LIFE19 NAT/IT/000264

Seagrass transplantation for transitional Ecosystem Recovery

(LIFE- TRANSFER)

Action D1: Monitoring of C1 action

First monitoring progress report

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EXECUTIVE SUMMARY

The D.1.2 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 Caleri and Barbamarco lagoons. As known, the transplantation activities at the Lagoons of Po Delta sites suffered a delay due to technical problems of the Veneto Park, which was in charge of the activity. With the transfer of the activity from the Veneto Park to the University of Ferrara and Cà Foscari Venice, the activities started regularly. However, all of this led to a delay of about a year on the roadmap. The monitoring programmed in Action D1 therefore started late, i.e. after the first transplant of seagrass in Caleri and Barbamarco.

This **First Monitoring Progress Report** is divided in three sections: the first section, edited by DAIS-UNIVE, concerns seagrass rooting and growth, water-sediment analyses, macroalgal sampling and determination, the ecological quality assessment by the application of the Macrophyte Quality Index (MaQI); the second and third sections, edited by UNIFE, concerns the benthic and the fish fauna sampling and determination, and the ecological quality assessment by the application of the multimetric Marine Biotic index (M-AMBI).

Briefly, concerning angiosperms growth at Barbamarco, a reduced amount of seagrasses was recorded, while at Caleri *Zostera noltei* tripled or quadrupled the size of the transplanted sods. Concerning water parameters, Salinity and the Oxygen Saturation on average were slightly higher at Caleri than at Barbamarco, and small differences were also recorded for Total Suspended Solids, slightly higher at Barbamarco. The ecological status determination by the application of MaQI during these 4 months was Poor in both stations.

Concerning the benthic fauna, 24 macrobenthic taxa were found at Caleri, and 24 at Barbamarco. The ecological quality status through M-AMBI was unsatisfactory at both lagoons, at both transplant and control sites; conversely, through the BITS index it was Moderate for both transplant and control site at Caleri, while at Barbamarco it resulted Good at the transplant site and moderate at the control. The majority of the macrobenthic taxa present at all the sites belongs to the tolerant (EG-III) and opportunistic (EG-IV, EG-V) groups at both lagoons.

Fish fauna sampled in February 2023 in Caleri and Barbamarco resulted in only two species (*Zosterisessior ophiocephalus* ;and *Gobius niger*), the reason is probably due to the fishing gear (cogollo) not performing at the sites with very shallow waters. HFBI index is null, in next sampling it was decided to change the fishing gear.

SubAction D.2.1 Monitoring angiosperm rooting and growth

Angiosperm transplants

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

On 6/6/2022 the Veneto Park resolved to transfer part of Action C1 (Caleri and Barbamarco seagrass transplantation) to the Universities of Ferrara (for the transplant part in the Delta lagoons) and Cà Foscari (for the explant part from Venice lagoon). Therefore, the first transplants in these lagoons were performed in November 2022, whereas the second ones occurred in June 2023. These lagoons have ecological conditions that are poorly suited for the transplant of *Ruppia cirrhosa,* which prefers choked environments; therefore our attention was focused on *Cymodocea nodosa, Zostera marina* and *Zostera noltei* which were explanted from the Venice lagoon where these species are very abundant (Sfriso et al., 2022).

In November 2022 the number of sod transplants was: 160 at Barbamarco and 144 at Caleri (**Table 2**).

Table 2*. Number of effective transplants in each lagoon*

Year	2022	2023	2023	Total
Lagoon	November	June	27-Jun	
		N° sods		
Barbamarco	160	144	50	**354**
Caleri	144	144		**288**
		Equivalent rhizomes		
Barbamarco	1600	1440	500	**3540**
Caleri	1440	1440		**2880**

Figure 1*. Sod trasplants at Barbamarco and Caleri in November 2022*

In June 2023 the number of sod transplant was: 144 at Barbamarco and 144 at Caleri (**Table 2**). In addition at Barbamarco in June 2023 approx. 500 rhizomes, accounting for 50 sods, were also transplanted by the staff of DAIS-UNIVE.

Figure 2*. Sod trasplants at Barbamarco in June 2023 by the staff of DAIS-UNIVE*

Angiosperm rooting and growth monitoring

In 26/04/2023 and 07/04/2023 during the monitoring of the environmental parameters the presence and the rooting of angiosperm transplants have also been verified. In April an abundant presence of macroalgae was recorded, however many sods were again present without showing a significant growth. In June at Barbamarco, a reduced amount of seagrasses was only recorded, however it was not possible to take photos due to the turbidity of the water and the high presence of macroalgae. At Caleri some photos were taken where macroalgae were absent (**Figure 2**). In this lagoon *Zostera noltei* and *Zostera* *marina* were mainly transplanted and the photos show the presence and growth of *Zostera noltei* which tripled or quadrupled the size of the transplanted sods (15 cm in diameter) (**Figure 3**).

***Figure 3**. Zostera noltei sod growth**.** The diameter was 40-60 cm.*

However, a more detailed control and an evaluation of the success rate of the transplants will be performed in September/October in the presence of lower algal biomass.

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 0.7μm). 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 (Ø=10cm). 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 <63μm), 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 (Ø=10cm). 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: Salinity = Cl^- x 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 (0.7μm) 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 μg 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 °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 N = 0.74%, art. no. 05 000 959 (Elementar Italia S.r.l.) and the standard used for carbon determination was "C2*", with 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 °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 °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 °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 4**) in order to determine the ecological status as required by Water Framework Directive (2000/60/EC).

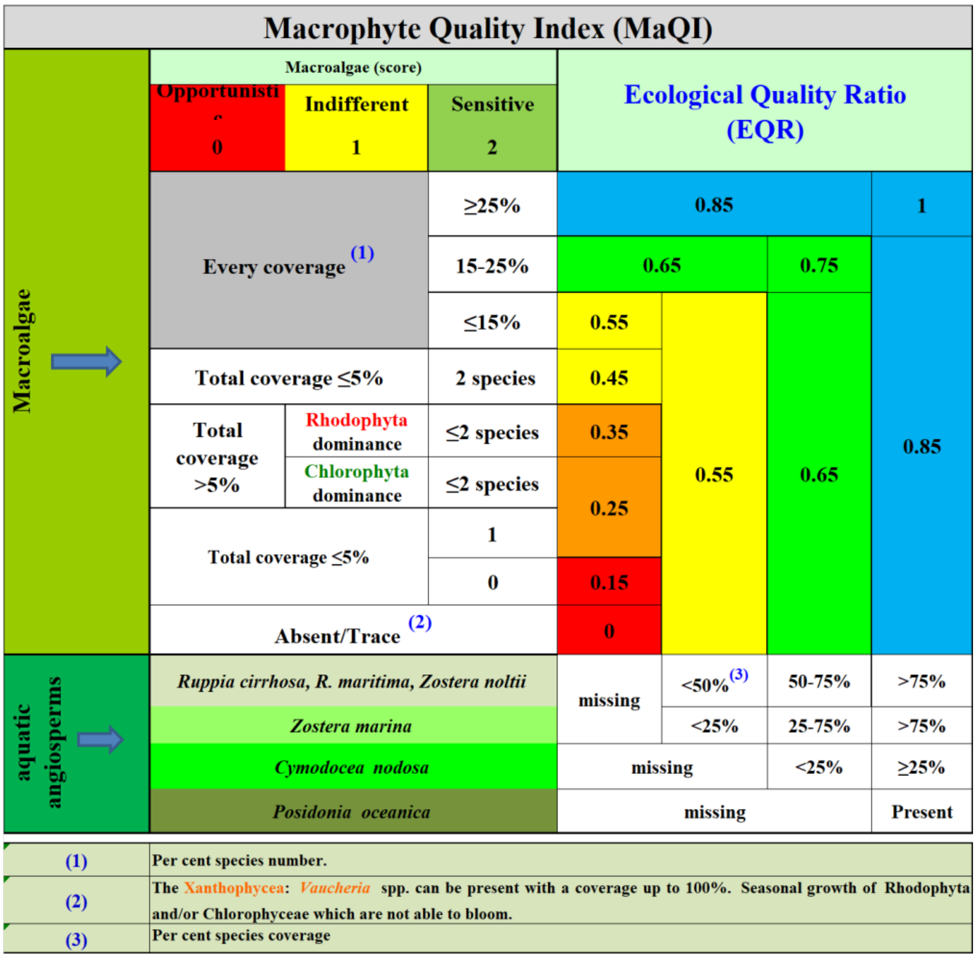

***Figure 4**. MaQI Scheme*

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 6 monthly samples both at Barbamarco and Caleri. The sampling period started the 26 January 2023 and will end in December 2023 (12 samples).

