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A Preliminary inventory of alien and cryptogenic species in Monastir Bay, Tunisia: spatial distribution, introduction trends and pathways

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Abstract

The Mediterranean Sea is a marine biodiversity hotspot under threat, with the invasiveness of non-indigenous species (NIS) presenting one of the major impacts on its biological resources and services, However, NIS monitoring programs in the south basin of the Mediterranean Sea are still in an early implementation stage. This study aims to describe NIS and cryptogenic species distribution in Monastir Bay (Tunisia) and to identify risk areas for the introduction and spread of invasive species, providing a baseline for future monitoring programs. To this end, a series of Rapid Assessment Surveys were carried out to identify NIS and cryptogenic species in one marina, five fishing ports, two aquaculture farms, and the Special Conservation Area of the Kuriat islands. 24 species were found, 11 of which constitute new records for Monastir Bay, representing 33.3% of the total NIS reported in this Bay. Assemblages differed between substrata types, with NIS being more abundant in artificial than in natural substrata. Regarding locations, Cap Monastir Marina was the most invaded site, the most transited by vessels, and the only one visited by international sailing. Hence, this marina constitutes the main risk area to be monitored, although the fishing ports and fishing farms in the semi-enclosed coastal lagoon of Monastir Bay can also be considered at risk areas. Nevertheless, more research effort is needed in Monastir Bay in order to update the records of NIS and cryptogenic species and increase insight into the ecological evolution of these species and their related impacts on natural communities and marine resources.

Keywords: Non-indigenous Species; Introduction Vectors; Risk Areas; Monastir Bay, Tunisia.

Introduction

The Mediterranean Sea is both a marine biodiversity hotspot and one of the most impacted ecoregions in the world (Coll *et al.*, 2010; Lejeusne *et al.*, 2010). This is mainly due to anthropogenic impacts such as pollution, habitat loss (i.e. that derived from coastal urbanization), the effects of climate change, and biological invasions (Vitousek *et al.*, 1997; Micheli *et al.*, 2013; Katsanevakis *et al.*, 2014; Katsanevakis *et al.*, 2016). Among those, invasive alien species are a primary threat to global biodiversity, natural resources, and human health (Occhipinti-Ambrogi, 2001; Galil, 2018). In the Mediterranean Sea, over 821 Non-Indigenous Species (NIS) have been reported to date, of which more than half are considered to be established and spreading (Zenetos *et al.*, 2010, 2012, 2017). After establishment, a fraction of the introduced NIS may become invasive. Invasive NIS exhibit aggressive behavior and may become natural enemies of the native species in an ecological sense (Streftaris & Zenetos, 2006; Boudouresque & Verlaque, 2010). Invasive NIS impacts are generally irreversible, especially in the marine environment, with prevention measures and early detection being the best options against this threat (Leung *et al.*, 2002; Occhipinti-Ambrogi & Savini, 2003).

Human activities such as shipping, aquaculture, and excavation of artificial waterways, have been prevalent vectors for the introduction of NIS (Carlton & Geller, 1993; Rilov & Crooks, 2009; Katsanevakis *et al.*, 2013). After their introduction, coastal urbanization, the effects of climate change, eutrophication, and pollution may assist in the spreading of the NIS in the hosting environment (Piola & Johnston, 2008, 2009; Boudouresque & Verlaque 2010; Crooks *et al.*, 2011; Ros *et al.*, 2013).

Artificial marine infrastructures such as harbors, marinas and aquaculture farms may increase niche opportunity for NIS and are considered stepping stones for NIS introduction and spread (Tilman, 1997; Bulleri & Airoldi, 2005; Glasby *et al.*, 2007; Ruiz *et al.*, 2009). These artificial infrastructures are frequently visited by vessels, increasing the chances of NIS propagules and larvae´s arrival and spread (Glasby *et al.*, 2007; Ros *et al.*, 2013; Martínez-Laiz *et al.*, 2019). They also expose novel substrata types to the sea environment (i.e. plastics, ropes, and concrete), which often lack the features that contribute to increasing microhabitat diversity in natural areas (i.e. roughness and spatial heterogeneity) (Bulleri & Chapman, 2010; Ostalé-Valriberas *et al.*, 2018; Sempere-Valverde *et al.*, 2018). Floating structures such as pontoons may also increase NIS abundance when compared with non-floating substrata (Megina *et al.*, 2016). Furthermore, the water stagnation that occurrs inside ports and marinas (but which is also naturally occurring inside coastal lagoons and bays) also contributes to habitat modification by increasing water temperature and salinity and reducing hydrodynamics (Verlaque, 2001; Marchini *et al.*, 2015; Ros *et al.*, 2015; Molina *et al.*, 2017). As a result, artificial substrata often support poorer benthic assemblages than natural substrata, which may reduce the biodiversity and ecological competitiveness of native species and increase fragmentation of the surrounding natural communities (Bulleri & Airoldi, 2005; Bulleri & Chapman, 2010; Molina *et al.*, 2017). This reduced competitiveness, coupled with the modified environment and novel substrata types, may favor the opportunistic establishment of NIS, which are generally more abundant on artificial substrata than in natural areas (Megina *et al.*, 2016; Ferrario *et al.*, 2017). Once colonized, artificial infrastructures can become sources of propagules for the secondary dispersal of NIS through both natural processes and secondary vectors (i.e. recreational boating and marine litter) (Glasby *et al.*, 2007; Ros *et al.*, 2013; Rech *et al.*, 2018).

