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A preliminary assessment of marine biodiversity to support the establishment of a marine protected area along the coast of Manduria (southeastern Italy, Ionian Sea)

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Abstract

The coast of Manduria, in the province of Taranto (southeastern Italy, Ionian Sea), characterized by important natural areas, has been recently proposed as a potential Marine Protected Area (MPA). With this aim, the Municipality of Manduria requested to A.B.A.P (Associazione Biologi Ambientalisti Pugliesi) a preliminary assessment of marine biodiversity along this coastline. Two benthic habitats of high conservation importance, *Posidonia oceanica* seagrass meadows and coralligenous formations, were selected because they have been designated as Mediterranean priority habitats by the EU Habitats Directive and/or the Barcelona Convention. Macroalgal flora and fish fauna, occurring in this poorly explored area, were also investigated to fill up the knowledge gap. Whenever possible, non-destructive sampling methods were used. The two surveyed *P. oceanica* meadows showed a fair state of conservation, since anthropic disturbance and degradation were only recorded near their upper limits. A total of 70 vegetal and animal *taxa* (33 macroalgae, 15 fish and 22 invertebrates) was reported and the presence of some high naturalistic value species was documented, revealing that the site would benefit from protection. The present baseline data represent an initial reference that may be useful to support the establishment and the sustainable management of the future MPA.

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Introduction

The Municipality of Manduria is located in the southwestern part of Apulia, at 35 km east of Taranto. The territory covers a surface of about 180 km² and extends over a coastline, about 15km long, bordered by the Ionian Sea. It is characterized by areas of ecological relevance such as the Regional Nature Reserve of the "Litorale Tarantino Orientale" (Eastern Taranto Coast), established by Regional Law 23/12/2002 n. 24, and two Sites of Community Importance (SCI, Habitat Directive 92/43/EEC) named "Torre Colimena" (IT9130001) and "Dune di Campomarino" (IT9130003) where meadows of *Posidonia oceanica* L. occur.

As regards the marine environment, no Marine Protected Area (MPA) exists along this coast, although a request was recently made by the local Municipality to the Italian Ministry of the Environment and Protection of Land and Sea. MPAs are important tools to achieve local and global marine conservation targets and can play a crucial role for the sustainable use of marine coastal resources (Guidetti *et al.*, 2008).

In 2015, A.B.A.P. (Associazione Biologi Ambientalisti Pugliesi) started an investigation along the Mandurian coastline between the two SCIs as a first contribution to the knowledge of marine biodiversity of this area, devoted to the establishment of the future MPA. Relevant habitats of this area are *P. oceanica* seagrass meadows and coralligenous, which represent the most important *hot-spots* of biodiversity in the Mediterranean Sea. Considering that *P. oceanica* is very sensitive to environmental changes, protection strategies for this species are recommended.

As a matter of fact, *Posidonia* meadows are included in Annex I (strictly protected species) of the Bern Convention, in Annex II (threatened species) of the ASPIM Protocol of the Barcelona Convention and in Annex I (Priority Habitats) of the Habitats Directive, transposed by Italian legislation with the DPR 08/09/1997 n. 357.

Coralligenous and other calcareous bio-concretions in the Mediterranean Sea are included in the EU Habitats Directive under the generic habitat type "Reefs". Furthermore, an Action Plan, aiming at their conservation, has been adopted by contracting parties of the Barcelona Convention (UNEP-MAP-RAC/SPA, 2008).

In the present study, we have focused on these benthic habitats of high conservation importance. Benthic macroalgal flora and fish fauna were also investigated. A few papers, reporting data on the distribution and bio-ecological features of *P. oceanica* meadows at Torre Colimena and Campomarino collected in 2004, are available from literature (AA.VV, 2006; Costantino *et al.*, 2010).

Coralligenous formations, phytobenthos and fish fauna have never been surveyed along this poorly explored coastline. The baseline information reported in this paper may represent a starting point to understand the current state of marine biodiversity and explore future modifications occurring over the medium- and long-term.

Material and methods

Study area

In the study area (Fig. 1) surveys were carried out during May-June 2015. Whenever possible, non-destructive sampling methods such as underwater visual census and photographic sampling were used for the investigated communities.

Posidonia oceanica meadows

A series of extensive explorations, by SCUBA diving, were carried out throughout the area in order to obtain some physiographic features of the meadows mainly concerning the bathymetric evolution of both upper and lower limits and the topology of the meadows. Two line transects were allocated in each SCI referred to as T1 for "Torre Colimena" and T2 for "Dune di Campomarino" (Fig. 2). Along each transect were selected 2 sampling sites near the upper limit (S1) and the lower limit of the meadow (S2), for a total of 4 sampling stations (Table 1).

Table 1. Data of sampling stations.

Transect	Station	Latitude North	Longitude East	Depth (m)
T1	T1S1	40° 17' 41,93"	17° 43' 01,87"	-12
(Torre Colimena)	T1S2	40° 17' 05,19"	17° 43' 00,59"	-25
T2	T2S1	40° 17' 25,50"	17° 35' 28,20"	-11
(Dune di Campomarino)	T2S2	40° 16' 50,42"	17° 35' 29,31"	-23

Fig. 1. Geographical location of the investigated coastline (red rectangle).

Fig. 2. Location of transects and sampling stations of *Posidonia oceanica*.