Figure 5. *Above: lagoon of Barbamarco with reeds indicating the transplant points of Ruppia cirrhosa. Below: lagoon of Caleri with reeds indicating the transplant points of Zostera noltei and Zostera marina..*

Currently, we have only 3-4 monthly samples of the physico-chemical analyses of the water column and surface sediments and the macrophyte determination collected during the first year (**Figures 5**).

Water Column

Figure 6 shows the variations of Temperature, pH, Eh, Salinity, Oxygen Saturation, Total Suspended Solids in the water column.

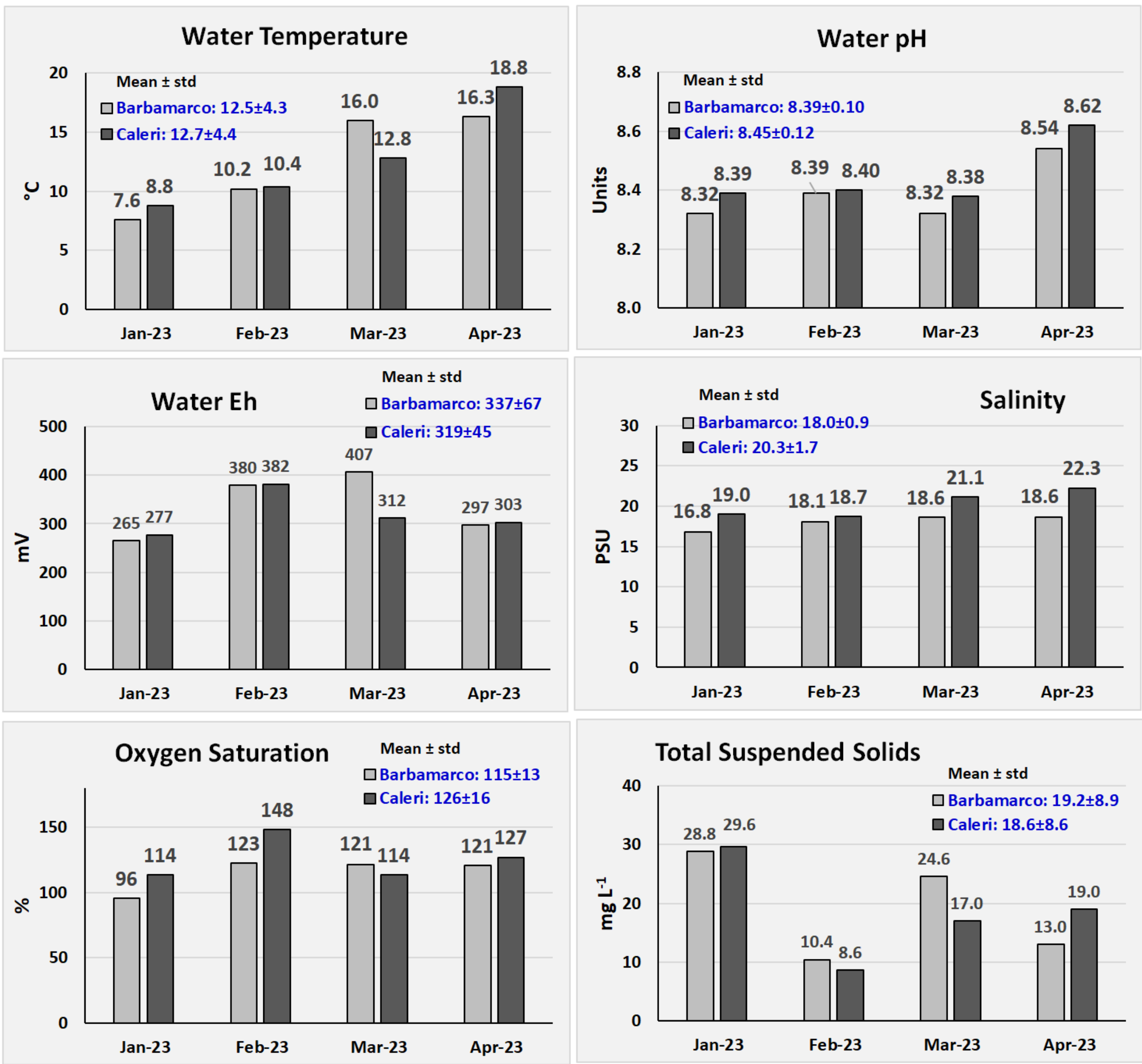

Figure 6. *Variation of some environmental parameters in the Water Column in the Barbamarco and stations.*

The mean water Temperature at Barbamarco and Caleri ranged from values of 7-8 °C in January to 16-18 °C in April with mean values quite similar. At Caleri the values of pH (8.45±0.12), on average, were higher than at Barbamarco (8.39±0.10), with the highest values in April. Conversely, the mean values of the redox potential (Eh) were higher at Barbamarco (337±67 mV) than at Caleri (319±45 mV) with the highest differences recorded in March.

Salinity and the Oxygen Saturation on average were slightly higher at Caleri (20.3±1.7 psu; 126±16%) than at Barbamarco (18.0±0.9 psu; 115±13%). Small differences were also recorded for Total Suspended Solids (TSS) which ranged from 18.6±8.6 mg L^{-1} at Caleri to 19.2 ±8.9 mg L^{-1} at Barbamarco.