Because of its location and geography, the Mediterranean Sea could be considered a surrogate of the world's oceans for the development of environmental and ecological studies on marine ecosystem resilience, climate warming impacts, and biological invasion spread (Bethoux *et al.*, 1999; Lejeusne *et al.*, 2010). In the Mediterranean Sea, the Tunisian coastline is about 1,400 km long, is located at the crossroads between the western and eastern Mediterranean basins (the Tuniso-Sicilian strait), and is close to vital shipping routes (Sghaier *et al.*, 2016; Deidun *et al.*, 2018). Therefore, Tunisian coasts may constitute a potential risk area for shipping-related impacts (i.e. collisions and spills) and for the spread of NIS between the west and east basins of the Mediterranean Sea. In this regard, alien diversity in the east Mediterranean basin is dominated by species introduced by corridors (Suez Canal) and shipping activities (fouling and ballast waters). On the other hand, the main introduction pathways in the west basin are shipping activities and aquaculture, contributing to a difference in alien species composition between basins (Zenetos *et al.*, 2010, 2012). Therefore, Tunisia is a transitional area that could be considered a risk area for the spread of NIS within the Mediterranean Sea. Furthermore, it is an area that is highly affected by cumulative human impacts (Micheli *et al.*, 2013). Nevertheless, the southern basin of the Mediterranean Sea has received low research efforts when compared with its northern basins and more efforts are required to increase our knowledge of NIS in this area (Zenetos *et al.*, 2010; Ulman *et al.,* 2019). To this end, in July 2017 Tunisia established a national monitoring program for biodiversity and non-indigenous species, in line with the requirements of the Integrated Monitoring and Assessment Program of the Mediterranean Sea and Coast and Related Assessment Criteria (IMAP) (UNEP/MAP, 2016a, b; PNUE-PAM-CAR/ASP, 2017). This national monitoring program proposes monitoring the trends in NIS spatial distribution, temporal occurrence and abundance, especially in risk areas, and focusing resources on invasive NIS, which are prone to causing environmental and economic problems (Streftaris & Zenetos, 2006).

The main objective of this study is to make an inventory of NIS and cryptogenic species and identify the main risk areas in Monastir Bay and the nearby Sensitive Coastal Area of the Kuriat islands (Tunisia). NIS and cryptogenic species occurrence is expected to be higher in artificial substrata than in natural substrata, and the most risk areas are expected to be inside ports, as vectors and propagule pressure are presumably concentrated in those areas and there are also environmental and ecological features that favor NIS colonization (Bulleri & Airoldi, 2005; Glasby *et al.*, 2007; Ruiz *et al.*, 2009). Additionally, the distribution of NIS and cryptogenic species in Monastir Bay, related to the main pathways of introduction and spread, will be described along with a bibliographic review of the current available data on the numbers of these species in Monastir Bay.

Materials and Methods

Study area

Monastir Bay is a semi-enclosed lagoon, extending for 38 km along the eastern coast of Tunisia, and one of the most important resources of marine biodiversity in Tunisia (Damak *et al.*, 2018). In 2017, Monastir Bay contributed to 22.24% of total fish production in Tunisia, and is the first in terms of production in this country, hosting 1069 active boats, 35 of which are dedicated to aquaculture farms (GDFA, 2018). However, this biodiversity hotspot has been impacted by several industrial, fishing, and fish-farming activities (Nouira *et al.*, 2013; Challouf *et al.*, 2017; Damak *et al.*, 2018). Additionally, Monastir Bay and its nearby areas are identified as risk zones for NIS introduction due to the presence of five fishing ports, eleven active offshore fish farms and a marina (Chebaane *et al.*, 2019). Within this biodiversity hotspot, the Sensitive Coastal Area (SCA) of the Kuriat archipelago is located in the eastern part of Monastir Bay (Mangos & Claudot, 2013). This SCA includes two islands: big Kuriat or Qûrya El Kabira and small Kuriat or Qûrya Essaghira and has been proposed as a future Marine and Coastal Protected Area of the Mediterranean (MCPA) (UNEP/MAP, 2018).

Data collection

Qualitative samplings were carried out during July and August of 2018 in Monastir Bay and nearby areas on (1) high-risk areas and (2) special conservation areas.

(1) High-risk areas were human-altered environments, with an abundance of artificial substrata. These comprise one marina (Cap Monastir), located north of Monastir Bay (35.77889°N, 10.83528°E) and designed to support 400 international recreational boats (Sghaier *et al.*, 2011a), five small fishing ports, and two fish aquaculture farms, that fatten European seabasses (*Dicentrarchus labrax*) and gilthead seabreams (*Sparus aurata*) (see Fig. 1A). Selection of the aquaculture farms was based on their proximity (about 4 km) from the SCA of the Kuriat archipelago. High-risk areas were sampled using the Rapid Assessment Survey methodology (RAS) described by Campbell *et al.* (2007). Samples were taken from substrata within arms-reach, up to a maximum depth of 2 meters (shallow sandy bottoms, floating docks, riprap structures and ropes that can be pulled out of the water).