Field activities as well as laboratory analyses (phenology and lepidochronology) were conducted according to standardized protocols (Buia *et al.*, 2004; ISPRA, 2008). For each station, three areas of about 400 m² were randomly located 10 m apart. For each area, 1 covering estimate (%) and 3 shoots counting (within a 40 x 40 cm square) were carried out. In addition, 5 shoots, avoiding to sample the dichotomous ones and choosing orthotropic rhizomes, were collected at a distance of approximately 100 cm from each other. In each station, 3 covering estimates, 9 replicates of shoots counting and the collection of 15 shoots were carried out (60 shoots in total). The number of sampled shoots was smaller than that suggested by standard protocols, in order to minimize the impact of

sampling in this preliminary investigation. All shoots were labelled and stored in a solution of seawater and 4% formalin for subsequent study in the laboratory. For each meadow, some bio-ecological data such as meadow continuity, presence of dead "matte", bottom type, presence of invasive algae, lower limit type and depth, were also collected and a detailed photographic and video documentation was provided.

For each station 10 shoots were used for morphological and biometrical analyses and 5 shoots were used for the lepidochronological analysis (Pergent, 1990; Pergent and Pergent-Martini, 1991; Pergent-Martini and Pergent, 1994). Leaf morphology, leaf biomass and epiphytes biomass were measured according to standard analytical methods (Buia *et al.*, 2004).

Leaf and epiphytes biomasses, expressed as dry weight (dw) were measured after drying the leaves and the epiphytes at 60°C in an oven until constant weight was reached. The data collected for each station were reported in Excel 2010 for processing.

Each leaf shoot was split into individual leaves, respecting the order of insertion (alternately distichous). The leaves were numbered from the center outward distinguishing them in young, intermediate and adult. The following parameters were measured: number of leaves per shoot; length and width of each leaf; length of the brown tissue, when present; apex state (intact or broken).

On the basis of these data the following phenological parameters were estimated: mean number of leaves per shoot; mean leaf area for each shoot (cm^2/ shoot); leaf area index (LAI) per station (m^2/m^2); Coefficient "A" (indicating the impact of the environment on the leaves) equal to the ratio between broken apices and the total number of leaves (expressed in per cent and based on mature and intermediate leaves); mean value of Coefficient "A" per station. Mean leaf biomass (mg dw $shoot^{-1}$) and mean epiphytes biomass (mg dw $shoot^{-1}$) were also calculated.

For each meadow, the lepidochronological study was carried out on 10 orthotropic rhizomes (5 per station) in order to determine, per each station, the average age of rhizomes, the average number of scales produced per annual cycle before the year 2015 and the annual rhizome production (mg dw $shoot^{-1}$ $year^{-1}$).

Macroalgal flora

Qualitative sampling was carried out by SCUBA diving, along three transects arrayed perpendicularly to the coastline (Table 2, Fig. 3), from the midlittoral zone to a maximum depth of 10 m. Sample collection was made by hand or with a chisel in order to remove basal portions, encrusting or mat-forming algae, attempting to gather as many species as possible. The collected material was preserved in 4% buffered formalin/seawater. In the laboratory, fresh material was observed under a Leica MZ 7.5 stereomicroscope (Leica, Wetzlar, Germany). Sections of thalli were obtained by hand or, if necessary, with a DSK-1000 vibratome (Dosaka, Kyoto, Japan) and properly stained. Photomicrographs and measurements were made using an Olympus BX-40 light microscope (Olympus, Melville, USA) fitted with an Olympus DP21 digital camera (Olympus, Melville, USA).

For nomenclature purposes, the following databases were used: Index Nominum Algarum (Silva, 2017) and AlgaeBase (Guiry and Guiry, 2017). After sorting, macroalgal taxa were identified according to the methodology of Cormaci *et al.* (2004).

Table 2. Sampling sites along the investigated coastline.

	Acronym	Latitude (°N)	Longitude (°E)
Torre Colimena	TC	40° 17' 56,5"	17° 44' 07,1"
Borraco	BO	40° 18' 11,1"	17° 38' 00,4"
Monaco Mirante	MM	40° 17' 56,0"	17° 35' 41,8"

In the floristic list (Table 6) for each species sampling stations and phytogeographic elements (according to Furnari *et al.*, 2010) were given. The Rhodophyta/ Phaeophyceae (Feldmann, 1937) and Rhodophyta +Chlorophyta/Phaeophyceae (Cheney, 1977) indices were calculated.

Fig. 3. Location of transects (→). TC = Torre Colimena; BO = Borraco; MM = Monaco Mirante.

Fish fauna

Underwater *visual census* (Jansson *et al.*, 1985) was the preferred method for non-destructively qualitative surveying of fish fauna. It was carried out along three linear transects in the same localities selected for macroalgal sampling (Table 2).

In Table 3 maximum depth, direction and observation time (minutes) for each survey transect are reported.

Table 3. Characteristics of visual census transects.

	Max. depth (m)	Route Direction	Observation time (mins)
Torre Colimena	6	S→N	13
Borraco	4,5	N→W	15
Monaco Mirante	5	N→S	18

Two independent SCUBA divers followed each transect annotating the individuals observed along a 200 m linear route. Notes were written with a lead pencil on a white plastic sheet. Observations were made between 11.00 am and 4.00 pm, in order to standardize as much as possible all surveys and to exclude the high activity periods of early morning and late afternoon (Halford and Thompson, 1994).

In order to record as many species as possible, each route was followed twice at different speeds. The first passage was carried out at a speed of about 30 m per minute to census the most mobile species; the second passage was performed at a lower speed searching for more cryptic and lower moving target species.

