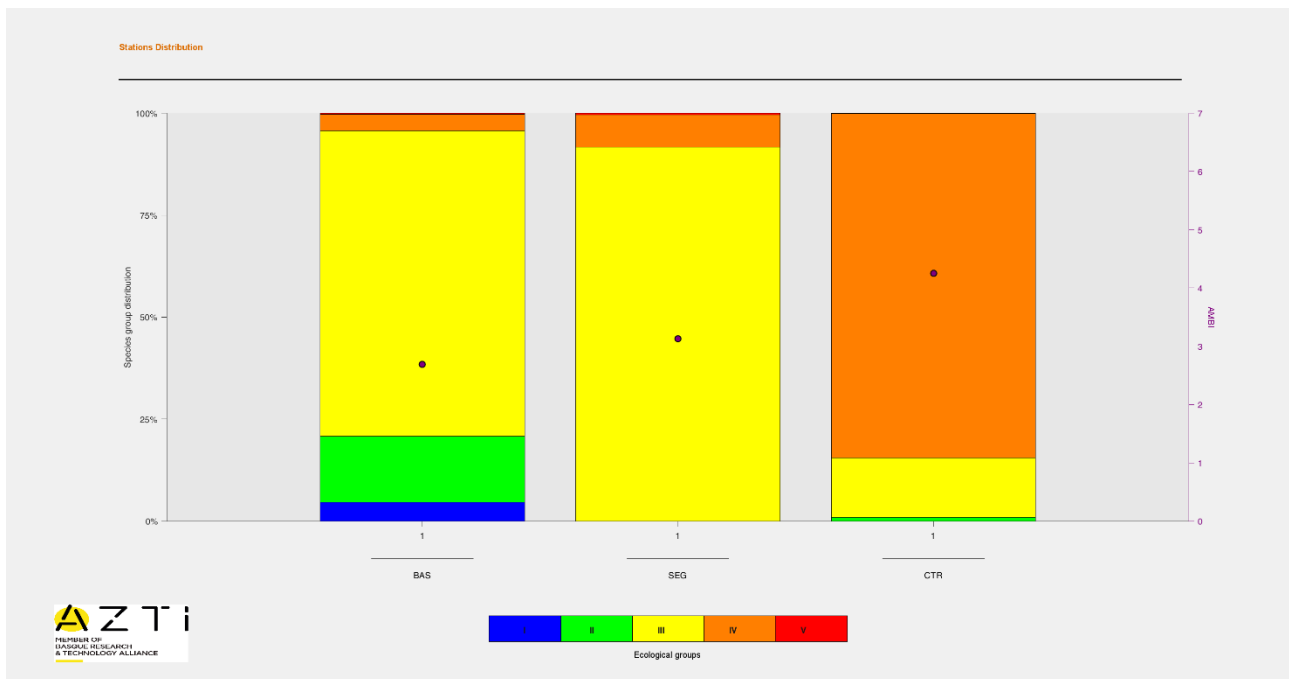


Fig. 4. Distribution of EGs at Goro

The majority of the taxa present at the two transplant sites belongs to the “tolerant” group (EG-III), while at the control site to the opportunist group (EG-IV). At BAS, however, are present also sensitive (EG-I) and indifferent (EG-II) species. It is also evident how, in the transplant sites, the benthic community is better structured, in terms of ecological groups, than in the control site.

The application of the BITS index gave a higher value at the Bassunsin transplant site, where the ecological status was High. Both transplant sites exhibited, however, a better ecological status than the control (i.e. not transplanted) site.

	BAS	SEG	CTR
<i>BITS</i>	3,13	1,78	0,84
<i>EQR</i>	0,92	0,52	0,25
<i>Status</i>	High	Moderate	Poor

Valle Fattibello

Overall, 20 macrobenthic taxa were found in the Valle Fattibello. **Table 4** shows the faunal list of the macrobenthic taxa collected during the monitoring campaign.