(2) Special conservation areas were those with reduced human impact and abundance of natural substrata (sandy bottoms and natural rock), with the sampling sites located in the SCA of the Kuriat archipelago. Because of the morphological and slope differences between natural and artificial substrata, and the bathing prohibitions in the latter, RAS samplings in the special conservation areas were carried out along snorkeling transects perpendicular to the shoreline (Rapid Assessment Snorkeling Survey - Corsini-Foka *et al.* 2015). Six snorkeling transects were sampled in the small Kuriat Island and four in the big Kuriat Island (see dotted lines in Fig. 1B).

Sampling efforts were standardized between high-risk and low-risk areas by having the recording of NIS and cryptogenic species larger than 1 millimeter last one hour and be by the same researcher. Species that could not be identified in-situ were hand-collected for their identification in the laboratory using a Leica MS5 Stereo Microscope and specific identification guides. Taxonomic experts were consulted for species identification whenever necessary and taxa names were checked in WoRMS (Horton *et al.*, 2019).

In order to understand the introduction pathways and to help in the assessment of risk areas for the introduction of aliens in Monastir Bay, the number of boats arriving from international ports to Marina Cap Monastir and the most common departure destinations from Marina Cap Monastir to domestic fishing ports were recorded. This data was collected from the captaincy administration of Marina Cap Monastir and the director of the Border and Foreign Police in Marina Cap Monastir. Data was mapped using the QGIS free-software.

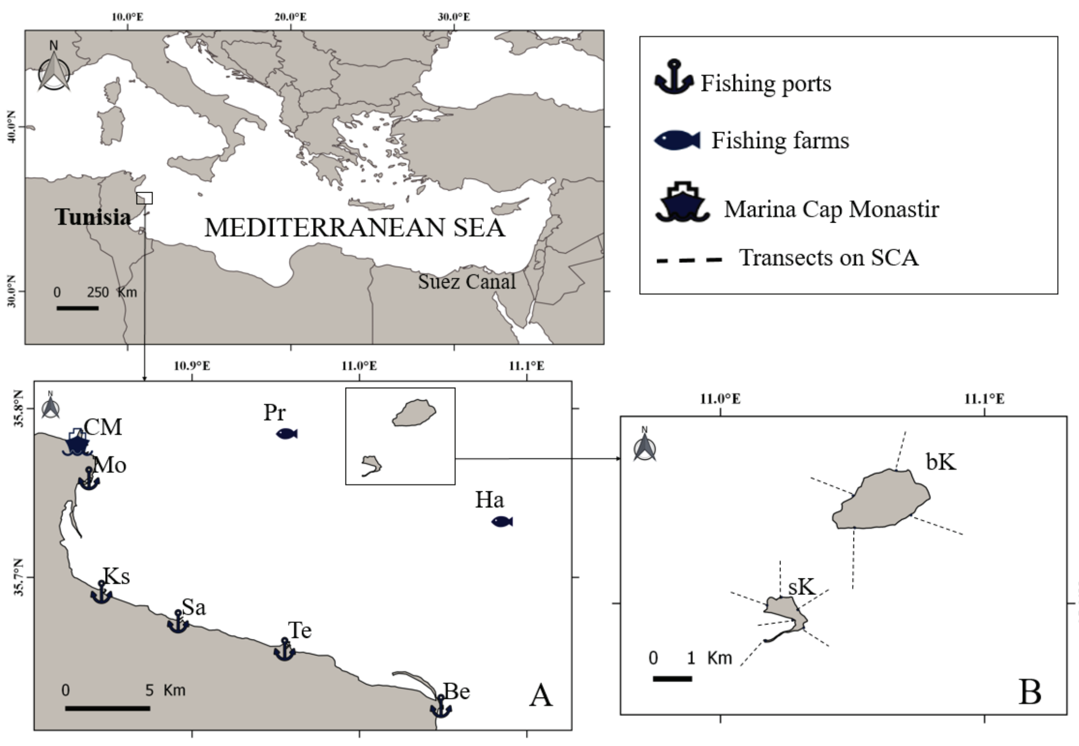

Fig. 1: Locations of sites and transects surveyed for NIS and cryptogenic species in Monastir Bay in July and August 2018. A: Risk Areas and B: Transects on the Special Conservation Area of the Kuriat islands (SCA). Fishing ports: Monastir: Mo; Ksebit El Mediouni: Ks; Sayada: Sa; Teboulba: Te; Bekalta: Be. Marina: Marina Cap Monastir: CM. Fishing farms: Prima Fish: Pr; Hanshia Fish: Ha. Special Conservation Areas: Small Kuriat transects; big Kuriat transects: bK.