A third diver, equipped with a digital camera (Sony HDR-AS100V, Tokyo, Japan) housed in a waterproof underwater case (Sony MpK AS3, Tokyo, Japan), recorded videos in order to create permanent records of observations that were later analyzed in the laboratory. Identification of species was made by referencing the photographic texts available (Tortonese 1975; Louysy and Trainito 2006).

Coralligenous and animal biodiversity

The photographic sampling was carried out in the same period and in the same areas investigated for both fish fauna and macroalgal flora (Table 2). Three sites were randomly selected in each area and two divers collected 3 photographic samples of 1600 cm^2 in each site, using a digital camera (Sony HDR-AS100V, Tokyo, Japan) housed in a waterproof underwater case (Sony MpK AS3, Tokyo, Japan) (Fig. 4)

Fig. 4. 40 x 40 cm frame for photographic sampling.

Using a total of 27 photographs, the main morphological groups or species were identified to the lowest possible taxonomic level, on a purely qualitative basis. These photographs, together with videos recorded for the census of fish fauna, also allowed collecting information on the animal biodiversity present on the investigated coast, with respect to sponges, cnidarians, annelids, gastropod mollusks, crustaceans, echinoderms and tunicates.

Results and discussion

Posidonia oceanica meadows

The meadow located in the SCI "Torre Colimena" develops in the bathymetric range between 12 (upper limit) and 25 m depth (lower limit). According to the evaluation scale of water transparency proposed by Pergent *et al.* (1995), based on the mean depth of the *P. oceanica* lower limit, the waters of this area are classified as "slightly transparent waters". The meadow is a "pure bed", i.e. monospecific, considering that neither invasive macroalgae (such as the genus *Caulerpa* J.V. Lamouroux) nor other phanerogams occur.

The transect T1 extended over a length of about 2500 m from the coastline, along the N-S direction. Results for the two sampling stations of this transect are summarized in Table 4. The upper limit (sampling station T1S1) was located about 650 m from the coast and presented a patchy distribution on sandy bottom. The meadow shows an absolute density of 286.8±72.0 shoots/m² and belongs to the type IV ("very sparse bed") of Giraud's classification (1977). Considering that the meadow in this station shows a total cover of 75%, its relative density is 215.1±54.0 shoots/m². According to Pergent *et al.* (1995) and Pergent-Martini and Pergent (1996) classification, it appears to be "disturbed" and with a "low sub-normal density" (LSD) in showing values ranging between 191 and 303 shoots/m² (the "normal" values for a "bed in equilibrium" at this depth are between 303 and 527 shoots/m²).

Table 4. Bio-ecological data (mean ± SD) recorded in transect T1 (SCI "Torre Colimena").

Sampling date: 23/05/2015	T1S1 (Upper limit)		T1S2 (Lower limit)	
Depth (m)	12		25	
Absolute density (shoots/m²)	286,8±72,0		313,3±39,5	
Cover (%)	75		77	
Relative density (shoots/m²)	215,1±54,0		241,2±30,4	
Meadow classification (Giraud, 1977)	class IV	Very sparse	class III	Sparse

Meadow classification (Pergent *et al.*, 1995; Pergent-Martini and Pergent 1996)	class LSD	Disturbed	class ND	In equilibrium
Young leaf mean length (cm)	4,3±0,3		3,0±0,2	
Intermediate leaf mean length (cm)	15,3±5,0		17,0±4,2	
Adult leaf mean length (cm)	28,0±3,9		38,9±11,4	
Young leaf mean width (cm)	0,7±0,1		0,7±0,1	
Intermediate leaf mean width (cm)	0,7±0,2		0,9±0,1	
Adult leaf mean width (cm)	0,8±0,1		0,8±0,03	
Mean number of leaves per shoot	4,7±1,2		4,8±0,4	
Intermediate leaf mean Coefficient A (%)	36,4		18,2	
Adult leaf mean Coefficient A (%)	29,4		61,1	
Total Coefficient A (%)	32,1		44,8	
Intermediate leaf mean brown tissue (%)	0		0	
Adult leaf mean brown tissue (%)	0		0	
Mean leaf biomass (mg dw shoot^{-1})	235,0±72,8		678,5±194,9	
Mean epiphytes biomass (mg dw shoot^{-1})	11,0±10,9		116,8±72,4	
Mean leaf area (cm^2 shoot^{-1})	84,3±25,6		124,4±35,9	
LAI (m^2/ m^2)	1,8		3,0	
Annual rhizome production (mg dw/shoot/year)	27,7±17,4		39,0±28,8	
Number of flower peduncles	0		0	

The lower limit (sampling station T1S2) is clear-cut on sandy sediment, sometimes characterized by a "matte" about 20cm high with a prevalence of plagiotropic (horizontally oriented) shoots. The soft bottom is often modelled by oscillation waves forming ripple-marks where vegetal matter and dead *Posidonia* leaves accumulate (Fig. 5). The meadow shows an absolute density of 313.3±39.5 shoots/m^2 and belongs to the type III ("sparse bed") of Giraud's classification (1977), while since in this station it shows a total cover of 77%, its relative density is 241.2±30.4 shoots/m^2. On the basis of Pergent *et al.* (1995) and Pergent-Martini and Pergent (1996) classification, the meadow of this station is a "bed in equilibrium", with a "normal density" (ND) in showing values ranging from 116 to 340 shoots/m^2.

Fig. 5. Ripple-marks seabed with vegetal matter and dead leaves of *Posidonia*.

