



A new record of the invasive seaweed *Caulerpa cylindracea* Sonder in the South Adriatic Sea



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ARTICLE INFO

Keywords:

Environmental science
Ecology
Aquatic ecology
Ecosystem change
Flora
Biological pollutants
Invasive macrophytes
Mediterranean sea
Caulerpa cylindracea
Bioinvasion
Tropicalisation

ABSTRACT

The green alga *Caulerpa cylindracea* Sonder is one of the most infamous and threatening invasive species in the Mediterranean Sea. Since 1985, it started rapidly spreading to all Mediterranean regions causing many ecological changes on natural communities. In the present study, we present an example of this proliferation with the first record in the Marine Protected Area of Tremiti Island (MPATI) in the South Adriatic Sea. Fifteen sites along the coast and 5 different depths have been investigated. Our results provide evidence of a wide invasion of this pest in three islands, San Domino, San Nicola and Capraia. This study fills a particular data gap in the ongoing bio-monitoring of invasive seaweeds in the Mediterranean Sea representing a base line of this invasive species for the MPATI.

1. Introduction

Ongoing climate change and impacts related to human population increases, including aquaculture, shipping and transportation are considered important driving forces behind the intensification of biological invasion phenomena worldwide (Strefaris et al., 2005; Occhipinti-Ambrogi, 2007; Schaffelke and Hewitt, 2007; Jauni et al., 2015). The scientific community has highlighted either positive, negligible and negative relationships between native biodiversity and invasions of exotic species (Lonsdale, 1999; McKinney and Lockwood, 1999; Byrnes et al., 2007; Fridley et al., 2007; Stachowicz et al., 2007; Wallentinus and Nyberg, 2007; Rilov and Crooks, 2009; Tamburello et al., 2015). It is however generally accepted that interactions between invasive species and native communities cause biotic and abiotic changes (Levine and D'Antonio, 1999; Ceccherelli and Sechi, 2002; Grosholz, 2002; Kennedy et al., 2002; Arenas et al., 2006; Beisner et al., 2006; Bulleri and Benedetti-Cecchi, 2008; Piazzini and Balata, 2009). Although invasive species affect marine ecosystems at the global scale, the Mediterranean Sea is amongst the most severely affected, with approximately 1000 introduced species, that now represent more than 5% of the known flora and fauna (Boudouresque et al., 2005; Occhipinti-Ambrogi and Shepard, 2007; Galil, 2008; Zenetos et al., 2012; Gorbi et al., 2014). There is

a pressing need to understand the mechanisms regulating species invasions both to predict pathways of invasion and to control their spread (Strefaris and Zenetos, 2006; Anderson, 2007; Gollasch et al., 2007; Hewitt and Campbell, 2007).

The alien green alga *Caulerpa cylindracea* Sonder is one of the most infamous and threatening invasive species in the Mediterranean Sea (Piazzini et al., 2005a,b; Montefalcone et al., 2015). This species was first found in the Mediterranean Sea along the Tunisia coast in 1985 (Sghaier et al., 2015), being introduced from south-western Australia (Famà et al., 2000; Verlaque et al., 2003; Belton et al., 2014). It was subsequently reported along the coastline of 12 Mediterranean countries. On the Italian coast, this species was first reported by Alongi et al. (1993), followed by the coasts of Greece (Panayotidis and Montesanto, 1994), Albania (Di Martino and Giaccone, 1995), Cyprus (Hadjichristophorou et al., 1997), France (Jousson et al., 1998), Spain (Ballesteros et al., 1999), Tunisia (Belkhiria, 1999), Turkey (Cirik, 1999), Malta (Stevens, 1999), Algeria (Verlaque et al., 2003; Ould-Ahmed and Meinesz, 2007), Croatia (Žuljević et al., 2003) and Montenegro (Mačić and Kaščelan 2006) also within many Marine Protected Areas (Katsanevakis et al., 2010; Felling et al., 2012). The species has been recorded on a variety of substrates and benthic assemblages, between 0 and 70 m depth, in both polluted and unpolluted areas, and proliferated rapidly showing high