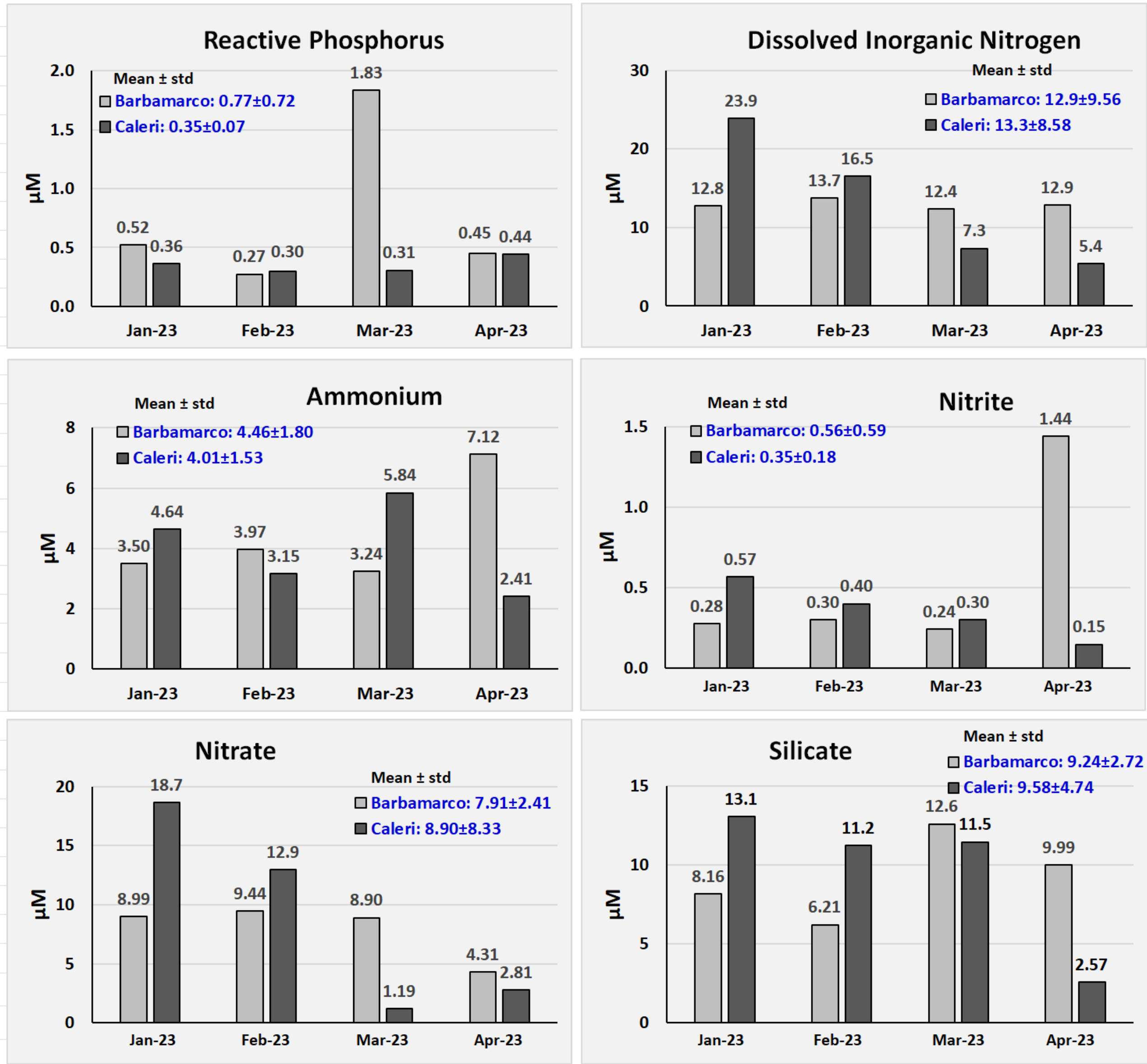

Figure 7. *Variation of nutrient concentrations in the Water Column at Barbamarco and Caleri.*

The mean concentrations of the Reactive Phosphorus (RP) at Barbamarco (0.77±0.72 μM) were twice higher than at Caleri (0.35±0.07 μM) due to the high concentration (1.83 μM) recorded in March (**Figure 7**), whereas the Total Dissolved Nitrogen (DIN) was quite similar ranging from 12.9±9.56 μM at Barbamarco to 13.3±8.58 μM at Caleri with the highest value (23.9 μM) recorded at Barbamarco in January. Among Ammonium, Nitrite and Nitrate the highest differences were recorded for Nitrite due to the higher (1.44 μM) concentration recorded in April at Barbamarco. Ammonium showed mean concentrations ranging from 4.01±1.53 μM at Caleri to 4.46±1.80 μM at Barbamarco. Similarly, Nitrate ranged from 8.90± 8.33 μM at Caleri to 7.91±2.41 μM at Barbamarco.

Silicates showed similar concentrations both at Caleri (9.58±4.74 μM) than at Barbamarco (9.24±2.72 μM).

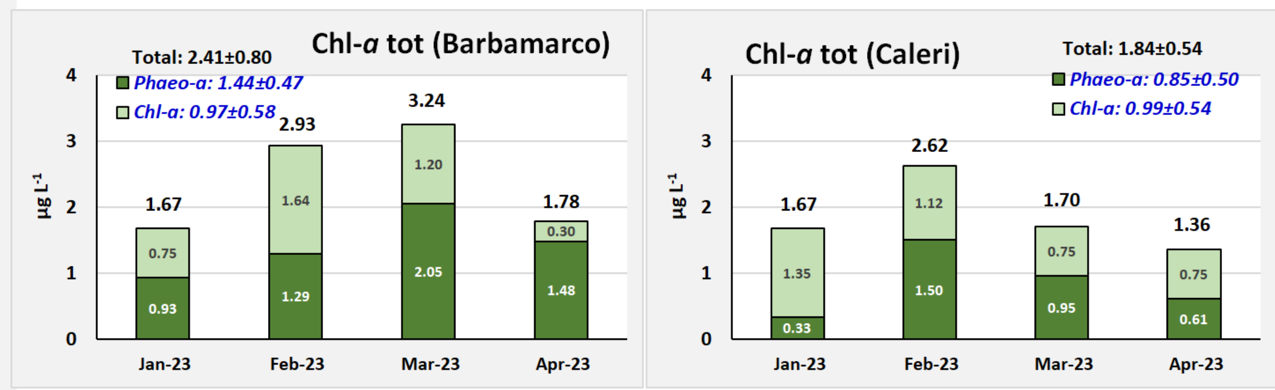

Figure 8. *Variation of the total Chlorophyll-a at Barbamarco and Caleri.*

On average the concentrations of total Chl-*a* was low in both stations (**Figure 8**) and ranged from 1.84±0.54 μg L^{-1} at Caleri to 2.41±0.80 μg L^{-1} at Barbamarco. Phaeopigments (degraded Chl-*a*) prevailed at Barbamarco and the active Chl-*a* at Caleri.

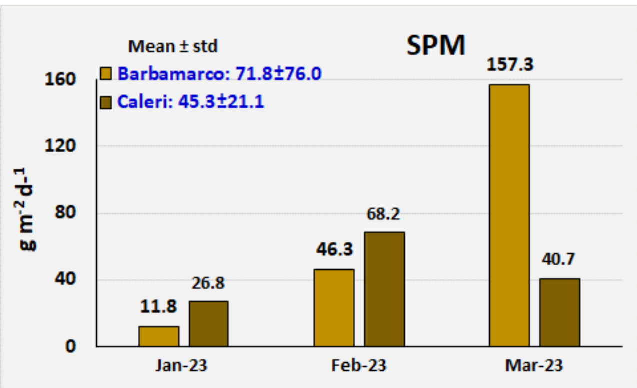

Figure 9. *Variation of sedimentation rates in the two stations*

Also the amount of particulate that settled in the sedimentation traps (SPM) was relatively low in both stations (**Figure 9**). On average, it was higher at Barbamarco (71.8± 76.0 g m^{-2} d^{-1}) than at Caleri (45.3±21.1 g m^{-2} d^{-1}). This difference was mainly due to the highest amount recorded at Barbamarco in March (153.3 g m^{-2} d^{-1}).

SURFACE SEDIMENTS

Some physical characteristics of the surface sediment 5-cm top layer are reported in **Figure 10**. Barbamarco was a sandy station with 6.1% of Fines only, whereas at Caleri Fines were 27.9%. Therefore, the amount of dry sediment for volume unit (Dry Density) was higher than at Barbamarco (1.54 g dwt cm^{-3}) than at Caleri (1.33 g dwt cm^{-3}). As expected, Moisture and Porosity were lower at Barbamarco (25.3% and 52%, respectively) than at Caleri (33.0% and 63.0%, respectively).