Statistical analyses

The non-indigenous and cryptogenic species presence-absence data was used to create a resemblance matrix based on Jaccard distance measures, which can be directly interpreted as the percentage of unshared species between two sample units (Anderson *et al.*, 2008). This resemblance matrix was used to perform a non-metric multidimensional scaling ordination (nMDS) and PERMANOVA and PERMDISP analyses, testing the unbalanced fixed factor substrata (at two levels: natural and artificial). The Primer-e v6 +PERMANOVA software was used for the statistical analyses, for which 1000 permutations were used (Clarke & Gorley, 2006; Anderson *et al.*, 2008).

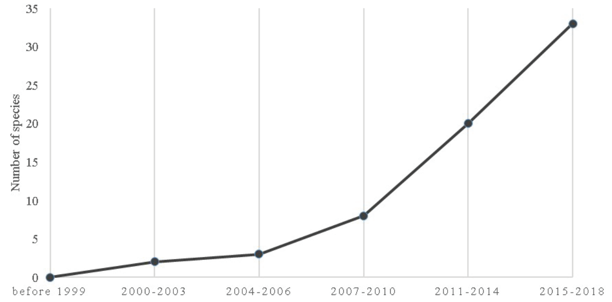

Fig. 2: Number of NIS and cryptogenic species recorded in Monastir Bay, based on this study and previous bibliography (Ben Mustapha *et al.*, 2002; Bradai *et al.*, 2004; Tlig-Zouari *et al.*, 2009; Ramos-Espla & Ben Mustapha, 2010; Sghaier *et al.*, 2011a; Sghaier *et al.*, 2011b, 2016; Bdioui, 2016; Zaafrane & Maatouk, 2016; Aissi *et al.*, 2018; Sghaier *et al.,* 2019).

Results

A total of 22 non-indigenous (NIS) and 2 cryptogenic species, belonging to nine phyla (Mollusca, Crustacea, Clorophyta, Bryozoa, Ascidiacea, Rhodophyta, Magnoliophyta, Porifera and Polychaeta), were identified in Monastir Bay and nearby areas (Suppl.file and Table 1). The species found in this study represent 72.7% of the total NIS and cryptogenic species reported in Monastir Bay (Fig. 2). The majority of these species (75%) have been reported from 2010 onwards and this study includes the first reports of 33.3% of the NIS and cryptogenic species found in Monastir: *Codium fragile*, *Magallana gigas*, *Amathia verticillata*, *Bugula neritina*, *Tricellaria inopinata*, *Hydroides elegans*, *Branchiomma bairdi*, *Paracerceis sculpta*, *Paradella dianae*, *Caprella scaura* and *Microcosmus exasperatus*.

Within the sampled areas, the most widespread phyla were Rhodophyta, with one species (*Lophocladia lallemandii*) present in 15 out of 18 sites (15/18), Mollusca (14/18), with five species, and Ascidiacea (13/18), with three species (see most common species in Fig. 3).

Regarding substrata types, all species (24) and phyla (9) were found in artificial substrata (harbors and fishing farms), while seven species from five phyla were present in the Kuriat natural areas. The NIS-cryptogenic assemblages composition varied between natural and artificial substrata (PERMANOVA: pseudo-$F_{1.17}$ = 6.33, P (perm) <0.01; PERMDISP: $F_{1.17}$ = 3.18, P (perm) = 0.16). For the artificial substrata, and with the exception of the Teboulba fishing port (which differed from all sampled locations), Marina Cap Monastir, the fishing ports, and the aquaculture farms were relatively similar in species composition despite the geographical distance between them (Fig. 4).

The highest number of NIS and cryptogenic species and phyla occurred in Marina Cap Monastir, with 18 species belonging to all of the registered phyla (9), followed by fishing ports, aquaculture farms, and natural areas (Fig. 5). The total number of species per location is shown in Table 1.

From January to September 2018, 218 boats docked in Marina Cap Monastir including 133 international boats and 85 national boats, with an average of 1 to 5 boats entering/leaving the Marina daily. As for international traffic, the largest fraction of boats arrived from Malta (31.6%), followed by Palermo, Sicily, Italy (22.6%); Trapani, Sicily, Italy (15.8%); Sardinia, Italy (12%); Toulon, France (7.5%); Baleares, Spain (6%); Greece (3.8%) and Novarossisk, Russia, from which a single boat arrived at Marina Cap Monastir in 2018 (see Fig. 6). National navigation from Marina Cap Monastir to other ports in Tunisia, shows that Mahdia is the most favoured destination for recreational boats followed, in order, by Chebba, Sayada, Monastir, Sousse, Beni Khiar, Hammamet, El Kantaoui, Teboulba, Kelibia, Bizerte, Tunis and Hergla (Fig. 7).