Considering the morphological parameters (Table 4) the mean number of leaves per shoot was similar for the two stations (4.7±1.2 for S1 and 4.8±0.4 for S2). The average length of young leaves resulted 4.3±0.3 cm in the upper limit and 3.0±0.2 cm in the lower limit. The mean length of intermediate leaves ranged between 15.3±5.0 cm in the upper limit and 17.0±4.2 cm in the lower limit.

The mean length of adult leaves varied between 28.0±3.9 cm in the upper limit and 38.9±11.4 cm in the lower limit. Brown tissue was absent on intermediate and adult leaves of both the stations. The leaf area per shoot showed mean values ranging between a minimum of 84.3±25.6 cm^2 in shallower stands and a maximum of 124.4±35.9 cm^2 at the lower limit.

Thus, considering that along the transect there were no significant variations in the mean number of leaves per shoot, the different mean leaf area was mainly due to the different mean leaf lengths that were calculated for different age classes (young, intermediate and adult leaves). In accordance with the shoot density data, the LAI value calculated at the lower limit (3.0 m^2/m^2) was higher than that observed at the upper limit (1.8 m^2/m^2). The mean leaf biomass per shoot ranged from 235.0±72.8 mg dw in the upper limit to 678.5±194.9 mg dw in the lower limit. The biomass of epiphytes per shoot showed mean values varying from a minimum of 11.0±10.9 mg dw at the upper limit to a maximum of 116.8±72.4 mg dw at the lower limit. The mean value of coefficient "A" (percentage of adult and intermediate leaves with broken apices) was higher at the lower limit (44.8%) than at the upper limit (31.2%).

The lepidochronological study focused on the identification of annual rhizomes cycles and the number of scales produced for each cycle. The number of cycles for rhizome, estimated within each bathymetric level, presented significant variations correlated to depth. It was growing from the upper limit (mean value of 8.7±5.0 cycles) to the lower limit (mean value of 9.5±4.0 cycles). The mean number of scales per rhizome, estimated within each sampling bathymetric level, did not present significant variations as a function of depth. It was found an average of 4.7±1.4 scales at the upper limit and 4.9±1.5 scales at the lower limit. In both the sampling stations the oldest rhizome was dated back to 2001 and no flower peduncles were found.

The rhizome production (mg dw $shoot^{-1}$ $year^{-1}$) showed an average value of 27.7±17.4 mg dw in the upper limit and 39.0±28.8 mg dw in the lower limit.

The meadow located in the SCI "Dune di Campomarino" develops in the bathymetric range between 11 (upper limit) and 23 m depth (lower limit). According to the evaluation scale of water transparency proposed by Pergent *et al.* (1995), the waters of this area are classified as "slightly transparent waters". The meadow is a "pure bed", not presenting interference by other phanerogams or invasive macroalgae.

The transect T2 extended over a length of about 2500 m from the coastline, along the N-S direction. Results for the two sampling stations of this transect are reported in Table 5. The upper limit (sampling station T2S1) was located about 900 m from the coast and presented a patchy distribution on a bottom showing an alternation of sandy and rocky horizontal tracts with a maximum height of about 2 m and a slight slope. The meadow shows an absolute density of 164.4 ± 40.6 shoots/m^2, thus belonging to the type IV ("very sparse bed") of Giraud's classification (1977), while since this station shows a total cover of 60%, its relative density is 98.6±24.4 shoots/m^2. According to Pergent *et al.* (1995) and Pergent-Martini and Pergent (1996) classification, the meadow appears to be "very disturbed" and with an "abnormal density" (AD) showing values below 213 shoots/m^2 (the "normal" values for a "bed in equilibrium" at this depth are between 325 and 549 shoots/m^2).

Table 5. Bio-ecological data (mean ± SD) recorded in transect T2 (SCI "Dune di Campomarino").

Sampling date: 09/05/2015	T1S1 (Upper limit)		T1S2 (Lower limit)	
Depth (m)	11		23	
Absolute density (shoots/m^2)	164,4±40,6		155,6±28,8	
Cover (%)	60		47	
Relative density (shoots/m^2)	98,6±24,4		70,8±13,5	
Meadow classification (Giraud, 1977)	Class IV	Very sparse	Class IV	Very sparse
Meadow classification (Pergent *et al.*, 1995; Pergent-Martini and Pergent 1996)	Class AD	Very disturbed	Class ND	In equilibrium
Young leaf mean length (cm)	2,5±0,2		4,1±0,2	
Intermediate leaf mean length (cm)	13,1±4,1		16,9±2,7	
Adult leaf mean length (cm)	30,2±4,9		42,4±11,9	
Young leaf mean width (cm)	0,7±0,06		0,8±0,05	

Intermediate leaf mean width (cm)	0,9±0,1	0,9±0,07
Adult leaf mean width (cm)	0,9±0,06	0,8±0,05
Mean number of leaves per shoot	4,7±0,8	8,7±5,0
Intermediate leaf mean Coefficient A (%)	50,0	13,3
Adult leaf mean Coefficient A (%)	62,5	43,2
Total Coefficient A (%)	57,1	34,6
Intermediate leaf mean brown tissue (%)	0	0
Adult leaf mean brown tissue (%)	0	0
Mean leaf biomass (mg dw shoot^{-1})	428,0±104,6	1150,7±737,5
Mean epiphytes biomass (mg dw shoot^{-1})	40,8±19,0	162,5±103,1
Mean leaf area (cm^2 shoot^{-1})	92,1±22,1	260,5±151,3
LAI (m^2/ m^2)	1,1	1,9
Annual rhizome production (mg dw/shoot/year)	35,2±25,9	37,2±28,8
Number of flower peduncles	0	0