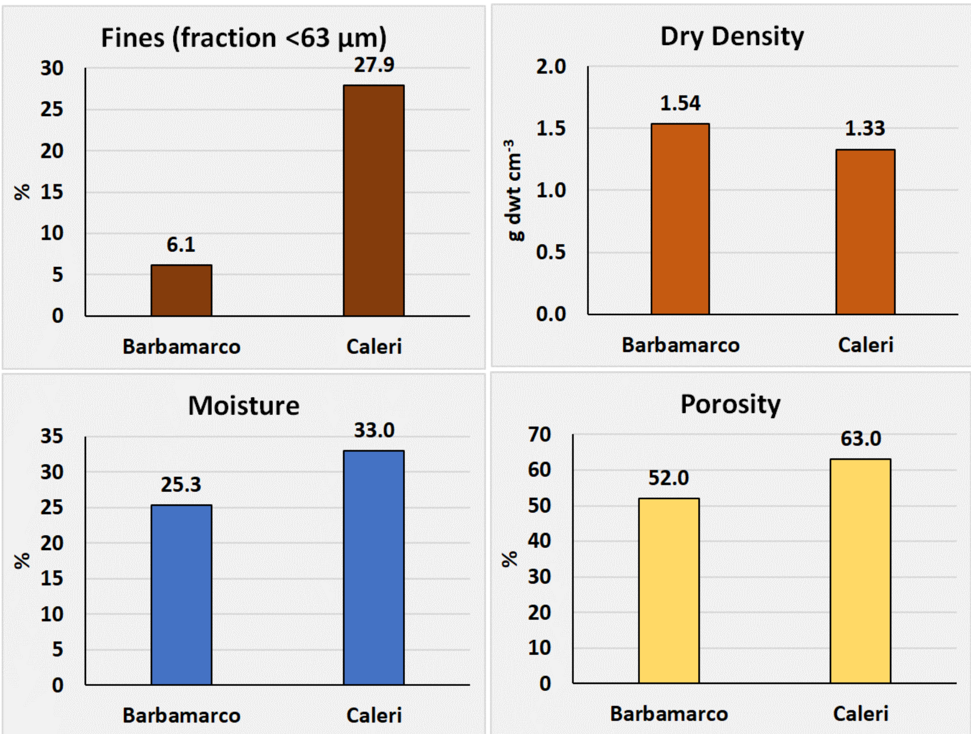

Figure 10. *Main sediment characteristics in the two lagoons.*

Nutrient concentrations and carbon compounds in surface sediments showed higher concentrations at Caleri where the amount of Fines was higher (**Figure 11**).

Total Phosphorus (Ptot) at Caleri (626±90 µg g^{-1}) was almost twice higher than at Barbamarco (347±11 µg g^{-1}). In both stations the inorganic fraction (Pinorg: 327±16 and

508±46 μg g^{-1}, at Barbamarco and Caleri, respectively) was significantly higher than the organic fraction (Porg: 20±11 and 118±61 μg g^{-1}).

Similarly, Total Nitrogen (Ntot) was more than twice higher at Caleri (1.03±0.12 mg g^{-1}) than at Barbamarco (0.4±0.1 mg g^{-1}) and Total Carbon (Ctot) at Caleri was approx. two-fold higher (32.9±5.04 mg g^{-1}) than at Barbamarco (15.4±1.29 mg g^{-1}) and the inorganic (Cinorg) and organic (Corg) fractions followed the same trend.

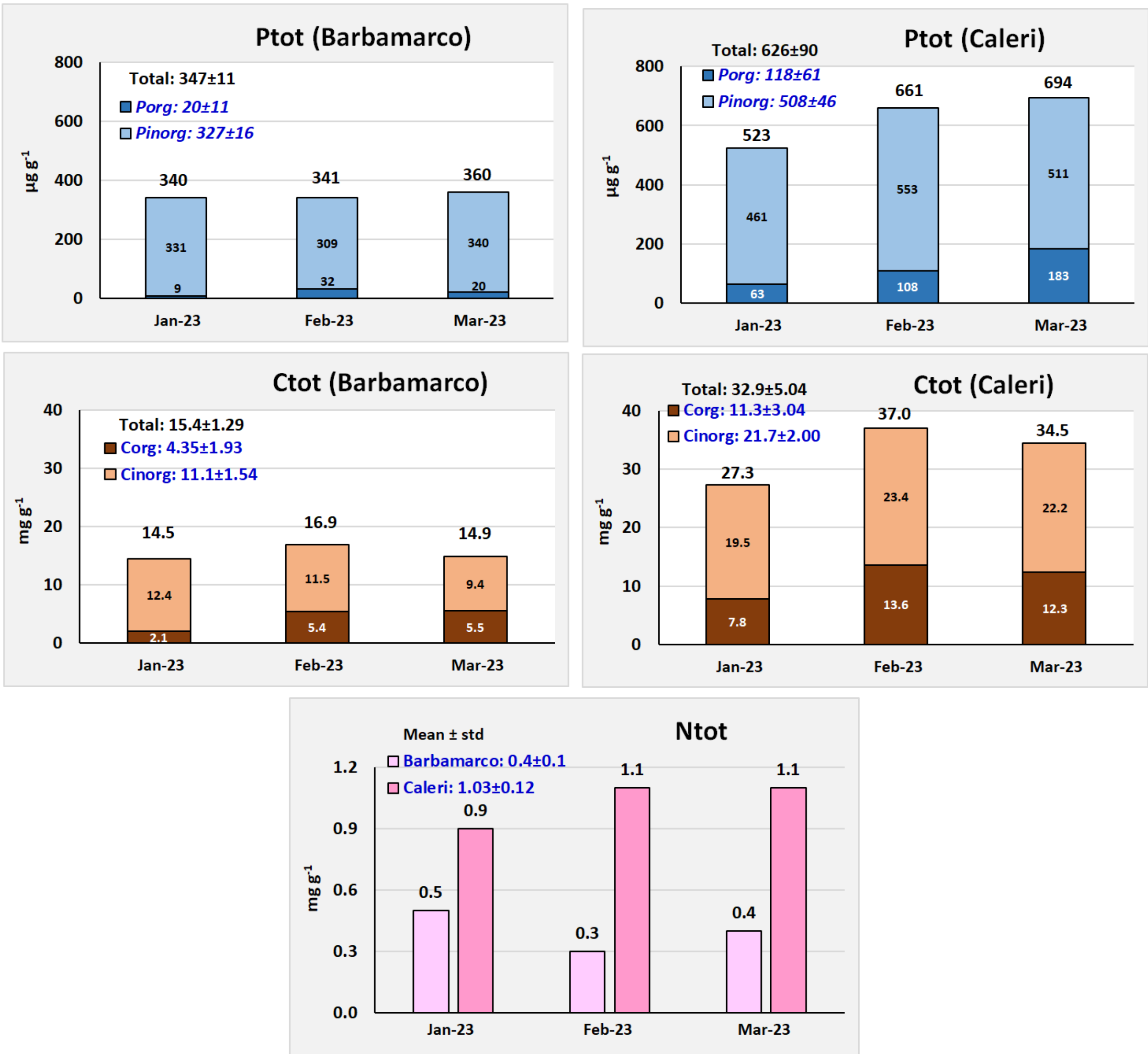

Figure 11. *Nutrient concentrations in the surface sediments of the two lagoons.*

Macrophytes

Macroalgae and MaQI determination

The quantitative and qualitative consistency of macroalgae present in the two stations is reported in **Figure 12.**