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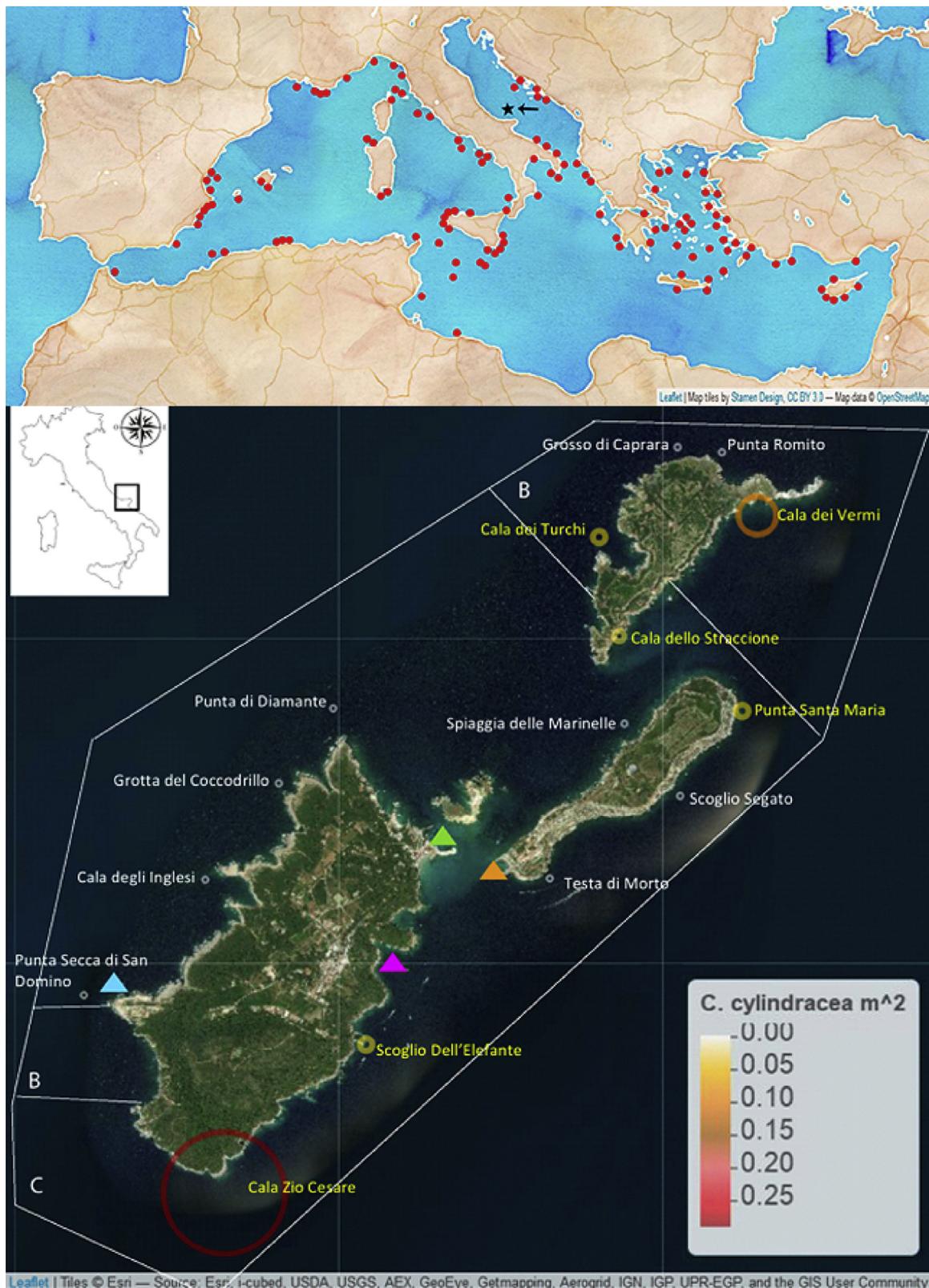


Fig. 1. a) Updated geographical distribution of *Caulerpa cylindracea* in the Mediterranean Sea. Black dots denote invaded locations cited in Verlaque et al., (2000); Ruiz et al., 2007; Sciberras and Schembri (2007); Klein and Verlaque (2008); Bouiadjra et al., (2010); Guillén et al., 2010; Rivera-Ingraham et al., (2010); Tsiamis et al., (2010); Bentaallah and Kerfouf (2013); Otero et al., (2013); Altamirano et al., (2014); star with black arrow indicates the new reported presence; b) Map of the MPA of Tremiti Islands with the 15 transects (7 labeled in yellow with presence of *C. cylindracea*; 8 labeled in white without); and the four alleged pollution sources; Gas station (sky blue triangle Site A); Port of San Domino (green triangle Site B); Port of San Nicola (orange triangle Site C); Water Tanker Vessel (fuchsia triangle Site D). The circle size and color refer to the cover of *C. cylindracea* as total sum in all quadrats and in all depths of the sites where it has been reported.

adaptability to physical stressors (Verlaque et al., 2000, 2003; Capiomont et al., 2005; Piazzini et al., 2005a,b; 2016; Strefataris and Zenetos, 2006; Tsiamis et al., 2008; Cebrian and Ballesteros, 2009; Piazzini and Balata, 2009; Altamirano et al., 2014; Bulleri and Malquori, 2015) displaying a maximum growth rate and yield at 27 °C and 25 °C, respectively, and maintaining an high eco-physiological rates between 25 °C and 29 °C (Sampeiro-Ramos et al., 2015). It can spread by fragmentation (Smith and Walters, 1999) sexual reproduction (Panayotidis and Žuljević, 2001) and its spherical branchlets can also act as propagules (Renoncourt and Meinesz, 2002).