<i>Polydora cornuta</i>	<i>Caprella scaura</i>
<i>Streblospio shrubsolii</i>	<i>Monocorophium acherusicum</i>
<i>Prionospio lighti</i>	<i>Cyathura carinata</i>
<i>Hediste diversicolor</i>	<i>Grandidierella japonica</i>
<i>Caulleriella alata</i>	<i>Microdeutopus gryllotalpa</i>
<i>Alitta succinea</i>	<i>Monocorophium insidiosum</i>
<i>Capitella capitata</i>	<i>Lentidium mediterraneum</i>
<i>Capitella minima</i>	<i>Arcuatula senhousia</i>
<i>Prionospio cirrifera</i>	<i>Ceratoderma glaucum</i>
<i>Corophium orientale</i>	<i>Platyhelminthes sp.</i>

Tab. 4. Taxonomic list of the macrobenthic taxa identified in the Valle Fattibello

In **Table 5** the values of community descriptors (diversity and species richness), together with AMBI/M-AMBI values and ES are reported. In the Table also the reference parameters are shown (in grey).

<i>Stations</i>	<i>AMBI</i>	<i>Diversity</i>	<i>Richness</i>	<i>M-AMBI</i>	<i>Status</i>
Bad	6	0	0	0	Bad
High	1,85	3,30	25	1	High
FAT	3,64	2,12	16	0,62	Moderate
CTR	3,64	2,29	17	0,65	Moderate

Tab. 5. Community parameters and AMBI/M-AMBI values at Fattibello

The ecological quality at Fattibello is also summarized in **Figure 5**. There is no difference between the ecological quality value at transplant and control sites.