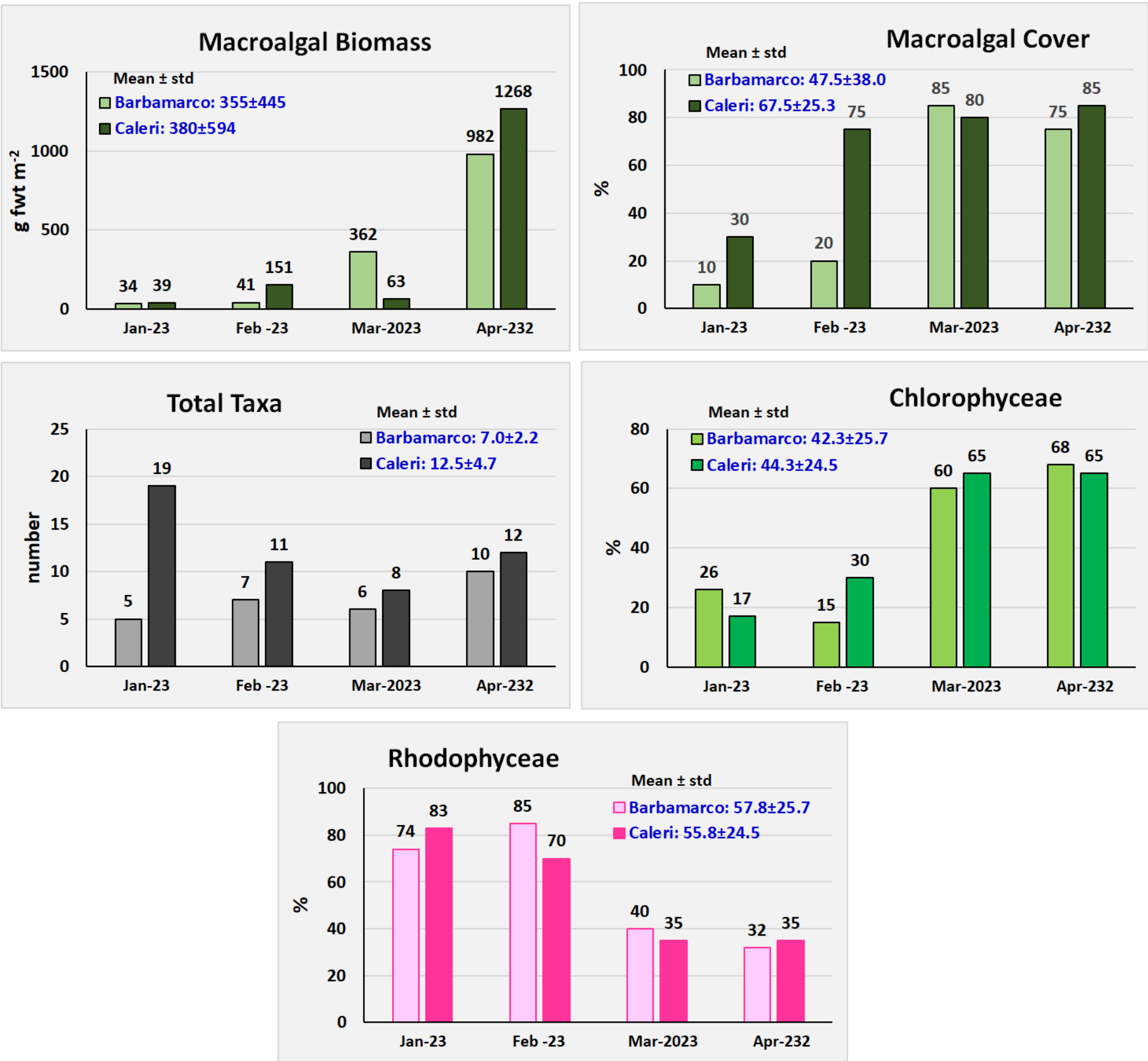

Figure 12. *Variation of macroalgae in the two stations.*

In both stations the biomass was very low in January (34 and 39 g fwt m^{-2} at Barbamarco and Caleri, respectively), and progressively increased up to 982 and 1268 g fwt m^{-2} in April. On average, the mean values were very similar fluctuating between 380±594 at Caleri and 355±445 at Barbamarco (**Figure 12**). Similarly, macroalgal cover increase from 10-30% in January to 75-85% in April with the highest value at Caleri (67.5±25.3%) in comparison to

Barbamarco (47.5±38.0). The number of taxa per sampling data at Caleri was almost double (12.5±4.7) than at Barbamarco (7.0±2.2). In both lagoons the percentage of Rhodophyceae (55.8-57.8) in term of biomass was higher than that of Chlorophyceae (42.3-44.3). However, Rhodophyceae were abundant especially in January and February than they decreased replaced from Chlorophyceae.

Total macroalgal records are shown by **Table 3** and **Table 4**.

On the whole Caleri was colonized by a higher number of species (35) in comparison to Barbamarco (25), due to a higher number of Rhodophyceae (18 instead 12) and Phaeophyceae (5 instead 2) recorded in this station.

In addition, at Caleri two sensitive taxa were recorded: the Rhodophyceae *Alsidium corallinum* and *Dasya punicea*.

However, the ecological status determination by the application of MaQI during these 4 months was Poor in both stations.

Table 3. *Macrophytes sampled at Barbamarco.*

Taxonomic list first year						
Barbamarco						
N°	**N°**	**Chlorophyceae**	**Jan**	**Feb**	**Mar**	**Apr**
1	1	***Blidingia dowsonii*** (Hollenberg & I.A.Abbott) S.C.Lindstrom, L.A.Hanic & L.Golden		X		
2	2	***Blidingia minima*** (Nägeli ex Kützing) Kylin			X	
3	3	***Bryopsis hypnoides*** Lamouroux		X		
4	4	***Chaetomorpha aerea*** (Dillwyn) Kützing				
5	5	***Cladophora albida*** (Nees) Kutzing				X
6	6	***Ulva australis*** Areschoug	X	X	X	X
7	7	***Ulva intestinalis*** Linnaeus				X
8	8	***Uva polyclada*** Kraft				
9	9	***Ulva prolifera*** O.F.Müller				X
10	10	***Ulvella viridis*** (Reinke) R.Nielsen, C.J.O'Kelly & B.Wysor				X
11	11	***Uronema marinum*** Womersley	X	X		
		Rhodophyceae				
12	1	***Caulachantus okamurae*** Yamada				
13	2	***Ceramium cimbricum*** H.E.Petersen				
14	3	***Chondria capillaris*** (Hudson) M.J.Wynne				X
15	4	***Erythrotrichia carnea*** (Dillwyn) J.Agardh				X
16	5	***Gracilaria bursa-pastoris*** (S.G.Gmelin) P.C.Silva	X			X
17	6	***Gracilaria gracilis*** (Stackhouse) Steentoft, L.M.Irvine & Farnham				
18	7	***Gracilariopsis longissima*** (S. G. Gmelin) Steentoft *et al.*	X			
19	8	***Gracilariopsis vermiculophylla*** Ohmi	X	X	X	X
20	9	***Neopyropia elongata*** (Kylin) L.-E.Yang & J.Brodie			X	
21	10	***Polysiphonia morrowii*** Harvey		X		
22	11	***Polysiphonia*** **sp.**				X
23	12	***Solieria filiformis*** (Kützing) P. W. Gabrielson				
		Phaeophyceae				
24	1	***Ectocarpus siliculosus*** (Dillwyn) Lyngbye			X	
25	2	***Ectocarpus siliculosus*** var. ***arctus*** (Kützing) Gallardo		X	X	
		Total Taxa	**5**	**7**	**6**	**10**
		Chlorophyceae N°	**2**	**4**	**2**	**5**
		Rhodophyceae N°	**3**	**2**	**2**	**3**
		Phaeophyceae N°	**0**	**0**	**0**	**0**
		Cover %	**10**	**20**	**85**	**75**
		Biomass g m^{-2}	**34**	**41**	**362**	**982**
		Chlorophyceae %	**26**	**15**	**60**	**68**
		Rhodophyceae %	**74**	**85**	**40**	**32**
		Chlorophyceae abundance %	**2.6**	**3**	**51**	**51**
		Rhodophyceae abundance %	**7.4**	**17**	**34**	**24**
		Sensitive species	**0**	**0**	**0**	**0**
		MaQI	**0.35**	**0.35**	**0.25**	**0.25**