Discussion

Within Monastir Bay, Marina Cap Monastir and the Monastir fishing port are the main risk areas for NIS introduction, from which they may spread to other areas in Tunisia. Consequently, these should constitute priority areas for the monitoring of potential invasions in Monastir Bay and the Special Conservation Area (SCA) of the Kuriat islands. Since biological invasion of the marine environment is a phenomenon that is generally irreversible on a human scale, the prevention of species introduction and early detection for eradication are both the top priority management strategies to be implemented and the most cost-effective, ecologically and economically (Boudouresque & Verlaque, 2010). In Monastir Bay, most of the previous observations of NIS were based on occasional or incidental findings (Sghaier *et al.*, 2016, 2019; Chebaane *et al.*, 2019), with the survey carried out by Sghaier *et al.* (2019) in the year 2013 in Marina Cap Monastir being the only previous existing survey. Furthermore, there has been a major increase in reported NIS and cryptogenic species in Monastir Bay from 2007 to 2018. This suggests that research efforts in this area have just started to be implemented and there may still be a high number of non-reported species in this area, taking into account that up to 163 marine NIS and cryptogenic species have been recorded in Tunisia (Ounifi-Amor

Table 1. NIS and cryptogenic species recorded between July and August 2018 in each surveyed site of Monastir Bay (presence of NIS in grey). **Fishing ports:** Monastir: Mo; Ksebit El Mediouni: Ks; Sayada: Sa; Teboulba: Te; Bekalta: Be. **Marina:** Marina Cap Monastir: CM. **Fishing farms:** Prima Fish: Pr; Hanshia Fish: Ha. **Special Conservation Areas:** small Kuriat transects 1 and 2: sK1-2; small Kuriat transect 3: sK3; small Kuriat transects 4 to 6: sK4-6; big Kuriat transects 1 to 4: bK. Presence. Origin: Atlantic: At; Australia: Au; Circumtropical: Ci; Cryptogenic: Cry; East Pacific: EP; Indian Ocean: IO; Indo-Pacific: IP; North East Pacific: NEP; North West Pacific: NWP; Pacific Ocean: PO; Pantropical: Pa; Red Sea: RS; Subtropical: Su; Tropical: Tr; West Atlantic: WA.

Taxa	Species	Origin	Mo	Ks	Sa	Te	Be	CM	Pr	Ha	sK1,2	sK3	sK4-6	bK
	Caulerpa chemnitzia (Esper) J.V. Lamououx,	RS/ Pa					■							
Chlorophyta	*Caulerpa cylindracea* Sonder,	IP	■	■	■		■	■			■	■	■	
	Codium fragile (Suringar) Hariot,	PO		■										
Rhodophyta	*Lophocladia lallemandii* (Montagne) F.Schmitz,	IP/ RS	■		■		■	■	■	■	■		■	■
Magnoliophyta	*Halophila stipulacea* (Forsskål) Ascherson,	IO/ RS	■			■		■						
Porifera	*Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004	IP/Au	■					■	■					
	Bursatella leachii Blainville, 1817	Ci		■		■		■			■	■		
Mollusca (Gastropoda)	*Melibe viridis* (Kelaart, 1858)	IP		■	■									
	Cerithium scabridum Philippi, 1848	IO/RS	■	■	■		■	■			■			
Mollusca (Bivalvia)	*Magallana gigas* (Thunberg, 1793)	NWP	■		■			■						
	Pinctada imbricata radiata (Leach, 1814)	IP/RS	■		■		■	■	■					■
Annelida (Polychaeta)	*Hydroides elegans* (Haswell, 1883)	Ci	■		■			■						
	Branchiomma bairdi (McIntosh, 1885)	WA/EP	■		■			■						
Crustacea (Isopoda)	*Paracerceis sculpta* (Holmes, 1904)	Su		■				■						
	Paradella dianae (Menzies, 1962)	NEP		■										
Crustacea (Decapoda)	*Percnon gibbesi* (H. Milne Edwards, 1853)	WA	■		■			■			■			
	Portunus segnis (Forskål, 1775)	IO					■							
Crustacea (Amphipoda)	*Caprella scaura* Templeton, 1836	IO							■					
	Amathia verticillata (delle Chiaje, 1822)	AO	■		■			■	■					
Bryozoa	*Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985	IP	■	■	■		■	■	■	■				
	Bugula neritina (Linnaeus, 1758)	Cry	■	■	■	■		■	■					
	Microcosmus exasperatus Heller, 1878	IP	■	■	■		■	■						
Ascidiacea	*Symplegma brakenhielmi* (Michaelsen, 1904)	Tr	■					■						
	Ecteinascidia turbinata Herdman, 1880	Cry						■				■	■	■
	Total number of NIS and cryptogenic species		15	10	13	3	8	18	7	2	5	3	3	3

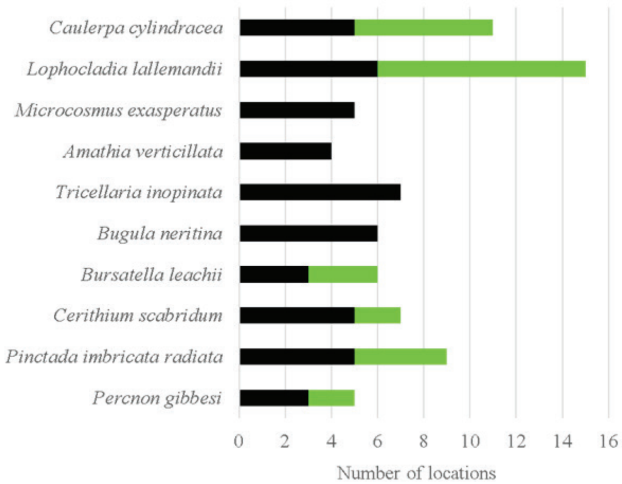

Fig. 3: Number of sites (artificial structures in black) and transects (natural Special Conservation Areas in green) in which species were present, for species occurring in more than three of the sampled sites/transects.