Caulerpa cylindracea exerts negative effects on marine macrophytes (Ceccherelli and Campo, 2002; Raniello et al., 2007), and can alter the behavior of native species, with putative adverse repercussions on patterns of fish growth and population dynamics (Magliozzi et al., 2017). This seaweed can exert relevant effects on composition of sedimentary organic matter (OM), and on the associated microbial populations (Rizzo et al., 2017). There are other well documented negative implications; on alpha diversity of benthic assemblages (Piazzini et al., 2001; Balata et al., 2004; Piazzini and Balata, 2008; Pacciardi et al., 2011); on Carbon turnover in invaded sediments (Pusceddu et al., 2016); on native macroalgal assemblages (Piazzini and Ceccherelli, 2006); and on macrofauna (Lorenti et al., 2011; Cantasano et al., 2017) such as, amphipods (Vazquez-Luis et al., 2008). The aim of the present study is to report a new record of *Caulerpa cylindracea* in the South Adriatic Sea reporting the magnitude of its invasion in the Marine Protected Area of Tremiti Island (MPATI).

2. Materials and methods

2.1. Sampling

The survey was conducted in the MPATI located in the southern Adriatic Sea (Figure 1a, b), founded in 1989. The MPATI is divided into three main management zones (A, B, C). The A Zone is the no entry-no take area and only few scientific activities with specific authorizations

are permitted. In the B Zone, anchoring, spearfishing and recreational fishing are forbidden. Scientific activities, navigation, diving and artisanal fishing are regulated by specific authorizations; swimming is permitted. In the C Zone, spearfishing is forbidden, artisanal fishing and scientific activities are regulated by authorizations. Navigation, swimming, anchoring, diving and recreational fishing are permitted.

Field work was conducted during August and September 2013. Five different depths (5, 10, 15, 20, and 25 m) were sampled at 15 sites along the coast of the three islands of MPATI (San Domino, San Nicola, and Capraia) by SCUBA diving. At each depth, 10 random quadrats 20 × 20 cm² were photographically sampled using a Canon G11 (CanonG11) (Klein and Verlaque, 2008; Baldacconi and Corriero, 2009; Katsanevakis et al., 2010; Cantasano et al., 2017). A total of 730 photos (in San Nicola all 5 depths are not present, see Table 1 and Fig. 2) were taken. Wherever present, thalli of *C. cylindracea* were scraped off from each quadrat and stored in individual plastic bags. Seawater temperature, seafloor slope and substrate main features were recorded (Tables 2, 3, and 4).

2.2. Data analyses

Presence/absence (P/A) of fauna and flora were recorded at the taxa level for all photos. *Caulerpa cylindracea* percentage cover was estimated with image analysis by Photoshop and SeaScape software. The sampled algae were dried and then weighed. Dry weight was obtained after drying at 60 °C to constant weight. Wind exposure was recorded as mean wind affecting each individual site. As corroborated by the frequency and velocity data gathered by the airport of Foggia-Gino Lisa (Foggia-Gino Lisa), the primary winds blowing across the study area are: Scirocco (South-East) and Maestrale (North-West). The wind direction was associated to each study site based on the site's exposure. The distance of each site from four alleged pollution sources: Gas Port of San Domino, Port of San Nicola, Water Tanker Vessel (Fig. 1b), was calculated as shortest path along the coastline separating the two. Data were analyzed using a constrained correspondence analysis CCA. All data used for this

Table 1

Sites and number of visual quadrats where *C. cylindracea* was recorded for each island, site and depth. Only the quadrats with *C. cylindracea* are reported. The cover is reported as cumulative cover surface in m² and the biomass as cumulative sum of dry-weight biomass in g/m² for each depth.