able 4. *Macrophytes sampled at Caleri.*

Taxonomic list first year						
Caleri						
N°	N°	Chlorophyceae	Jan	Feb	Mar	Apr
1	1	*Blidingia dowsonii* (Hollenberg & I.A.Abbott) S.C.Lindstrom	X			
2	2	*Bryopsis hypnoides* J.V.Lamouroux				X
3	3	*Cladophora glomerata* (Linnaeus) Kützing	X			
4	4	*Cladophora sericea* (Hudson) Kützing	X			
5	5	*Ulothrix implexa* (Kützing) Kützing	X			
6	6	*Ulva australis* Areschoug				
7	7	*Ulva flexuosa* Wulfen			X	
8	8	*Ulva pilifera* (Kützing) Škaloud & Leliaert		X		X
9	9	*Ulva laetevirens* Areshoug	X	X		X
10	10	*Uva polyclada* Kraft				
11	11	*Ulva prolifera* O. F. Müller	X			
12	12	*Ulva rigida* C. Agardh	X		X	
		Rhodophyceae				
13	1	*Alsidium corallinum* C.Agardh	X	X		
14	2	*Agardhiella subulata* (C. Agardh) Kraft et M. J. Wynne		X	X	X
15	3	*Callithamnion corymbosum* (Smith) Lyngbye	X			
16	4	*Antithamnion nipponicum* Yamada & Inagaki		X		
17	5	*Bangia fuscopurpurea* (Dillwyn) Lyngbye	X			
18	6	*Ceramium polyceras* (Kützing) Zanardini		X		
19	7	*Dasya punicea* (Zanardini) Meneghini			X	X
20	8	*Erythrotrichia carnea* (Dillwyn) J.Agardh		X		X
21	9	*Gracilaria bursa-pastoris* (S.G.Gmelin) P.C.Silva				
22	10	*Gracilariopsis longissima* (S. G. Gmelin) Steentoft et al.	X	X	X	X
23	11	*Gracilariopsis vermiculophylla* Ohmi			X	X
24	12	*Kapraunia schneideri* (Stuercke & Freshwater) Savoie & G.\	X			
25	13	*Melanothamnus harveyi* (Bailey) Díaz-Tapia & Maggs	X			
26	14	*Polysiphonia morrowii* Harvey	X	X	X	X
27	15	*Polysiphonia sp.*				
28	16	*Radicilingua mediterranea* Wolf, Sciuto & Sfriso	X	X	X	X
29	17	*Sahlingia subintegra* (Rosenvinge) Kornmann	X			
30	18	*Spyridia filamentosa* (Wulfen) Harvey	X			
		Phaeophyceae				
31	1	*Acinetospora crinita* (Carmichael) Sauvageau	X			
32	2	*Dictyota linearis* (C.Agardh) Greville	X			
33	3	*Ectocarpus siliculosus* var. *arctus* (Kützing) Gallardo		X		
34	4	*Ectocarpus siliculosus* (Dillwyn) Lyngbye				X
35	5	*Scytosiphon dotyi* M.J.Wynne				X
		Total Taxa	19	11	8	12
		Chlorophyceae N°	7	2	2	3
		Rhodophyceae N°	10	8	6	7
		Phaeophyceae N°	2	1	0	2
		Cover %	30	75	80	85
		Biomass g m^{-2}	39	151	62	1268
		Chlorophyceae %	17	30	65	65
		Rhodophyceae %	83	70	35	35
		Chlorophyceae abundance %	5.1	23	52	55
		Rhodophyceae abundance %	24.9	53	28	30
		Sensitive species	1	1	1	1
		MaQI	0.35	0.35	0.25	0.25

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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 2 sites (Caleri, Barbamarco), the monitoring action is carried out at 2 stations at each lagoon, i.e. in the transplant site and in a control site (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**).

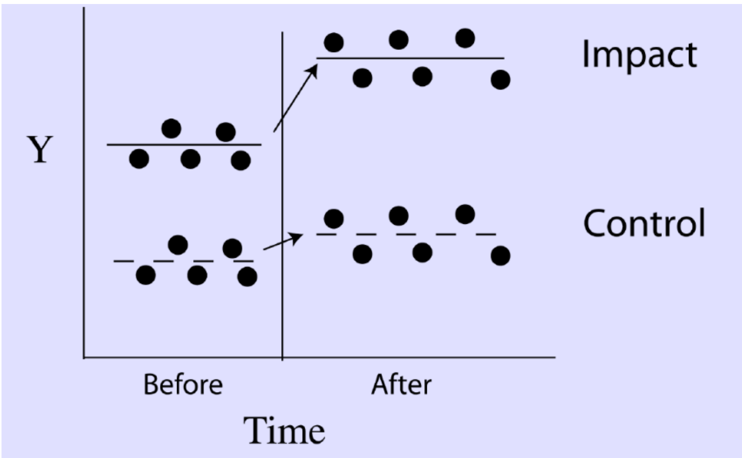

Fig. 1. Before-after-control-impact (BACI) design

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 January 2023.

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 2 (M-AT-2), to which Caleri and Barbamarco belong.

Valori di riferimento e limiti di classe

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

Rapporto di Qualità Ecologica			
Elevato/Buono	*Buono/Sufficiente*	*Sufficiente/Scarso*	*Scarso/Cattivo*
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:

$$BITS = \log[(6fI+fII) / (fIII+1)+1] + \log[nI/(nII+1) + nI/(nIII+1) + 0,5nII/(nIII+1) +1]$$

where *fI* 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), *fII* 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 *fIII* 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 *fIII* is null, and to prevent the eventuality of a log of zero if *fI* and *fII* are null. The second term of the BITS model allows to weight the number of sensitive families respect the tolerant and the opportunistic ones: *nI* is the number of sensitive families, *nII* is the number of tolerant and *nIII* 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

Caleri lagoon

A total of 24 macrobenthic taxa were found in the Caleri Lagoon. **Table 1** shows the faunal list of the macrobenthic taxa collected during the ex-ante monitoring campaign.

Capitella minima	*Streblospio eridani*
Capitella capitata	*Streblospio shrubsolii*
Caulleriella alata	*Oligochaeta sp.*
Armandia cirrhosa	*Monocorophium insidiosum*
Hediste diversicolor	*Monocorophium acherusicum*
Polydora cornuta	*Penaeus kerathurus*
Phyllodoce lineata	*Microdeutopus gryllotalpa*
Alitta succinea	*Chironomus salinarius*
Glycera sp.	*Ophiura sp.*
Prionospio cirrifera	*Bittium reticulatum*
Spio filicornis	*Lentidium mediterraneum*
Malacoceros fuliginosus	*Ruditapes philippinarum*

Tab. 1. Taxonomic list of the macrobenthic taxa identified in the Caleri Lagoon

In **Table 2** the values of community descriptors (diversity and species richness), togerther 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	High
C-CON	4,92	2,79	17	0,56	Poor
C-TR	3,99	3,03	19	0,69	Moderate

Tab. 2. Community parameters and AMBI/M-AMBI values in the Caleri Lagoon (C-CON: control site; C-TR: transplant site)

The ecological quality at Caleri is also summarized in **Figure 3**. It is evident how the ecological quality is slightly better at the transplant site CAL respect to the control site.