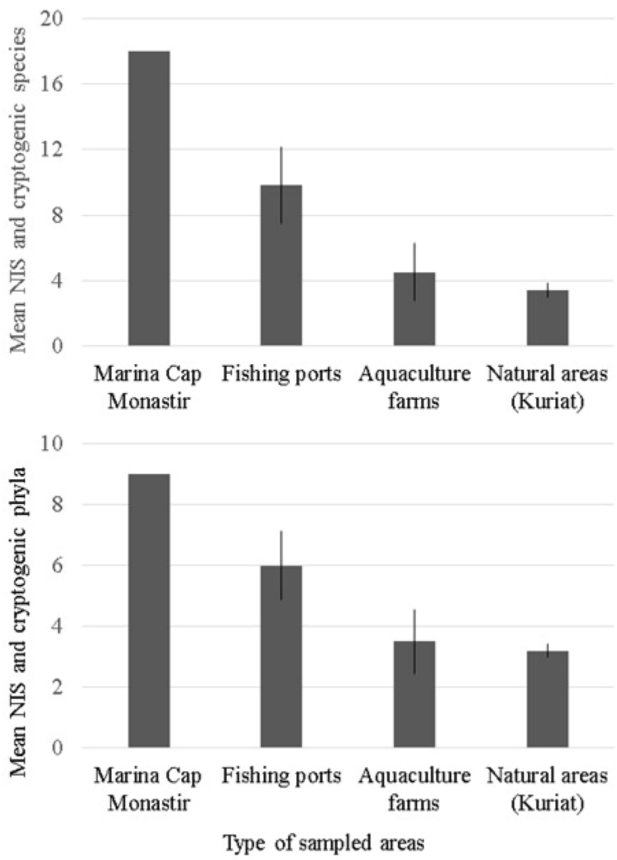

Fig. 4: Mean number of NIS and cryptogenic species with phyla and standard deviation error bars for each of the different types of sampled areas (Marina Cap Monastir, the five fishing ports, two aquaculture farms and transects in the Special Conservation Area of the Kuriat Islands).

et al., 2016; Sghaier *et al.*, 2016). Therefore, this study could provide a baseline for a future long term monitoring programme and early-detection of NIS in this bay, its coastal semi-enclosed lagoon, and the nearby SCA of the Kuriat islands.

In this regard, the monitoring should focus on artificial structures (such as marinas and fishing ports) as these are the most invaded habitats in the studied area and have already been identified as risk areas, usually hosting higher number and coverage of NIS than other coastal areas (Occhipinti Ambrogi, 2001; Verlaque, 2001; Marchini *et al.,* 2015).

The Rapid Assessment Survey (RAS) used in this study allows for informal comparison with similar studies. In the Porto Turistico di Roma (Ostia, Rome, Italy), nine NIS were found in a sampling carried out for 6 hours inside the marina by one researcher (Ferrario *et al.* 2016). Seemingly, 13 NIS were found in Greece in Faliraki Marina and five in Mandraki harbor during samplings of up to two hours, carried out by groups of 4 to 6 students led by marine taxonomy experts (Corsini-Foka *et al.* 2015). Furthermore, Ulman *et al.* (2019) found 11±5.6 NIS on average in 34 marinas (2 to 27 NIS per marina), sampled by RAS throughout the Mediterranean basin, using eight hours of sampling efforts by one or two researchers per marina. Taking into account the relatively lower efforts used in this study, the number of NIS found raises concerns about the status of Monastir Bay, especially Marina Cap Monastir, where 18 NIS and cryptogenic species were found. Therefore, Marina Cap Monastir may be within the most invaded marinas of the Mediterranean Sea (Corsini-Foka *et al.* 2015; Ferrario *et al.* 2016; Ulman *et al.*, 2017, 2019).

Marina Cap Monastir is an attractive destination for coastal holiday tourism. Traffic of recreational boats is most likely the primary vector of introduction of NIS, which could explain the higher occurrence of NIS in the marina and the adjacent fishing port of Monastir (Glasby *et al.*, 2007; Ros *et al.*, 2013; Martínez-Laiz *et al.*, 2019). In this regard, in-water cleaning of fouling on international boats could have played an important role in the liberation of propagules inside the marina (Hopkins & Forrest, 2008; Woods *et al.*, 2012). The process of removing boats from the water for cleaning may also release mobile fauna from boats fouling inside ports (Coutts *et al.*, 2010). Finally, the liquid and solid waste resulting from the cleaning of boats outside water, if it ends up in the marina water mass, has also been identified as an issue related to the cleaning of domestic boats (Floerl *et al.*, 2005; Woods *et al.*, 2012). All these practices, which are usually regulated in most countries, have been observed in the studied area by the authors of this study. Nevertheless, NIS could have also reached Monastir Bay by natural processes (i.e. spawning from nearby areas) or different anthropogenic vectors such as marine litter or aquaculture activities (Katsanevakis *et al.*, 2013; Rech *et al.*, 2018).

