Preliminary evaluation of the effects of *Caulerpa cylindracea* on *Posidonia oceanica* through the analysis of primary production and morphometric characteristics

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INTRODUCTION

Today, biological invasions represent a threat to endemic animal and plant communities and a major cause of biodiversity loss worldwide. In the Mediterranean Sea, there are about 100 species of macrophytes, introduced intentionally or accidentally, most of which are highly invasive. Among these, the macroalga *Caulerpa cylindracea* Sonder, 1845, entered in the Mediterranean basin since 1990 through the Suez Canal and now it is widespread along the Italian coasts. This species is able to colonize a high number of coastal substrates and it can affect the density of some seagrasses, such as *Cymodocea nodosa* (Ucria) Ascherson, 1870 and *Posidonia oceanica* (Linnaeus) Delile, 1813 . Its colonization ability is enhanced in environments with a high concentration of nutrients and its growth can modify the redox potential of the substrate making it unsuitable for the establishment of other seagrasses and algae. This work aimed to analyse and describe the potential interaction between the *C. cylindracea* and *P. oceanica* in the coastal area of Civitavecchia. The potential effects of this interaction were studied inside of two different *P. oceanica* patches, located at a depth of 3-5 m and characterized by the presence/absence of the invasive alga, through the morphostructural analysis of the two species. In particular, the seagrass growth and primary production were analysed using some direct and indirect techniques (phenology and lepidochronology), while for the alga were analysed the phenological characteristics and the percentage of coverage of the substrate. The sampling campaigns were carried out in two different months of the same year, June and October 2019, in order to observe both the growth phase and the maximum bloom phase of the *C. cylindracea*.

MATERIALS AND METHODS

C. cylindracea and *P. oceanica* were sampled within the project PO-FEAMP 2014-2020 "Study of the effects of the invasive alien species *Caulerpa racemosa* on Natura 2000 sites and impact on fishing activities " in the SCI IT60000005 (seabed between Punta S. Agostino and Punta della Mattonara), following the sampling plan and technical details of Piazzi and Ceccherelli (2006) as well as the ecology of the species (Ruitton et al., 2005) for *C. cylindracea*, while for *P. oceanica* shoots were collected according to ICRAM-ISPRA standard protocols (Cicero and Di Girolamo, 2001; Buia et al., 2003). Monitoring and sampling operations were performed by SCUBA divers in July and October 2019, respectively the peak of proliferation and the end of the reproductive period of the seaweed. *C. cylindracea* samples were taken by scraping a 400 cm^2-metal reference square in triplicate, which had been photographed/filmed for subsequent in-lab image analysis, and storing *C. cylindracea* specimens in 80% ethanol-filled plastic containers. Cover is expressed as a percentage of seabed surface covered by vertically-projected vegetative material (Boudouresque, 1971).

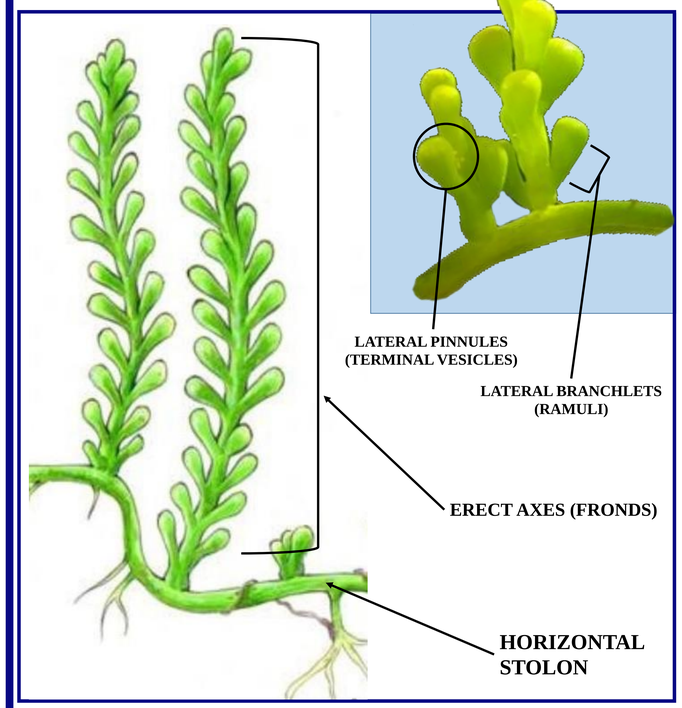
For each replicate, the following phenological parameters were measured: thickness of the horizontal stolons, length of the erect axes (fronds), number of fronds per stolon, length of lateral branchlets (ramuli), diameter of the lateral pinnules (terminal vesicles) and number of vesicles per stolon (Buia et al., 2001). At the same time, the seagrass bed density was estimated using a 40 cm side square (three replicates), and 15 orthotropic shoots were sampled. After being transferred to the laboratory, phenological and lepidochronological analyses were carried out on the shoots according to the protocol described by Buia et al. (2003). For each replicate shoot, the following parameters were concerned: leaf width and length, total leaf area per shoot, n° of leaves per shoot, leaf and rhizome biomass and primary production. Sediment samples were collected to determine the concentrations of nutrients in the investigated areas. In the selected sites, 1 sediment sample was collected for each sub-area (invaded area and non-invaded area) by using pre-labeled sterile bottles and then analyzed according to the method described in DM 13/09/1999 OJ No. 248 21/10/1999.