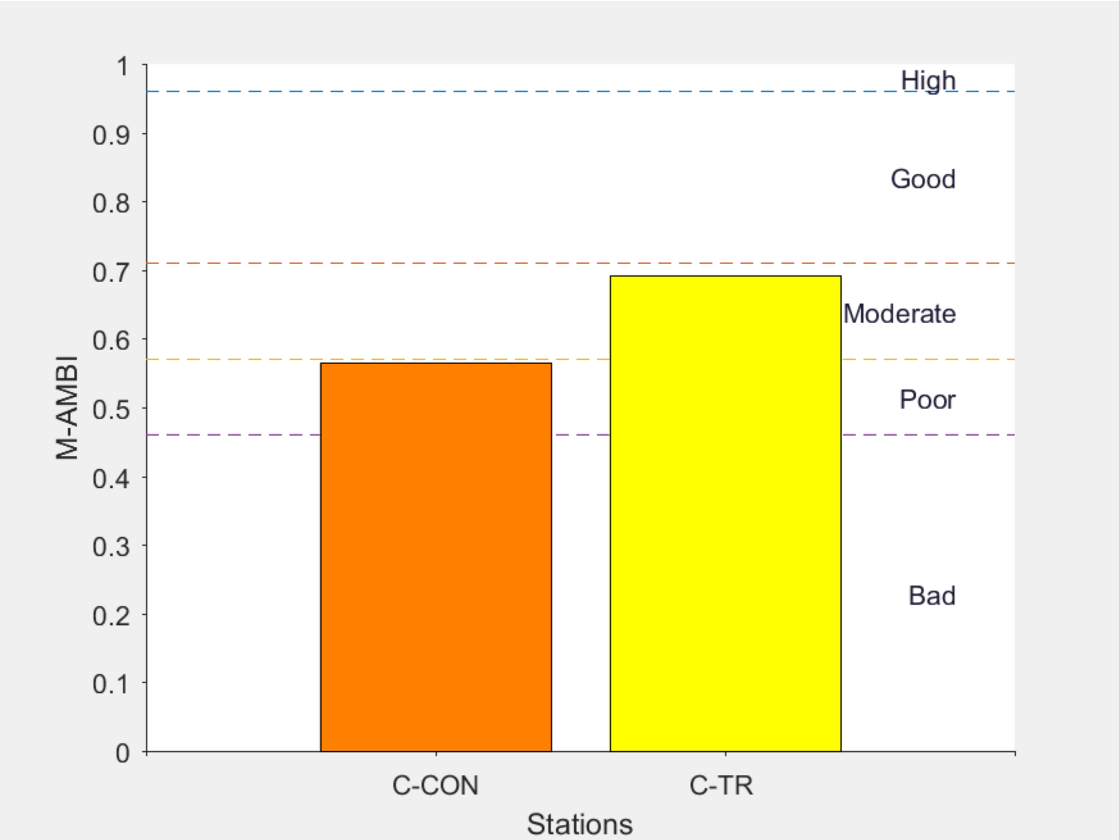

Fig. 3. Ecological quality through M-AMBI at Caleri (C-CON: control site; C-TR: transplant site).

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(%)*
C-CON	0	3,1	20,6	21,6	54,7
C-TR	0,6	0,2	53,4	24,1	21,7

Tab. 3. Sensitivity-tolerance distribution of macrobenthos in Caleri (C-CON: control site; C-TR: transplant site).

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

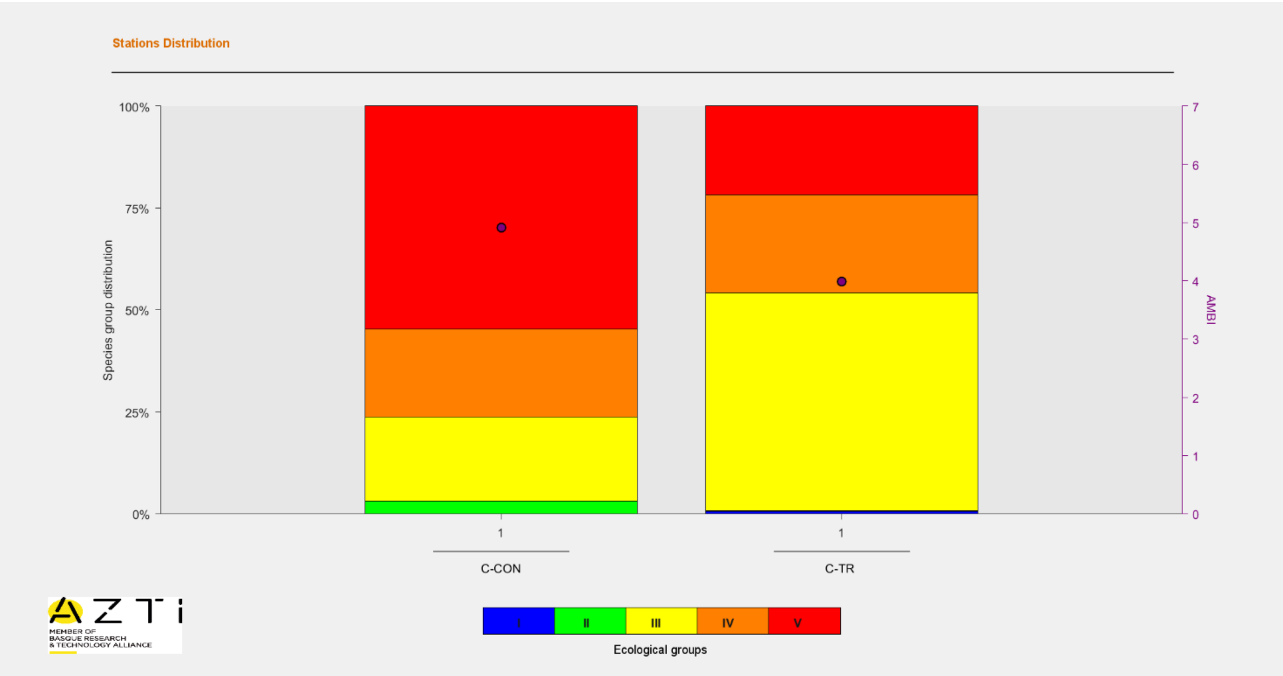

Fig. 4. Distribution of EGs at Caleri

The majority of the taxa present at the two transplant sites belongs to the opportunist (EG-V, EG.IV) and tolerant group (EG-III). At the control site there are also a few indifferent (EG-II) species. It is evident, however, that in the transplant site the benthic community is slightly better structured than in the control site.

The application of the BITS index gave similar values at transplant and control site, where the ecological status was Moderate.

	C-TR	C-CON
BITS	1,80	1,66
EQR	0,53	0,49
Status	Moderate	Moderate

Barbamarco Lagoon

Overall, 24 macrobenthic taxa were found in the Barbamarco Lagoon. **Table 4** shows the faunal list of the macrobenthic taxa collected during the January monitoring campaign.

Capitella minima	*Monocorophium acherusicum*
Capitella capitata	*Idotea balthica*
Caulleriella alata	*Gammarus aequicauda*
Caulleriella mediterranea	*Microdeutopus gryllotalpa*
Hediste diversicolor	*Chironomus salinarius*
Polydora cornuta	*Bittium reticulatum*
Malacoceros fuliginosus	*Hydrobia acuta*
Streblospio shrubsolii	*Lentidium mediterraneum*
Oligochaeta sp.	*Arcuatula senhousia*
Tanais dulongii	*Haminoea hydatis*
Corophium orientale	*Cerastoderma glaucum*
Monocorophium insidiosum	*Ruditapes philippinarum*

Tab. 4. Taxonomic list of the macrobenthic taxa identified in the Barbamarco Lagoon

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).

Stations	*AMBI*	*H'*	*S*	*M-AMBI*	*Status*
Bad	6	0	0	0	Bad
High	2,14	3,40	28	1	High
B-CON	4,24	2,71	16	0,60	Moderate
B-TR	4,53	2,68	19	0,61	Moderate

Tab. 5. Community parameters and AMBI/M-AMBI values at Barbamarco (B-CON: control site; B-TR: transplant site).