As occurs in other coastal areas, NIS may have taken advantage of the low hydrodynamics, substrata features, and the low ecological competition occurring inside ports and marinas for the successful colonization of artificial substrata (Bulleri & Airoldi, 2005; Bulleri & Chapman, 2010). Consequently, these ecological and environmental similarities among ports and marinas could be responsible for the similarities in the community compositions found

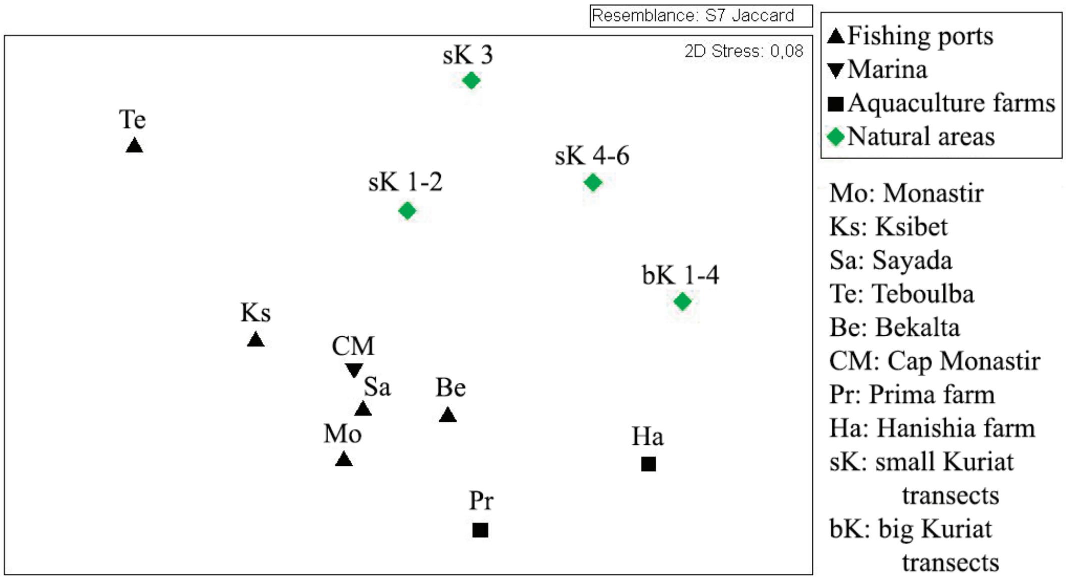

Fig. 5: nMDS based on Jaccard similarities for each of the sites surveyed in Monastir Bay and nearby areas. Black: artificial substrata.

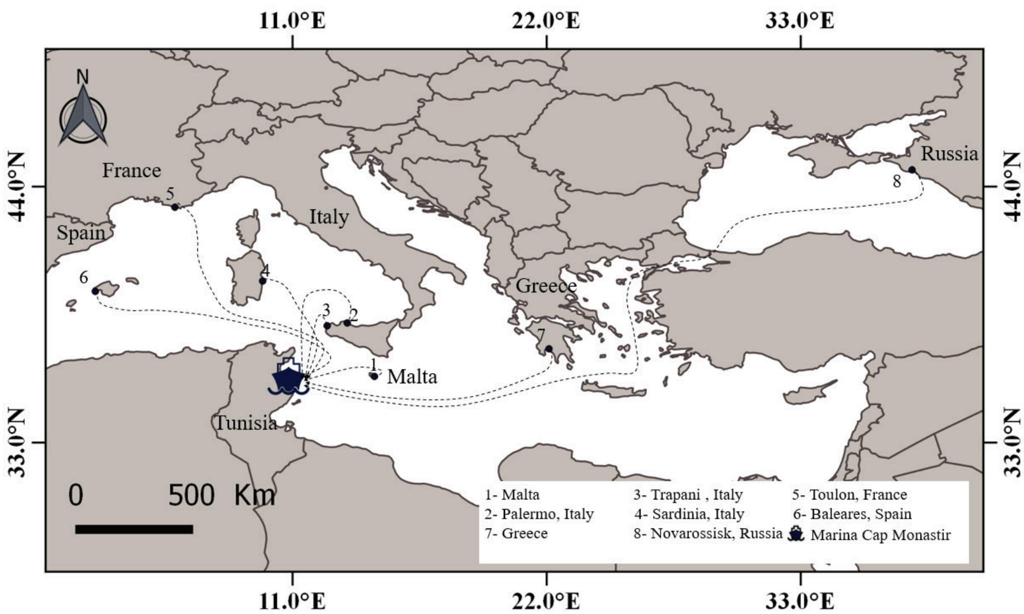

Fig. 6: Map of the Mediterranean Sea showing the incoming boat traffic from different destinations to Marina Cap Monastir, Tunisia (2018 data).