Statistical analyses

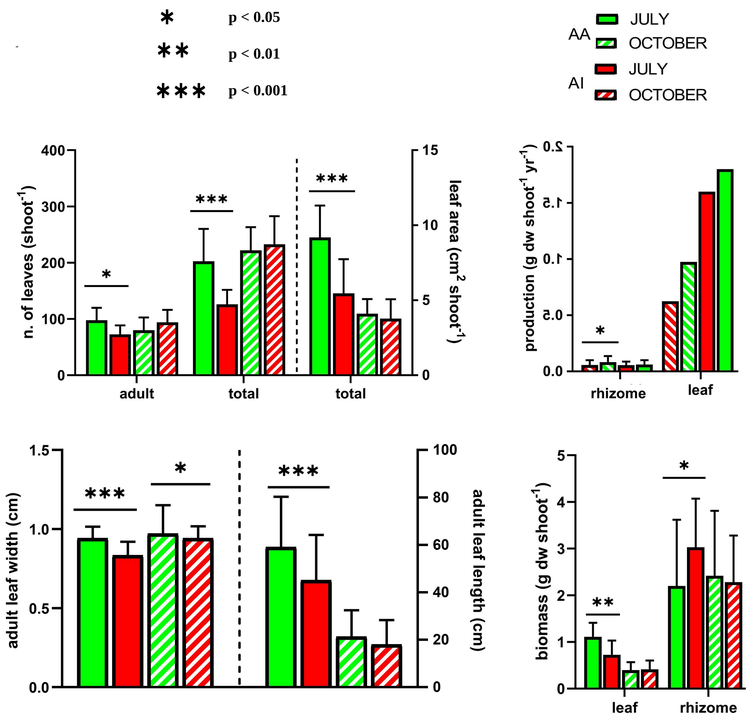
Both *C. cylindracea* and *P. oceanica* datasets were firstly verified to fulfill parametric conditions with a Shapiro-Wilk test. *C. cylindracea* phenology data were statistically analyzed with a two-way ANOVA followed by a Sidak posthoc multiple comparison test for statistically significant differences between sampling periods. *P. oceanica* morphological parameters were statistically analyzed with a one-way ANOVA when the assumption of normal distribution was confirmed, otherwise Kruskal-Wallis test was applied.

RESULTS

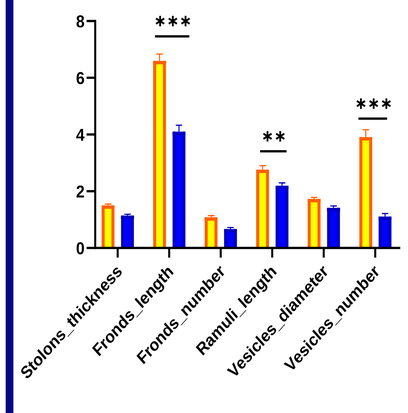
Caulerpa cylindracea Sonder, 1845



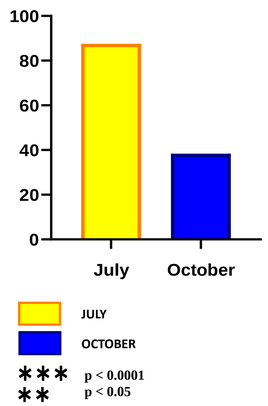
Posidonia oceanica morphological parameters