Island	Site	Latitude	Longitude	Depth (m)	Quadrats	<i>C.cylindracea</i> m ²	Dry weight (g/m ²)	
SD	Cala Zio Cesare	42.10378° N	15.48297° E	5/10	0	0	0	
				15	6	2.9·10 ⁻²	6·10 ⁻⁷	
				20	10	0.1	1.7·10 ⁻⁶	
	Scoglio Dell'Elefante	42.11045° N	15.49262° E	5/10/15	0	0	0	
				20	3	2.8·10 ⁻²	4.6·10 ⁻⁷	
				25	0	0	0	
	Punta Di Diamante	42.12730° N	15.49037° E	5/10/15/20/25	0	0	0	
				Grotta Del Coccodrillo	42.12359° N	15.48668° E	5/10/15/20/25	0
	Cala Degli Inglesi	42.11872° N	15.48172° E	5/10/15/20/25	0	0	0	
				Punta Secca Di San Domino	42.11291° N	15.47351° E	5/10	0
					15	3	2.7·10 ⁻³	6.3·10 ⁻⁸
					20/25	0	0	0
					5/10/15/20/25	0	0	0
	SN	Testa Di Morto	42.11877° N	15.50503° E	5/10/15/20/25	0	0	0
					5/10/15/20/25	0	0	0
Scoglio Segato		42.12296° N	15.51393° E	5/10/15	0	0	0	
				20	2	1.9·10 ⁻²	3.4·10 ⁻⁷	
Punta Santa Maria		42.12720° N	15.51814° E	5/10/15	0	0	0	
CA	Spiaggia Delle Marinelle	42.12655° N	15.51009° E	5/10/15/20	0	0	0	
				Cala Dello Straccione	42.13094° N	15.50980° E	5/10	0
	Cala Dei Vermi	42.13757° N	15.51910° E	15	4	2.5·10 ⁻²	6.8·10 ⁻⁷	
				5	0	0	0	
				10	1	4.5·10 ⁻³	7.4·10 ⁻¹²	
				15	5	4·10 ⁻²	5.9·10 ⁻⁷	
				20	4	2.1·10 ⁻²	3.3·10 ⁻⁷	
	Punta Romito	42.14018° N	15.51670° E	5/10/15/20/25	0	0	0	
				Grosso di Caprara	42.14044° N	15.51374° E	5/10/15/20/25	0
	Cala Dei Turchi	42.13593° N	15.50845° E	5/10/15	0	0	0	
20				4	3.1·10 ⁻²	8·10 ⁻⁷		
25				0	0	0		