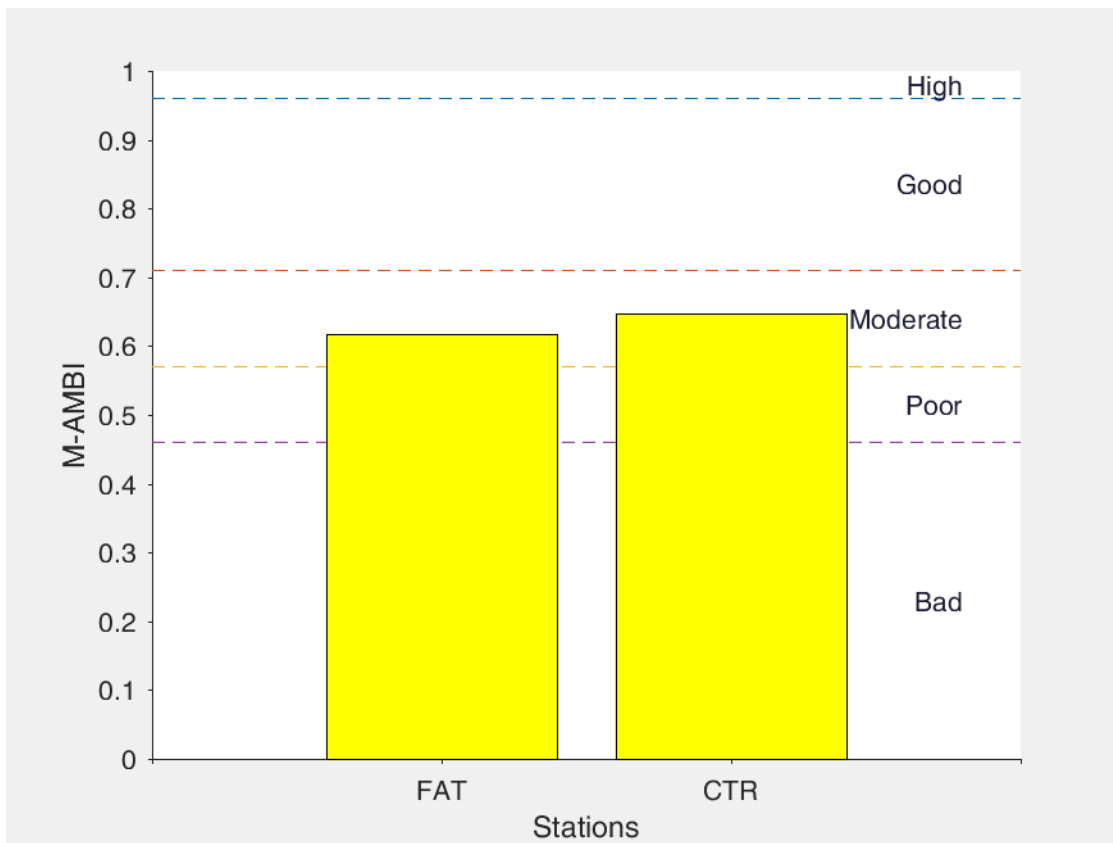


Fig. 5. Ecological quality through M-AMBI at Fattibello.

Table 6 shows the percentage of invertebrates belonging to the five sensitivity-tolerance groups: EG-I sensitive, EG-II indifferent, EG-III tolerant, EG-IV and EG-V opportunistic of second and first order respectively; the higher the percentage of EG-I (or the lower the percentage of EG-IV and EG-V), the better the ecological quality.

Stations	I(%)	II(%)	III(%)	IV(%)	V(%)
FAT	0	7,8	42,7	48,5	0,9
CTR	0	7,7	43,8	46,5	1,9

Tab. 6. Sensitivity-tolerance distribution of macrobenthos at Fattibello

Figure 6 shows the distribution of EGs at the transplant site.