The lower limit (sampling station T2S2) is clear-cut on sandy/rocky bottom and it is characterized by a sudden interruption of the meadow. It appears as a sparse bed among the formations of platform coralligenous, representing the dominant element. The meadow shows an absolute density of 155.6±28.8 shoots/m^2 and belongs to the type IV ("very sparse bed") of Giraud's classification (1977). Considering that the meadow in this station shows a total cover of 47%, its relative density is 70.8±13.5 shoots/m^2. On the basis of Pergent *et al.* (1995) and Pergent-Martini and Pergent (1996) classification, the meadow appears as a "bed in equilibrium" with a "normal density" (ND) in showing values between 137 and 361 shoots/m^2.

Considering the morphological parameters (Table 5), the mean number of leaves per shoot presented a minimum value of 4.7±0.8 leaves at upper limit and a maximum value of 8.7±5.0 leaves at lower limit. The mean length of leaves, calculated for different age classes, increased with depth. The average length of young leaves resulted 2.5±0.2 cm in the upper limit and 4.1±0.2 cm in the lower limit. The mean length of intermediate leaves ranged between 13.1±4.1 cm in the upper limit and 16.9±2.7 cm in the lower limit. The mean length of adult leaves varied between 30.2±4.9 cm in the upper limit and 42.4±11.9 cm in the lower limit. Brown tissue was absent on intermediate and adult leaves of both the stations. The leaf area per shoot showed mean values ranging between a minimum of 92.1±22.1 cm^2 in shallower stands and a maximum of 260.5±151.3 cm^2 at the lower limit.

The LAI value calculated at the lower limit (1.9 m^2/m^2) was higher than that observed at the upper limit (1.1 m^2/m^2). The mean leaf biomass per shoot ranged from 428.0±104.6 mg dw in the upper limit to 1150.7±737.5 mg dw in the lower limit. The biomass of epiphytes per shoot showed mean values varying from a minimum of 40.8±19.0 mg dw at the upper limit to a maximum of 162.5±103.1 mg dw at the lower limit. The mean value of coefficient "A" was higher at the upper limit (57.1%) than at the lower limit (34.6%).

In the lepidochronological study, the mean number of cycles for rhizome, estimated within each bathymetric level, did not present significant variations correlated to depth and varied between 9.7±4.1 cycles in the upper limit and 9.5±4.0 cycles in the lower limit. The mean number of scales per rhizome, estimated within each sampling bathymetric level, showed similar values of 5.0±1.1 scales at the upper limit and 4.9±1.5 scales at the lower limit. In both the sampling stations the oldest rhizome was dated back to 2001 and no flower peduncles were found.

The rhizome production (mg dw shoot^{-1} year^{-1}) showed an average value of 35.2±25.9 mg dw in the upper limit and 37.2±28.8 mg dw in the lower limit.
Analyzing the total results for the two investigated meadows, on the basis of the values of absolute density, according to Giraud's classification (1977), the meadow of "Torre Colimena" resulted "very sparse" at the upper limit and "sparse" at the lower limit, while the meadow of "Dune di Campomarino" resulted "very sparse" at both limits.

On the basis of Pergent *et al.* (1995) and Pergent-Martini and Pergent (1996) classification, at the upper limit the meadow of "Torre Colimena" resulted "disturbed" with "low sub-normal density", while the meadow of "Dune di Campomarino" resulted "very disturbed" with "abnormal density".

At the lower limit both the meadows resulted "in equilibrium" with "normal density". In studies carried out in 2004 (AA.VV., 2006; Costantino *et al.*, 2010), both meadows showed higher density values and were classified as "sparse" at the upper limit and "very sparse" at the lower limit, according to Giraud (1977), and "in equilibrium" with "normal density" according to Pergent *et al.* 1995 and Pergent-Martini and Pergent (1996), but anthropic disturbance and degradation, mainly related to the upper limit of the meadows were already reported.

Results of this study have confirmed the previous findings, showing, in both the meadows, the suffering state of upper limits that could be principally affected by the reduced transparency of the water, due to the presence of suspended material.

The LAI values at the lower limit of the two meadows fell within the normal range of 1.1 and 2.6 m^2/m^2 for meadows at this depth (Buia *et al.*, 2003). According to Buia *et al.* (2003) the number of leaves per shoot should be, normally, 8. The average number of leaves per shoot found in this study for both the meadows was generally below the normal value. However, other authors described the annual production of leaves as a not programmed, but casual event (Dolce *et al.*, 1996; Di Dato *et al.*, 2000). The coefficient "A", which expresses the combined effect of the mechanical action of hydrodynamism and the *grazing* of herbivores on the leaves, increased according to depth for the meadow of "Torre Colimena", while showed the opposite trend for the meadow of "Dune di Campomarino".

The longevity of the rhizomatous system of *Posidonia* resulted homogeneous in the two meadows, with an average age of about 9 years and no flower peduncles were found in all the analyzed samples. Hence, at least in the last 9 years, the environmental conditions were not suitable for *P. oceanica* flowering. The turnover of the meadow was probably assured only by vegetative reproduction, as it is also suggested by the high number of plagiotropic shoots observed in the field. Invasive macroalgae of the genus *Caulerpa* never occurred in both the meadows. It should be pointed out that well-structured *banquettes*, about 1 m high, located at Torre Colimena, were observed. These piles of dead *P. oceanica* leaves are a sign of good water quality and indicate the nearby presence of vaste healthy meadows. In the SPAMI Protocol of the Barcelona Convention (on Specially Protected Areas and Biodiversity in the Mediterranean) *banquettes* represent "priority habitats" worthy of protection.