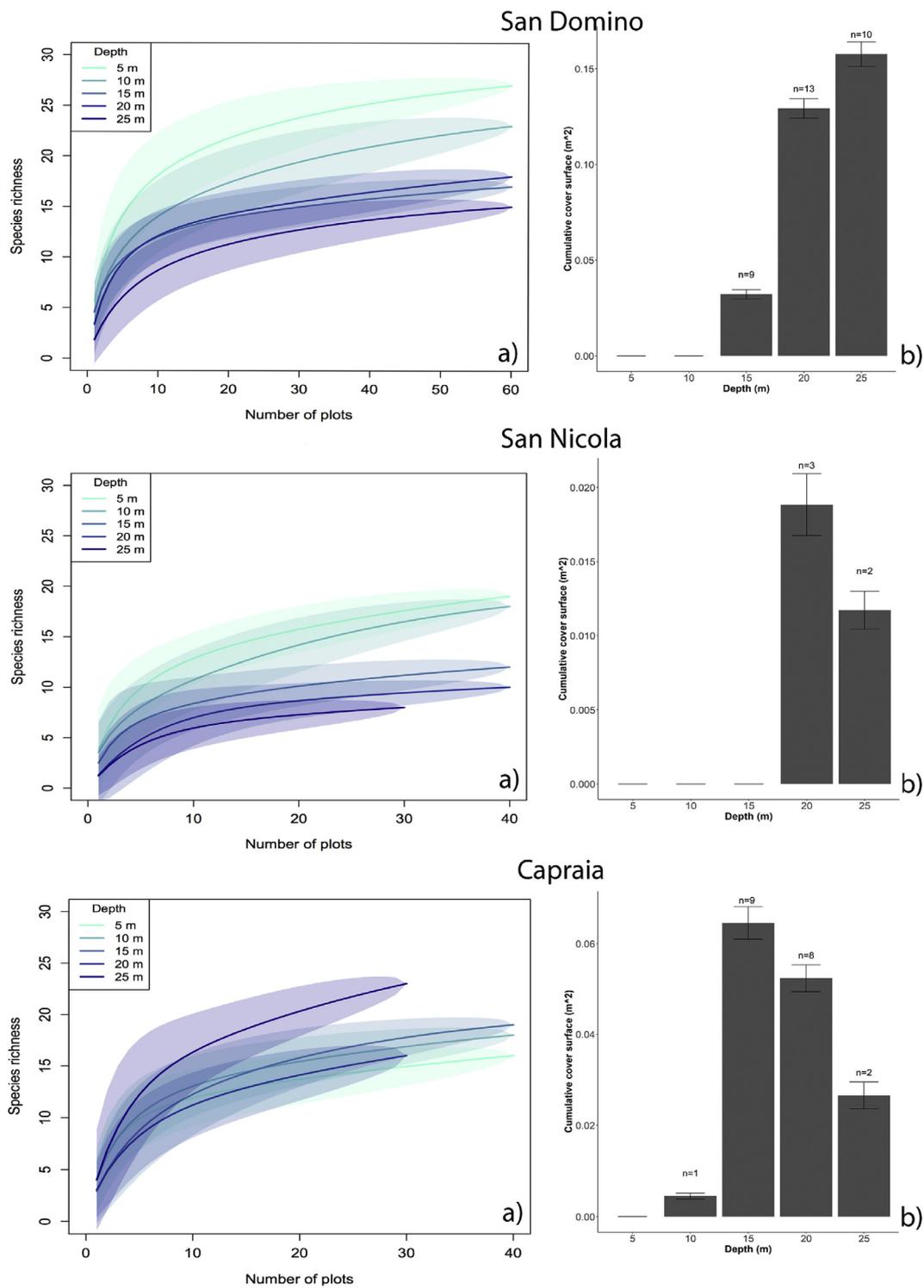


Fig. 2. a) Species accumulation curves and b) bar plots illustrating the abundance of *C. cylindracea* as cumulative cover and standard deviation in all quadrats where it was recorded, grouped by depth and isle; n = number of plots with *C. cylindracea*.

study are provided in Supplementary Tables 1, 2, and 3.

3. Results

Our results show a predominance invasion along the south west coasts of the three islands with a peak of *C. cylindracea* in Cala Zio Cesare (Figs. 1 and 2 and Table 1). The invasion was recorded in all the three islands (3 sites in San Domino, 1 in San Nicola and 3 in Capraia. Fig. 1b) and in all the five sampling depths except at 5 m (Fig. 1b and Tables 1 and

4). Fifty-seven quadrats reported the presence of *C. cylindracea* (Tables 1, 2, 3, and 4; Fig. 1b) with a cumulative cover surface of 0.5 m² and a cumulative sum of dry-weight biomass of 1·10⁻⁵ g/m² for a sampling surface of 2.3 m², representing a percentage cover of 22%. The most invaded depths were 15, 20 and 25 m. At 15 m depth we reported a wide range of cumulative cover and cumulative sum of dry-weight biomass from only 2.7·10⁻³ m² and 6.3·10⁻⁸ g/m² in Punta Secca di San Domino (Isle of San Domino) to 4·10⁻² m² and 5.9·10⁻⁷ g/m² in Cala dei Vermì (Isle of Capraia). The highest cover and biomass were collected in Cala

Table 2

Sampling sites in San Domino with depth (m); slope and type of substrate expressed as M = mixed; R = rock; S = sand; water temperature (C°); main wind, M = Maestrone (North-West) and S = Scirocco (South-East); presence (+) absence (-) of *C. cylindracea* (*C. cyl*) and mean species richness by depth and site.

San Domino									
Site	Depth (m)	Slope	Substrate (%)			T (C°)	Wind	C. cyl	S.R.
			M	R	S				
Cala Zio Cesare	5	L	–	20	80	26.1	S	–	4
	10	M	30	70	–	24.9	S	–	5
	15	H	50	40	10	23.5	S	+	5
	20	L	–	80	20	22.2	S	+	3
	25	L	10	80	10	21	S	+	4
Scoglio Dell'Elefante	5	H	–	100	–	26.6	S	–	4
	10	M	–	100	–	26.2	S	–	5
	15	M	–	90	10	25.6	S	–	4
	20	L	20	60	20	25.2	S	+	4
	25	L	40	–	60	24.7	S	–	1
Punta Di Diamante	5	M	–	100	–	26.8	M	–	6
	10	M	–	100	–	25	M	–	6
	15	M	–	100	–	23.3	M	–	5
	20	M	–	100	–	21.4	M	–	5
	25	L	60	–	40	18.5	M	–	3
Grotta Del Coccodrillo	5	L	–	100	–	28	M	–	6
	10	H	–	100	–	26.5	M	–	4
	15	H	–	100	–	25.3	M	–	5
	20	M	80	20	–	23.6	M	–	5
	25	L	20	20	60	22	M	–	3
Cala Degli Inglesi	5	L	–	100	–	28	M	–	6
	10	L	–	100	–	26.5	M	–	4
	15	L	–	100	–	25.3	M	–	5
	20	M	–	100	–	23.6	M	–	3
	25	M	–	40	60	22	M	–	2
Punta Secca Di San Domino	5	L	–	100	–	26.8	M	–	8
	10	H	20	80	–	24.6	M	–	5
	15	H	80	20	–	22.5	M	+	4
	20	H	30	40	30	20.3	M	–	4
	25	L	10	–	90	18.2	M	–	2