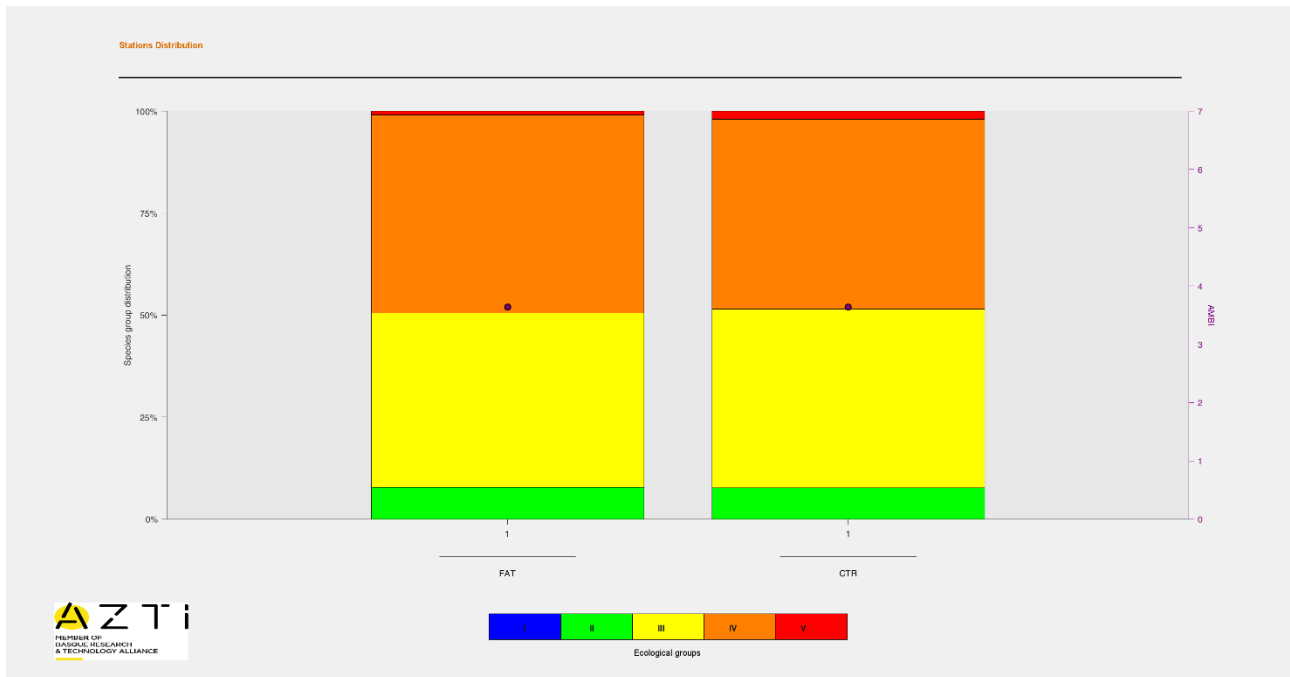


Fig. 6. Distribution of EGs at Fattibello

The majority of taxa present at the two sites belongs to the “tolerant” (EG-III) and opportunistic (EG-IV) group, and the composition of the two communities is almost identical.

The application of the BITS index gave the same value at the transplant (FAT) and the control (i.e. not transplanted) site.

	FAT	CTR
<i>BITS</i>	1,67	1,76
<i>EQR</i>	0,60	0,63
<i>Status</i>	Moderate	Moderate

References

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SECTION 3 - FISH FAUNA MONITORING

The HFBI (Habitat Fish Bio Indicator) index

The HFBI is an empirically derived multi-metric index, composed of six metrics expressed as ecological quality reports. The index is calculated by combining various ecological descriptors including species richness and biomass, but also characteristics related to the belonging of each species to the different functional groups. They contribute to the calculation of the metrics that describe the characteristics of the functional groups, exclusively species belonging to the following ecological guilds: estuarine residents (ES), diadromes (Di) and marine migratory (MM). These groups were included due to their high susceptibility to environmental degradation, being highly dependent on the integrity of the habitats for the purposes of reproduction, nourishment and growth.

The fishing tool we adopted has such a selectivity as to allow a representative sampling of the communities fish present in the sampled area and associated with the particular type of habitat to be monitored. The mesh size (internode equal to 2 mm) of the tool allow to capture even small species such as *Aphanius fasciatus* and *Syngnathus abaster*, often associated with environments in good health. Furthermore, since in the lagoon environments the distribution of fish species is strongly influenced by some environmental variables, such as water temperature, salinity, oxygen dissolved as well as vegetation cover, and has a strong seasonal variability, the index was designed in such a way as to be able to evaluate the structure of the fishing communities according to the type of water body, seasonality (spring and autumn) and habitats (vegetated or non-vegetated environment).

Results

Sampling of fish was carried out in November 2022 in Goro and Fattibello. **Table 7** shows the fish taxonomic list and the HFBI index value at Goro and Fattibello. At Goro the fish community is richer (both in species number and abundance) and more diversified respect to Fattibello.

<i>Anguilla anguilla</i>	<i>Pomatoschistus canestrini</i>
<i>Atherina boyeri</i>	<i>Zosterisessor ophiocephalus</i>
<i>Sardina pilchardus</i>	<i>Dicentrarchus labrax</i>
<i>Alosa fallax</i>	<i>Liza aurata</i>
<i>Sprattus sprattus</i>	<i>Liza ramada</i>
<i>Aphanius fasciatus</i>	<i>Solea solea</i>
<i>Engraulis encrasicolus</i>	<i>Syngnathus abaster</i>
<i>Knipowitschia panizzae</i>	<i>Mullus barbatus</i>
<i>Gobius niger</i>	

HBFI = 1,0

<i>Atherina boyeri</i>	<i>Knipowitschia panizzae</i>
<i>Sardina pilchardus</i>	<i>Gobius niger</i>
<i>Sprattus sprattus</i>	<i>Pomatoschistus canestrini</i>
<i>Aphanius fasciatus</i>	<i>Zosterisessor ophiocephalus</i>
<i>Engraulis encrasicolus</i>	<i>Solea solea</i>

HBFI = 0,4

Table 7. Taxonomic list and fish index value at Goro (above) and Fattibello (below)