among these structures. On the other hand, the semi-enclosed water mass inside Monastir Bay could also concentrate propagules and larvae released from colonized areas and contribute to this similarity of the NIS community within the studied marina and fishing ports (Verlaque, 2001; Ros *et al.*, 2015; Molina *et al.*, 2017). In spite of that, a low number of NIS and different community compositions were found in Teboulba fishing port. Teboulba port was heavily polluted and fouling communities were not abundant in its substrata, which could explain the low number of NIS in this site, as heavy pollution can have an adverse effect on the settlement of both natural and exotic communities (Kenworthy *et al.*, 2018; Ramalhosa *et al.*, 2019). Nevertheless, some NIS could also take advantage of the native species in polluted areas (Piola & Johnston, 2008, 2009). In any case, *Halophila stipulacea* was only found in the Teboulba and Monastir harbors, which could suggest that several factors are involved in the secondary spread, as this plant could be transported from the fishing port of Monastir in the boats' anchors and fishing nets (Sghaier *et al.*, 2011a).

Regarding aquaculture farms, the highest number of NIS in the Prima Fish farm may be due to its proximity to the Monastir fishing port which is also the base harbor for the boats of this farm. In this regard, fouling NIS associated with aquaculture equipment such as ropes and buoys could be transported offshore from the fishing port (Campbell *et al.*, 2017). Therefore, the aquaculture farms of Monastir Bay may be acting as stepping-stones for the spread of NIS from Monastir to nearby natural areas, such as the SCA of the Kuriat Islands (Mesel *et al.*, 2015; Chebaane *et al.*, 2019). Among the natural areas (the SCA

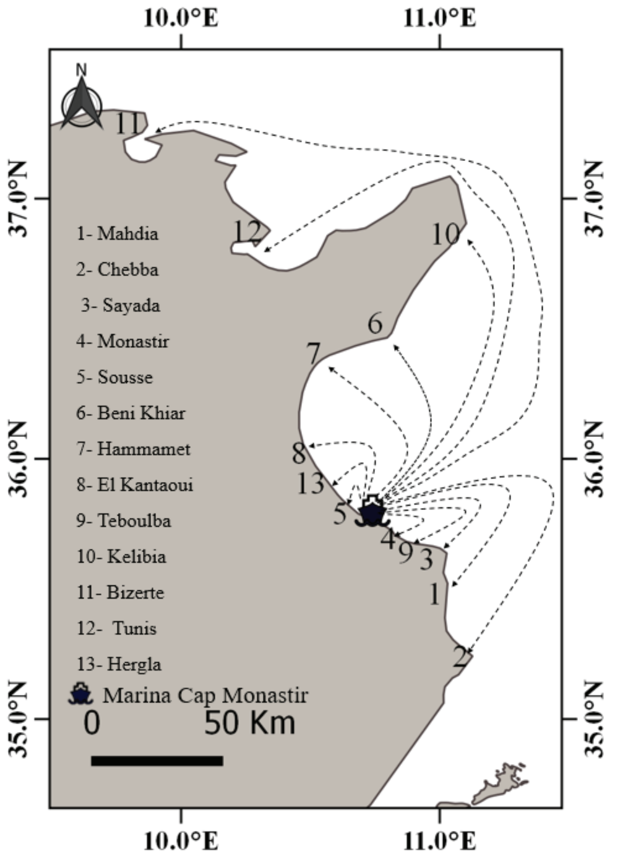

Fig. 7: Map of the Tunisian coast showing the outgoing traffic from Marina Cap Monastir to different ports of Tunisia (2018 data).

of the Kuriat Islands), big Kuriat is the site with the lowest number of NIS and the area least frequented by boats, since it is a military zone where visits are prohibited.

Overall, the main corridor of NIS introduction in Monastir Bay seems to be navigation and its associated risks, which include hull biofouling, standing waters of recreational boats (e.g. in ballast tanks, bilge, anchor boxes and engine cooling systems), and biota tangled in fishing nets and anchor chains (Sghaier *et al.*, 2011a; Minchin *et al.* 2009; Doll, 2018). Regarding ballast waters, recreational boats with inboard engines, such as medium to big watercrafts, generally have ballast tanks for stabilization that never dry completely and live organisms can be found in the residual water (Campbell *et al.* 2016; Doll, 2018).

Additionally, the National Program for Monitoring Marine Biodiversity and NIS in Tunisia identified 44 invasive NIS species to be monitored (PNUE-PAM-CAR/ASP, 2017). Of these species, 11 were recorded in this study: *Caulerpa cylindracea*, *Codium fragile*, *Halophila stipulacea*, *Bursatella leachii*, *Cerithium scabridum*, *Pinctada imbricata radiata*, *Tricellaria inopinata*, *Percnon gibbesi*, *Portunus segnis*, *Amathia verticillata* and *Hydroides elegans.* Therefore, Monastir Bay should be periodically monitored in order to update the records of NIS and cryptogenic species and increase insight into the ecological distribution of these species and their related impact on Tunisian natural communities and marine resources.

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