Table 3

Sampling sites in San Nicola with depth (m); slope and type of substrate expressed as M = mixed; R = rock; S = sand; water temperature (C°); main wind, M = Maestrone (North-West) and S = Scirocco (South-East); presence (+) absence (-) of *C. cylindracea* (*C. cyl*) and mean species richness by depth and site.

San Nicola									
Site	Depth (m)	Slope	Substrate (%)			T (C°)	Wind	C. cyl	S.R.
			M	R	S				
Testa Di Morto	5	M	–	100	–	26.8	S	–	6
	10	M	20	80	–	24.5	S	–	3
	15	M	30	60	10	22.2	S	–	4
	20	M	40	30	30	20	S	–	1
	25	M	20	20	60	17.8	S	–	0
Scoglio Segato	5	M	40	60	–	26.6	S	–	1
	10	M	10	80	10	24.8	S	–	4
	15	M	40	40	20	22.6	S	–	2
	20	M	20	20	60	20.8	S	–	1
	25	M	40	–	60	19.3	S	–	2
Punta Santa Maria	5	M	–	100	–	25.9	S	–	5
	10	M	–	100	–	24.7	S	–	4
	15	M	–	100	–	23.9	S	–	3
	20	M	20	40	40	23.1	S	+	2
	25	M	20	50	30	22.6	S	+	2
Spiaggia Delle Marinelle	5	M	–	100	–	26.5	M	–	4
	10	M	10	90	–	25.9	M	–	4
	15	L	100	–	–	25.4	M	–	1
	20	L	100	–	–	24.8	M	–	0

Zio Cesare at 25 m depth, where all the 10 random quadrats presented *C. cylindracea* with a cumulative cover of 0.2 m² and a cumulative sum of dry-weight biomass of 3.9·10⁻⁶ g/m². However, only in Cala dei Vermi (Isle of Capraia), *C. cylindracea* was recorded at all depths except at 5 m (Fig. 1b and Tables 1 and 4). During the sampling, seawater temperature at the five depths ranged between 18 °C (at 25 m of Punta Secca di San Domino, where the lowest abundance was recorded) and 27 °C (at 5 m in Cala Degli Inglesi, where the species was absent).

Species accumulation curves were also produced for each depth, separately for each island. A different pattern emerges in Capraia compared to the other islands (Fig. 2). CCA has been performed on fauna and flora P/A data collected at Capraia, San Domino and San Nicola from 5, 10, 15, 20 and 25 m depth. In CCA1 the explained variability is 40% and for CCA2 is 18% (Fig. 3).

Table 4

Sampling sites in Capraia with depth (m); slope and type of substrate expressed as M = mixed; R = rock; S = sand; water temperature (C°); main wind, M = Maestrane (North-West) and S = Scirocco (South-East); presence (+) absence (-) of *C. cylindracea* (*C. cyl*) and mean species richness by depth and site.

Capraia									
Site	Depth (m)	Slope	Substrate (%)			T (C°)	Wind	C. cyl	S.R.
			M	R	S				
Cala Dello Straccione	5	L	-	100	-	26.8	S	-	5
	10	L	10	90	-	25	S	-	6
	15	L	50	20	30	24	S	+	3
Cala Dei Vermi	5	M	-	100	-	26.5	S	-	4
	10	M	90	-	10	25.1	S	+	2
	15	L	100	-	-	24	S	+	2
	20	L	40	-	60	22.8	S	+	2
Punta Romito	25	L	-	-	100	21.6	S	+	2
	5	H	-	100	-	26.2	M	-	4
	10	H	-	100	-	24	M	-	4
	15	H	-	100	-	22.2	M	-	4
	20	M	-	100	-	20.5	M	-	5
Grosso di Caprara	25	M	-	100	-	18.6	M	-	7
	5	H	-	100	-	26.4	M	-	4
	10	H	-	100	-	23.6	M	-	5
	15	H	-	100	-	22	M	-	5
	20	M	-	100	-	20.2	M	-	4
Cala Dei Turchi	25	M	-	100	-	18.4	M	-	4
	5	L	-	100	-	26	M	-	4
	10	H	-	100	-	24.2	M	-	5
	15	H	-	100	-	22.5	M	-	5
	20	L	40	60	-	20.5	M	+	4
25	L	30	70	-	18.8	M	-	4	