Annex I



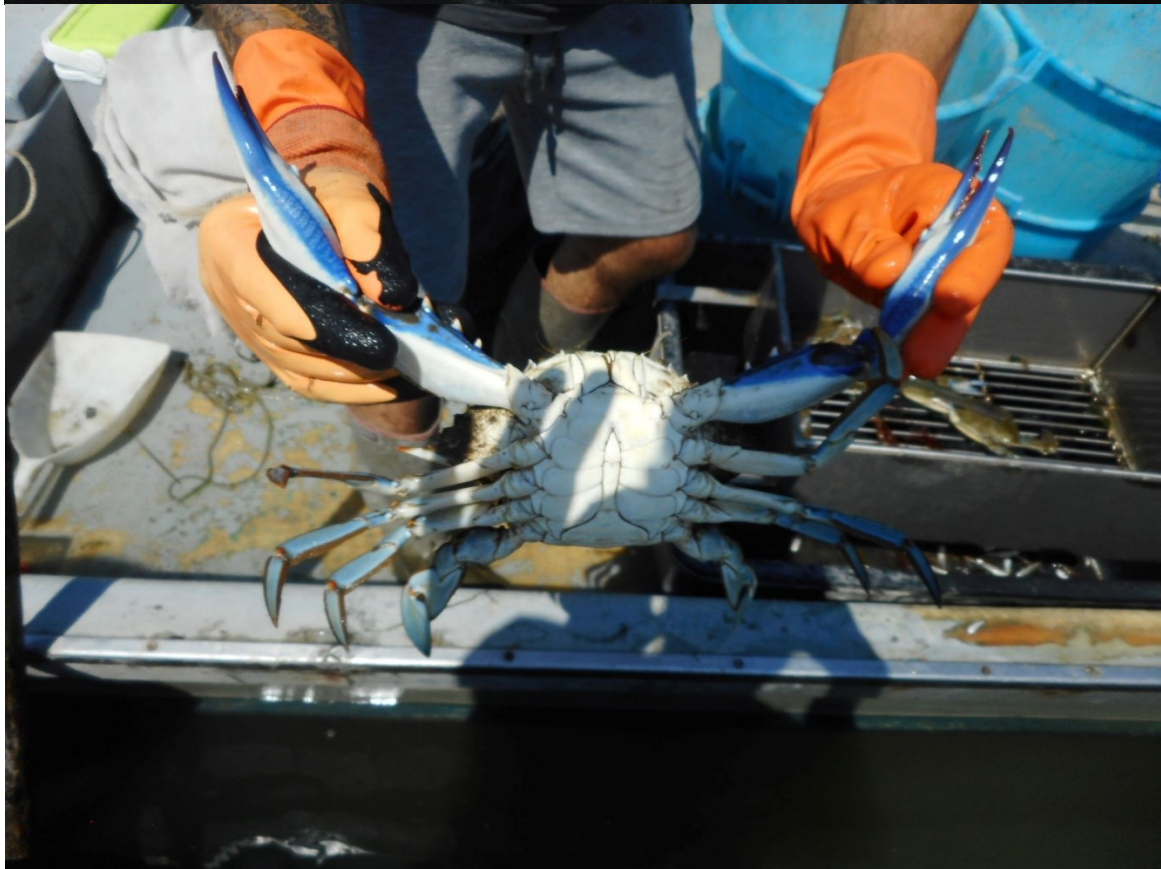
Sampling the benthos in Bassunsin



Sampling the benthos in Seganda



Sampling fish fauna in Seganda



A fish sample from Goro (above) and a new host (below, the blue crab *Callinectes sapidus*) in Goro and Fattibello lagoons



Sampling fish fauna in Fattibello



Fish samples: *Aphanis fasciatus* (above), *Atherina boyeri* (below)



Fish samples: *Engraulis encrasicolus* (above), *Sardina pilchardus* and *E. encrasicolus* (below)