Macroalgal flora

A total of 33 species (Table 6) were collected along the surveyed coastline, including 17 Rhodophyta (51.5%), 7 Phaeophyceae (Ochrophyta) (21.2%) and 9 Chlorophyta (27.3%). The most abundant algal orders were Ceramiales and Corallinales among Rhodophyta (47% and 29% respectively), Dictyotales and Ectocarpales among Ochrophyta-Phaeophyceae (43% and 29% respectively) and Cladophorales, Bryopsidales and Dasycladales among Chlorophyta (44.5%, 33.3% and 22.2% respectively).

From a biogeographic point of view, the flora was characterized by a high incidence of Atlantic (36.3%) and Cosmopolitan (33.3%) elements, followed by Circumtropical elements (18.2%). A lower incidence was shown by the Mediterranean (6.1%) and Indo-Pacific (6.1%) elements (Fig. 6). The R/P index was 2.4 and the R + C/P index was 3.7. Both indices showed intermediate values, characteristics of temperate regions.

Table 6. Floristic list. In the column chorology, phytogeographical elements are indicated according to Furnari *et al.* (2010): Ab = Boreo-Atlantic; At = Tropical Atlantic; IA = Indo-Atlantic; IAtf = Indo-Atlantic cold-temperate; C = Cosmopolite; SC = Sub-cosmopolitan; M = Mediterranean; IP = Indo-Pacific; CT = Circumtropical. The presence of each species in the 3 investigated stations (TC = Torre Colimena; BO = Borraco; MM = Monaco Mirante) is indicated with +.

Chorology	Taxa	TC	BO	MM
Rhodophyta				
SC	*Amphiroa rigida* J.V. Lamouroux	+	+	+
M	*Ceramium bertholdii* Funk	+		
SC	*Ceramium cimbricum* H.E. Petersen f. *flaccidum* (H.E. Petersen) G. Furnari & Serio	+	+	
IA	*Ceramium deslongchampsii* Chauvin ex Duby	+		
At	*Chylocladia verticillata* (Lightfoot) Bliding	+	+	
C	*Erythrotrichia carnea* (Dillwyn) J. Agardh		+	
CT	*Herposiphonia secunda* (C. Agardh) Ambronn	+	+	+
IA	*Hydrolithon cruciatum* (Bressan) Y.M. Chamberlain	+	+	+
C	*Jania rubens* (Linnaeus) J. V. Lamouroux v. *rubens*		+	
IAtf	*Jania virgata* (Zanardini) Montagne	+	+	+
IP	*Laurencia microcladia* Kützing	+	+	+
C	*Laurencia obtusa* (Hudson) J.V. Lamouroux	+	+	+
SC	*Palisada thuyoides* (Kützing) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii	+	+	+
IA	*Rhodothamniella floridula* (Dillwyn) Feldmann	+		
M	*Peyssonnelia squamaria* (S.G. Gmelin) Decaisne	+		+
IP	*Spongites fruticulosa* Kützing	+	+	
CT	*Wrangelia penicillata* (C. Agardh) C. Agardh	+	+	+
Ochrophyta (Phaeophyceae)				
Ab	*Cystoseira compressa* (Esper) Gerloff & Nizamuddin		+	
C	*Dictyota dichotoma* (Hudson) J.V. Lamouroux v. *dichotoma*	+	+	+
SC	*Dictyota dichotoma* (Hudson) J.V. Lamouroux v. *intricata* (C. Agardh) Greville	+	+	+
C	*Feldmannia irregularis* (Kützing) G. Hamel	+	+	
Ab	*Myriactula stellulata* (Harvey) Levring		+	
CT	*Padina pavonica* (Linnaeus) Thivy	+	+	
SC	*Sphacelaria cirrhosa* (Roth) C. Agardh	+	+	+
Chlorophyta				
IA	*Acetabularia acetabulum* (Linnaeus) P.C. Silva	+	+	+
CT	*Anadyomene stellata* (Wulfen) C. Agardh		+	
IA	*Cladophora dalmatica* Kützing	+		
IA	*Cladophora prolifera* (Roth) Kützing	+	+	+
At	*Dasycladus vermicularis* (Scopoli) Krasser	+	+	+
SC	*Derbesia tenuissima* (Moris & De Notaris) P.L. Crouan & H.M. Crouan		+	
At	*Flabellia petiolata* (Turra) Nizamuddin	+		
CT	*Halimeda tuna* (J. Ellis & Solander) J.V. Lamouroux	+	+	+
CT	*Valonia utricularis* (Roth) C. Agardh	+	+	+

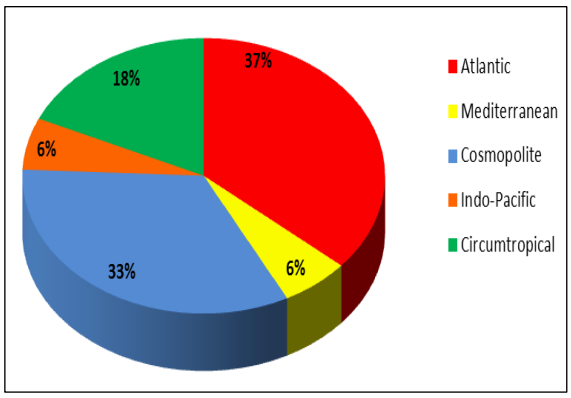

Fig. 6. Chorological *spectrum* of the macroalgal flora.