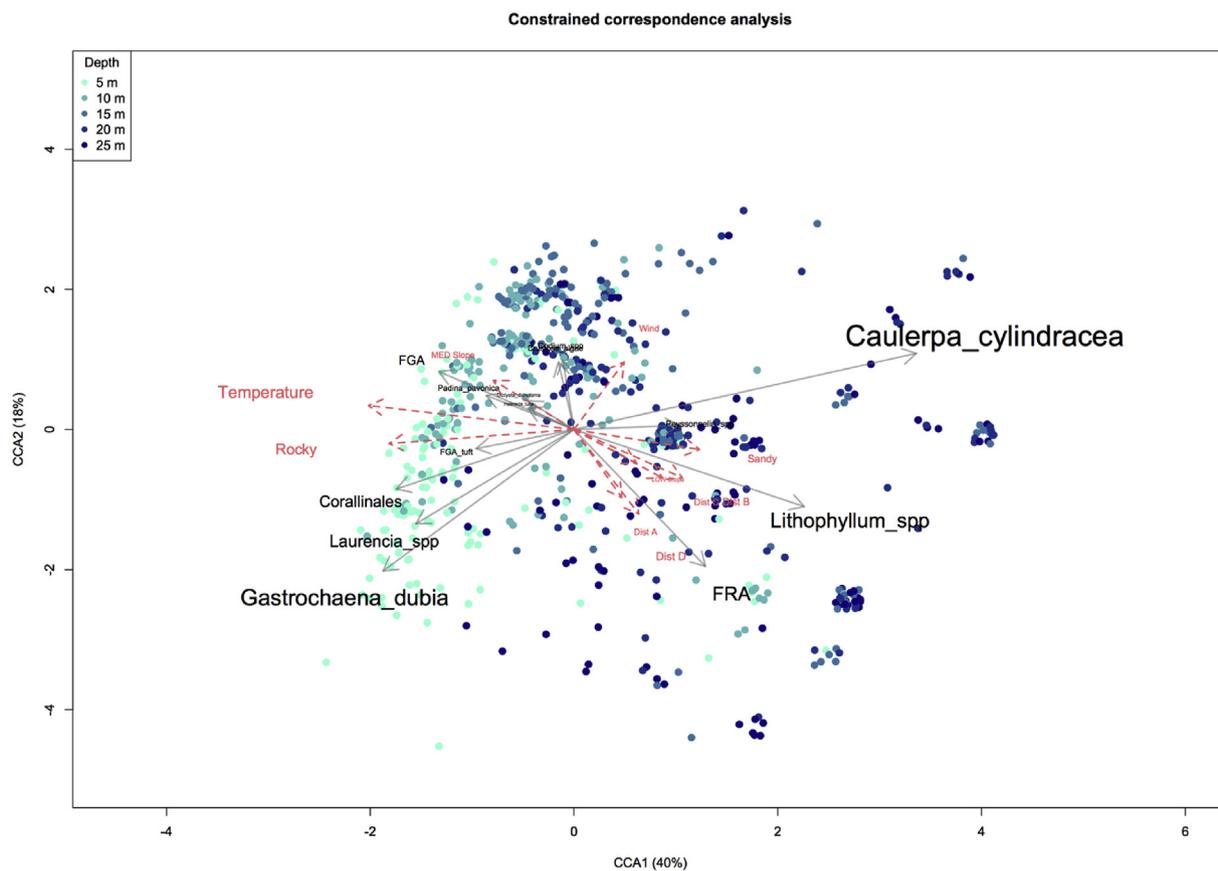


Fig. 3. Constrained correspondence analysis (CCA) on fauna and flora P/A data collected at Capraia, San Domino and San Nicola from 5, 10, 15, 20 and 25 m depth. Species vectors are indicated in grey, and species names in black. Environmental variables vectors and names are shown in red. Each point refers to a single replicate quadrat.