The ecological quality at Barbamarco is also summarized in **Figure 5**. There is no difference between the ecological quality value (“moderate”) at transplant and control sites.

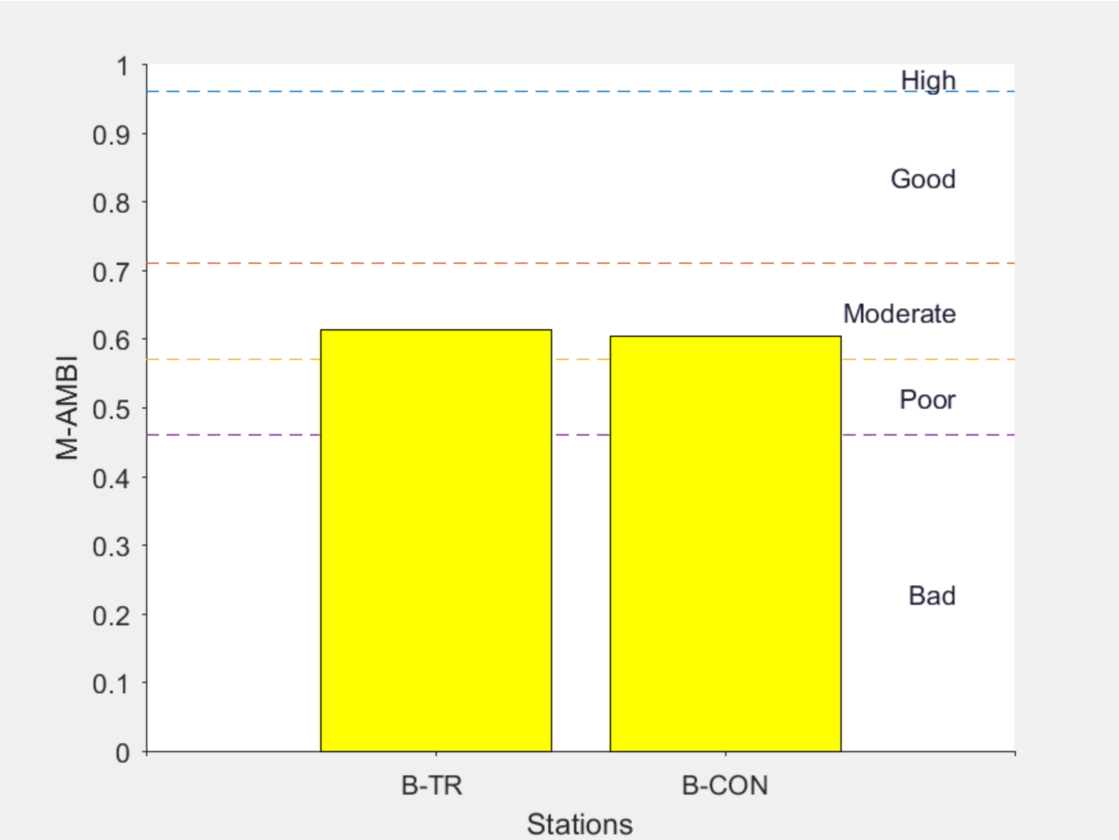

Fig. 5. Ecological quality through M-AMBI at Barbamarco (B-CON: control site; B-TR: transplant site).

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(%)*
B-CON	0,2	0,8	39,3	35,5	24,2
B-TR	0,3	0,2	28,8	38,7	32,1

Tab. 6. Sensitivity-tolerance distribution of macrobenthos at Barbamarco (B-CON: control site; B-TR: transplant site).

Figure 6 shows the distribution of EGs at the transplant site. The majority of taxa present at the two sites belongs to the “tolerant” (EG-III) and opportunistic (EG-IV, EG-V) group, and the composition of the two communities is very similar.

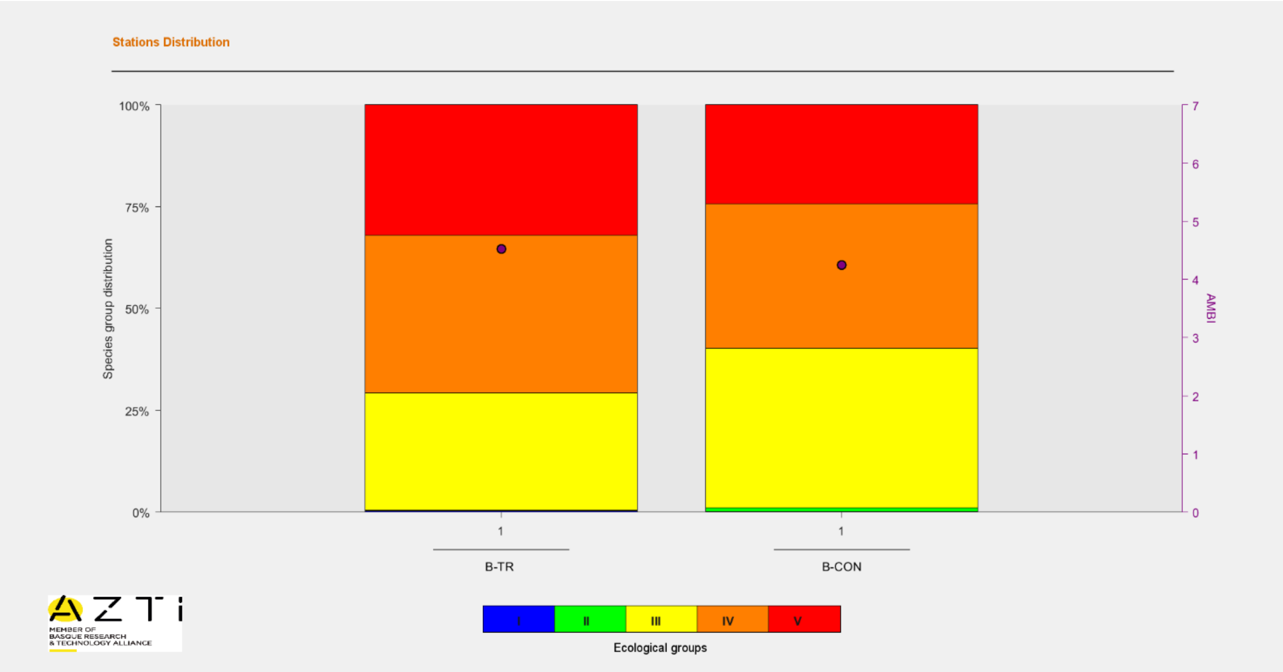

Fig. 6. Distribution of EGs at Barbamarco (B-CON: control site; B-TR: transplant site).

The application of the BITS index gave a higher value at the Barbamarco transplant site, where the ecological status was Good. The control (i.e. not transplanted) site resulted as Moderate.

	B-TR	**B-CON**
BITS	2,43	2,25
EQR	0,72	0,66
Status	Good	Moderate

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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 we were convinced it would be suitable 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 (it turns out we were wrong). 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 fauna was carried out in February 2023 in Caleri and Barbamarco. From the sampling campaigns, it seems that at both lagoons the fish community is quite poor, since only a few gobies (*Zosterisessior ophiocephalus* and *Gobius niger*) were caught. However, we believe that the reason for this is probably to be found in the fishing gear used in both lagoons (cogollo), which is probably not the best performing at the monitored transplant sites, which are characterized by very shallow waters. For this reason it was decided to change the fishing gear, buying a hand seine, which will be used from the next monitoring. Due to insufficient catch, applying the fish index doesn't make any sense.

Annex I

Sampling site at Caleri

Sampling site at Barbamarco

Sampling the benthos in Barbamarco

Fish sample (gobies) from Caleri and Barbamarco