The TC station (Torre Colimena) has a sandy bottom, with evident *ripple marks*, which continues with a rocky platform at about 200 m from the coast. In this station, 27 *taxa* including 15 Rhodophyta, 5 Ochrophyta-Phaeophyceae and 7 Chlorophyta were found. Single orthotropic shoots of *P. oceanica* were also present at a depth of about 6 m.

The BO station (Borraco) is located near the mouth of the river Borraco, approximately 0.4 km long and fed by underwater springs.

The seabed is characterized by a flat rocky platform, covered by sandy debris. In total, in this station, 27 *taxa* including 13 Rhodophyta, 7 Ochrophyta-Phaeophyceae and 7 Chlorophyta were collected. Single orthotropic shoots of *P. oceanica* at a depth of 4.5 m and rhodoliths of *Spongites fruticulosa* Kützing, about 2 cm in diameter, occurred.

The MM station (Monaco Mirante) has a rocky bottom. A total of 17 *taxa*, including 9 Rhodophyta, 3 Ochrophyta-Phaeophyceae and 5 Chlorophyta were found. In the investigated coastal area, the infralittoral fringe is populated by photophilous algae such as red (*Laurencia* J.V. Lamouroux, *Palisada* K.W. Nam, *Jania* J.V. Lamouroux and encrusting Corallinales) and brown algae mainly belonging to the order Dictyotales.

Photophilous algae dominate infralittoral rocks also at a major depth. The most representative species are the brown *Dictyota dichotoma* (Hudson) J.V. Lamouroux, *D. dichotoma* var. *intricata* (C. Agardh) Greville and *Padina pavonica* (Linnaeus) Thivy, the green *Acetabularia acetabulum* (Linnaeus) P.C. Silva and *Halimeda tuna* (J. Ellis & Solander) J.V. Lamouroux and the red *Laurencia microcladia* Kützing, *Laurencia obtusa* (Hudson) J.V. Lamouroux, *Palisada thuyoides*, (Kützing) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii, *Jania virgata* (Zanardini) Montagne and *Amphiroa rigida* J.V. Lamouroux.

Some photophilous and thermophilous species, represented in shallow waters by the green *Anadyomene stellata* (Wulfen) C. Agardh and *Dasycladus vermicularis* (Scopoli) Krasser also occur. At Monaco Mirante evidence of *overgrazing* by sea urchins (*Paracentrotus lividus* Lamarck 1816) and the subsequent formation of bare rocks or substrates exclusively colonized by articulated (*Amphiroa* J.V. Lamouroux and *Jania*) or encrusting (*Hydrolithon* (Foslie) Foslie) calcareous algae were observed. Throughout the area, structure-forming species of the genus *Cystoseira* C. Agardh were not found. Some isolated thalli of *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, a species considered more tolerant to pollution (Ballesteros *et al.* 2007) were only collected at Borraco station.

Similarly, opportunistic and nitrophilous macroalgae such as Ulvales among Chlorophyta and Gelidiales among Rhodophyta were absent The presence of single orthotropic shoots of *P. oceanica* in shallow waters at Torre Colimena and Borraco, is an indicator of a good ecological state of coastal waters.

The 33 records found in this study are all new to this coastline, never investigated before. The considerable occurrence of Indo-Pacific and Circumtropical elements in the chorological spectrum, even if referred to a single seasonal sampling, with percentage values much higher than those of the Apulian (Cormaci *et al.*, 2001) and Italian (Furnari *et al*, 2010) floristic contingent, emphasises the affinities with floras of the eastern Ionian Sea. On the other hand, both the Ionian Sea and the southern Aegean Sea are considered as a wide refuge area for Circumtropical and Indo-Pacific species (Giaccone and Geraci, 1989).

Fish fauna

The visual census of fish fauna led to the identification of 15 species of bony fish belonging to 8 families (Table 7). The family Sparidae was represented by the largest number of species (4 species, 27%), followed by Gobidae (3 species, 20%), Labridae (2 species, 13%), Tripterygidae (2 species, 13%). The remaining families were each represented by one species (7%). At Monaco Mirante the highest number of species (11) was found, followed by Borraco (6) and Torre Colimena (3). Juvenile stages were observed in all the three stations, in particular at Monaco Mirante for *Umbrina cirrosa* Linnaeus 1758, a species considered as vulnerable in the IUCN Red List (2015). Both male and female individuals of *Coris julis* Linnaeus 1758 were found in the 3 stations. Males of *Tripterygion tripneronotum* Risso 1810 in reproductive livery were observed at Borraco station. Noteworthy is the presence of male and female individuals of *Thalassoma pavo* Linnaeus 1758 at Monaco Mirante station. This species is considered non-native of the Mediterranean Sea.

During the sampling of *P. oceanica* at "Dune di Campomarino", near the lower limit of the meadow about two dozen of individuals of the species *Apogon imberbis* Linnaeus 1758, commonly known as "Cardinal fish", were observed. Since there are no previous data on this area, it is not possible to make a comparison with other *checklists* of fish fauna.