4. Discussion

Although, the effect of water motion on this species is unclear and it has been found on exposed shores as well as in sheltered areas (Klein and Verlaque, 2008), *C. cylindracea* was recorded mostly in sites protected from the main wind, Maestrale (from North West): in 5 out of 7 sheltered sites and in only 2 out of 8 exposed sites (Fig. 1). Our results show two interesting patterns: i) San Domino and San Nicola similarly display a higher species richness (SR) at lower depths (5 and 10 m) where *C. cylindracea* was not recorded (Fig. 2); ii) by contrast, the island of Capraia, where *C. cylindracea* invaded four out of the five investigated depths (10, 15, 20 and 25 m), shows a higher SR distribution at 25 m (Bar plots in Fig. 2). Similar patterns of colonization are reported by Cebrian and Ballesteros (2009) in the Archipelago of Cabrera National Park (Western Mediterranean). Our results show a lower SR in shallow water (5–20 m) associated with presence of *C. cylindracea* as similarly reported by Piazzi and Balata (2008) on the rocky coast of Tuscany (north-western Mediterranean Sea). Also Baldaconi and Corriero (2009) report a concomitant significant decrease in sponge structure community and cover caused by the spread of *C. cylindracea* in a nearby area along Apulia coast. In a close area, along the Calbiran Tyrrhenian coasts Cantasano et al. (2017) report as well a gradual decrease of crustose species directly associated with the presence of *C. cylindracea*.

Contrarily to what reported by Mifsud and Lanfranco (2007), the CCA analysis (Fig. 3) illustrates a low sensitivity to the four anthropic alleged pollution sources (Fig. 2b) and to the seafloor slope. These variables exert a low relevance in the dynamics of this invasive species, while conversely, temperature and type of substrate exert a larger effect, confirming the role of seawater temperature increase in the Mediterranean on the spread of this alga (Argyrou et al., 1999; Ruitton et al., 2005b; Ivesa et al., 2015). Interestingly, although dead matte of the seagrass *Posidonia oceanica* and rock covered with photophilic algae are often reported as favorable substrates for the spread of this alga (Piazzi and Cinelli, 1999; Piazzi et al., 2001; Ceccherelli et al., 2002; Piazzi et al., 2003; Ruitton et al., 2005a, b; Bulleri and Benedetti-Cecchi, 2008; Katsanevakis et al., 2010; Infantes et al., 2011), in the study area a larger abundance on sand and detrital substrata was recorded (Tables 2, 3, and 4). It is consistent with a recent review by Sghaier et al. (2015) along the coast of Tunisia, who report a higher presence of *C. cylindracea* on sand substrata instead of rock and *P. oceanica* meadow (0.68 % of sites observed). By contrast, Piazzi and Cinelli (1999) and Infantes et al. (2011) show a high density of *C. cylindracea* in shallow waters 0–3 m and <8 m depth, respectively. Moreover, De Biasi et al. (1999) observed a decrease in the *C. cylindracea* cover from 5–10 m to 15–20 m depth in a different pattern to that reported in MPATI.

Although, many studies show clear effects of this seaweed on benthic communities, and a recent review of Piazzi et al. (2016) underlines ten main direct and indirect factors affecting the spread of this species, many others are still poorly known. For example, the relevance of depth, water movement, herbivores and other invaders in dispersal dynamic of this pest are still not clear. Comparing our data with other studies available from *C. cylindracea* populations observed in different nearby areas and depths of the Mediterranean Sea, no general patterns can be clearly defined. However, as for *C. taxifolia* (Boudouresque and Verlaque, 2012) also for *C. cylindracea* the invasion might be summarized in four main steps: (1) arrival, (2) settlement, (3) expansion, (4) persistence. The expansion process can be very long (Montefalcone et al., 2015; Ivesa et al., 2015) showing that only with long-term monitoring studies coupled with a better ecophysiological knowledge of *C. cylindracea* and through manipulative experiments, it could be possible to better understand key factors driving the invasion of this species in the Mediterranean Sea.

This first record shows a remarkable presence and distribution of this invasive alien species in the MPATI in different areas, depths and substrates. Additional studies of particular biological interest are necessary to evaluate the spread, invasion speed, and impact of this seaweed.

Further monitoring activities will thus improve actual knowledge about the interaction of this seaweed with native Mediterranean communities.

Declarations

Author contribution statement

Andrea Pierucci: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gina De La Fuente: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Rita Cannas, Mariachiara Chiantore: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the MARLINTREMITI Laboratorio del mare Scholarship (logistic and dive equipment).

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2019.e02449>.

Acknowledgements

The authors thank the MARLINTREMITI Laboratorio del mare and park authorities of the Marine Protected Area of Isole Tremiti. The authors also thank Massimo Ponti, Carlo Cerrano, Giorgio Bavestrello, Stefano Schiaparelli and Danilo Pecorino for valuable comments and suggestions that improved the quality of this paper. A. Pierucci would like to gratefully thank Andrea Magnai, Roberto De Camillis, Nicola Filocamo, Martina Leporatti, Andrea Burini, Federica Montesanto and Giuseppe Suaria for field assistance. Finally, we are indebted with the reviewers, for providing useful and highly constructive comments that greatly improved this manuscript.

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