Caulerpa morphological parameters



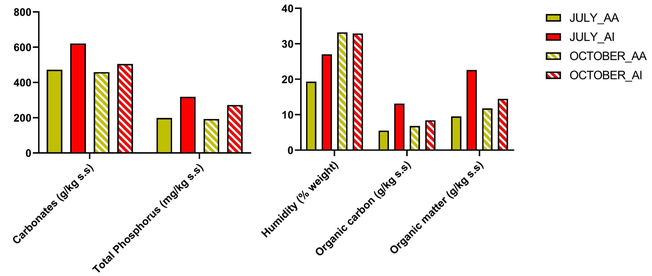
Caulerpa percentage cover



Multiparametric probe Idronaut 316 plus; Turbidimeter Cyclops 7™ Turner Design

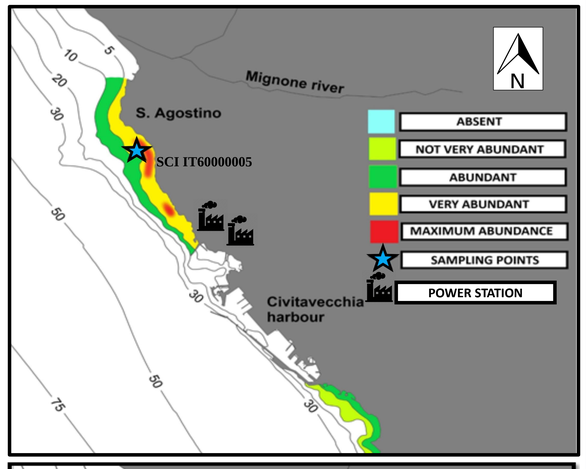
Wather column						
Period	**Temp (C°)**	**Sal (psu)**	**O2(% sat)**	**pH**	**CHL a (µg/l)**	**Turbidity (ntu)**
July	26.98	39.96	100.5	8.4	1.4	14.95
October	20.95	38.06	97.0	8.07	0.24	14.87

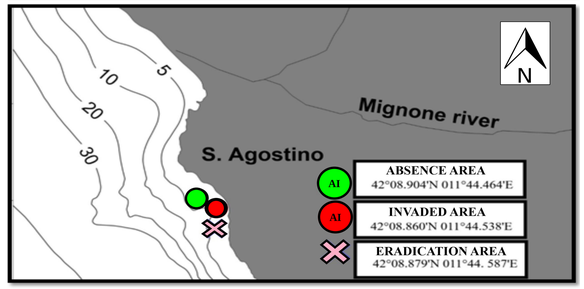
Sediment Nutrient analysis



STUDY AREA

The coastal area of the northern Tyrrhenian Sea (Italy), provides different scenarios due to its natural and anthropogenic features: the area around Civitavecchia, the most urbanized city in central Italian coast, is characterized by several wastewater discharges and by the presence of the Mignone river which is the main source of shore sediments. Moreover, the city of Civitavecchia hosts industrial activities of national and international interest such as the Port of Civitavecchia and the Torrevaldaliga Nord coal-fired power plant (TVN). The sampling locality (named "La Frasca") is only 2 km from the commercial Port of Civitavecchia, at the study site, the bed of *P. oceanica* occurs at depths between 1 m and about 20 m, and its architecture shows a fragmented coverage that is characterized by the presence of several circular sandy patches and mixed rocks.





CONCLUSIONS

Several studies have evidenced that *C. racemosa* mostly colonizes fragmented or low shoot density meadows of *P. oceanica* (Ceccherelli et al., 2000; Katsanevakis et al., 2010; Buller et al., 2011), where it is generally encountered at the edge or within disturbed meadows (Katsanevakis et al., 2010). To date, very few studies have described the vegetative cycle and primary production of *P. oceanica* concerning *C. cylindracea* presence (Dumay et al., 2002), and our work represents the first study in the coastal sector of Civitavecchia. We observed that the mean adult leaf length and leaf surface area per shoot were significantly higher in the absence area ($p < 0.001$ and $p < 0.001$, respectively), confirming the expected trend to increase with decreasing level of competitive interaction (Dumay et al., 2002; Villèle & Veralque, 1994, 1995). Our analysis has evidenced a significant difference between the two areas for several parameters which resulted higher in the absence area, such as the adult leaf width during both sampling periods ($p < 0.001$ and $p < 0.05$, respectively in July and October 2019), number of adult and total leaves ($p < 0.05$ and $p < 0.001$ respectively), and, leaf and rhizome biomass ($p < 0.01$ and $p < 0.05$ respectively) in July 2019.The parameters observed in the two areas showed only significant differences in summer. Seasonal variations (July-October) are also highlighted by Villèle & Verlaque (1995), and constitute symptoms of regression. In conclusion, our study showed the presence of a direct interaction between *C.cylindracea* and the *P.oceanica,* located in SCI IT60000005, which could lead to a decrease in the biological and structural qualities of the seagrass meadow. Further studies will be useful to analyze the level of this interaction and to develop a management and conservation plan.

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