Coralligenous and animal biodiversity

In the investigated area, some calcareous algae that characterize the coralligenous biocenosis were identified. These sciaphilous populations generally occur in the circalittoral, but, in this case, discontinuously colonized the infralittoral poorly illuminated rocky substrates as well as rhizomes of *P. oceanica*. The characteristic species of this biocenosis are calcareous habitat forming algae, such as *Halimeda tuna* (J. Ellis & Solander).

J.V. Lamouroux and *Spongites fruticulosa* Kützing, and species of the genus *Peyssonnelia* Decaisne, such as *Peyssonnelia squamaria* (S.G. Gmelin) Decaisne. Bryozoans, sedentary polychaetes, sponges and other sessile animals also contribute to the biogenic construction. As above mentioned, the observation of images for the coralligenous sampling and of videos recorded for the census of fish fauna has also allowed to collect information on animal biodiversity along the investigated coastline. A *checklist* of the main *taxa* is reported in Table 8. A total of 22 taxa were identified, four of which only at a genus level, represented by Porifera (4 species), Cnidaria (3), Gastropod Mollusks (4), Crustaceans (1), Echinoderms (5), Annelids (3) and Tunicates (2). Five species (4 invertebrates and the fish *U. cirrosa*) recorded in this study are of particular relevance to the Mediterranean Biological Diversity (UNEP-RAC/SPA 2002) and deserve conservation under the Berne Convention and the ASPIM Protocol of the Barcelona Convention (Tables 7, 8).

Table 7. List of fish species found in the 3 stations (TC = Torre Colimena; BO = Borraco; MM = Monaco Mirante). The presence is indicated with +. (Species of conservation interest: *UNEP-RAC/SPA 2002; °Annex 3 ASPIM, Barcelona Convention; ·Annex 3 Bern Convention).

Family	Species	TC	BO	MM
Centracanthidae	*Spicara maena* Linnaeus 1758		+	
Gobidae	*Gobius bucchichi* Steindachner 1870			+
	Gobius couchi Miller & El-Tawil 1974			+
	Gobius geniporus Valenciennes 1837			+
Labridae	*Coris julis* Linnaeus 1758	+	+	+
	Thalassoma pavo Linnaeus 1758			+
Pomacentridae	*Chromis chromis* Linnaeus 1758			+
Sciaenidae	*Umbrina cirrosa**,°,· Linnaeus 1758			+
Serranidae	*Serranus cabrilla* Linnaeus 1758		+	+
Sparidae	*Diplodus annularis* Linnaeus 1758	+	+	+
	Diplodus sargus Linnaeus 1758	+		
	Diplodus vulgaris Geoffroy Saint-Hilaire 1817			+
Tripterygidae	*Oblada melanura* Linnaeus 1758		+	
	Tripterygion delaisi Cadenat & Blache 1970		+	
	Tripterygion tripneronotum Risso 1810		+	+

Table 8. List of animal *taxa* (species of conservation interest: *UNEP-RAC/SPA 2002; °Annex 3 Bern Convention; Annex 2, 3 ASPIM, Barcelona Convention).

Phylum	Species
Porifera	*Aplysina aerophoba**,°,· Nardo 1833
	Crambe crambe Schmidt 1862
	Ircinia variabilis Schmidt 1862
	*Spongia officinalis**,°,· Linnaeus 1759
Cnidaria	*Actinia striata* Quoy & Gaimard 1833
	Anemonia viridis Forsskal 1775
	Condylactis aurantiaca Delle Chiaje 1825
Mollusca	*Cerithium vulgatum* Bruguière 1792
	Hexaplex trunculus Linnaeus 1758

Phylum	Species
Crustacea	*Nassarius* sp.
	Tarantinaea lignaria Linnaeus 1758
	*Scyllarides latus**,º,· Latreille 1802
Echinodermata	*Echinaster sepositus* Retzius 1805
	Holothuria forskali Delle Chiaje 1841
	Holothuria tubulosa Gmelin 1791
	Marthasterias glacialis Linnaeus 1758
	*Paracentrotus lividus**,º,· Lamarck 1816
Annelida	*Arenicola* sp.
	Protula sp.
	Sabella spallanzani Viviani 1805
Tunicata	*Microcosmus* sp.
	Polycitor adriaticus Drasche 1883

Conclusion

This preliminary investigation, primarily based on visual sampling methods, assessed the occurrence of 70 vegetal and animal *taxa*. It is clear that the lists reported in this paper cannot be considered exhaustive, but they represent a real "snapshot" of the present biodiversity. Generally, the surveyed coastline has the following principal characteristics: i) benthic habitats of high conservation importance such as *P. oceanica* seagrass meadows and coralligenous formations, allowing colonization by many invertebrates and promoting an increasing biodiversity; ii) occurrence of species that need protection measures already suggested by some European Community guidelines (Bern Convention 19/09/1979, Habitats Directive 92/43/EEC, ASPIM Protocol of Barcelona Convention 10/06/1995, ratified in Italy by Law 25/05/1999 n. 175); iii) limited presence of species with a wide ecological valence; iv) none record of non-native or invasive species, with the only exception of *Thalassoma pavo*, which has become settled in the Mediterranean. All the valuable elements recorded in the investigated area can benefit from the protection provided by the establishment of the future MPA that could represent a very successful way to preserve marine biodiversity, also supporting a general increase in the abundance and availability of the already existing benthic and bentho-pelagic resources. Moreover, the Mandurian area seems to be subjected to limited anthropic pressure, mainly related to its seaside tourism activities, and is therefore suited to the adoption of conservation measures of the marine coastal environment.

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