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

Sahar CHEBAANE1,2, Juan SEMPERE-VALVERDE2, Sahbi DORAI1, Adnen KACEM3 and Yassine RAMZI SGHAIER4

1 Association Notre Grand Bleu (NGB, NGO), Monastir, Tunisia
2 Laboratorio de Biología Marina (LBM), Facultad de Biología, Universidad de Sevilla, Sevilla, Spain
3 Research Laboratory LR14ES06 “Bioresources: Integrative Biology and Valorization”, Higher Institute of Biotechnology of Monastir, Avenue Tahar Hadded, BP 74, 5000 Monastir, Tunisia
4 Centre d’Activités Régionales pour les Aires Spécialement Protégées, Boulevard du leader Yasser Arafat, B.P.337 -1080, Tunis Cedex, Tunisie

Corresponding author: sahar1994ch@gmail.com
Handling Editor: Agnese MARCHINI

Received: 4 April 2019; Accepted: 2 September 2019; Published on line: 26 November 2019

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 environ-
ment (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-Lai 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 occurs 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 (Be thoux 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 (G DFA, 2018). However, this biodiversity hotspot has been impacted by several industrial, fishing, and fish-farming activities (Nouira et al., 2013; Challoul 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 Kuri-
at archipelago is located in the eastern part of Monastir Bay (Mangos & Claudot, 2013). This SCA includes two islands: big Kuriat or Qȗrya El Kabira and small Kuriat or Qȗrya 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 (Sghaijer 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 were 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).

Results

A total of 22 non-indigenous (NIS) and 2 cryptogenic species, belonging to nine phyla (Mollusca, Crustacea, Clorophyta, Bryozoa, Ascidiae, 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, Tricelaria inopinata, Hydrodies elegans, Branchiommia bairdi, Paracercis sculpia, Paradella dianae, Caprella scura 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 Ascidiae (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-F1.17 = 6.33, P (perm) <0.01; PERMDISP: F1.17 = 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%); Tapani, 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 Kantaucui, 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 et al., 2012).
| Taxa                        | Species                                                                 | Origin       | Mo | Ks | Sa | Te | Be | CM | Pr | Ha | sK1,2 | sK3 | sK4-6 | bK |
|-----------------------------|-------------------------------------------------------------------------|--------------|----|----|----|----|----|----|----|----|-------|-----|-------|----|
| Chlorophyta                 | *Caulerpa chemnitzia* (Esper) J.V. Lamououx,                           | RS/ Pa       |    |    |    |    |    |    |    |    |       |     |       |    |
|                             | *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* Blairville, 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           |    |    |    |    |    |    |    |    |       |     |       |    |
| Crustacea (Isopoda)         | *Branchionoma bairdi* (McIntosh, 1885)                                  | WA/EP        |    |    |    |    |    |    |    |    |       |     |       |    |
| Crustacea (Decapoda)        | *Paracerceis sculpta* (Holmes, 1904)                                   | Su           |    |    |    |    |    |    |    |    |       |     |       |    |
| Crustacea (Amphipoda)       | *Portunus segnis* (Forskål, 1775)                                      | WA           |    |    |    |    |    |    |    |    |       |     |       |    |
|                             | *Caprella scura* 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 brochienhelmi* (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     | 3  |
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; Martinez-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. 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).
among these structures. On the other hand, the semi-en-
closed water mass inside Monastir Bay could also con-
centrate propagules and larvae released from colonized
areas and contribute to this similarity of the NIS commu-
nity 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 com-
positions 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 as-
sociated 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. 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).

---

622 Medit. Mar. Sci., 20/3 2019, 616-626
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.

Acknowledgements

The surveys were conducted within the framework of supporting the management of the marine and coastal protected area of the Kuriat Islands executed by SPA/RAC in partnership with the Coastal Protection and Management Agency (APAL) and Notre Grand Bleu (NGB) NGO and funded by the MAVA Foundation. This work would not have been possible without the support of the Association Notre Grand Bleu. For taxonomic help, we would like to thank Prof. Alfonso A. Ramos Espla, Mr. Egidio Trainito and Dr. Rym Zakhma-Sraieb. The authors wish to express their gratitude to the handling editor and the two anonymous referees, who greatly contributed to improve the quality of the manuscript.

References

Aissi, M., Ben Amer, IB., Moslah S., Sghaier, Y.R., 2018. First record of the spotted sea hare Aplysia dactylomela Rang, 1828 in the south Mediterranean coast (Kuriat Islands, Tunisia). Journal of the Black Sea / Mediterranean Environment, 24 (2), 16368.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA + for PRIMER. Guide to software and statistical methods. PRIMER-E Ltd, Plymouth, UK.

Bдиou, M., 2016. Premier signalement du crabe bleu Portunus segnis (Forskal, 1775) dans le sud du golfe de Hammamet (centre-est de la Tunisie). Bulletin de l’Institut National des Sciences et Technologies de la Mer de Salammbo, 43,18387.

Ben Mustapha, K., Komatsu, T., Hattour, A., Sammari, Ch., Zarrouk, S. et al., 2002. Tunisian mega benthos from infra (Posidonia meadows) and circalittoral (coralligenous) sites. Bulletin de l’Institut National des Sciences et Technologies de la Mer de Salammbo. 29, 23-36.

Bethoux, J.P., Gentili, B., Morin, P., Nicolas, E., Pierre, C. et al., 1999. The Mediterranean Sea: a miniature ocean for climatic and environmental studies and a key for the climatic functioning of the North Atlantic. Progress in Oceanography, 44 (1-3), 131-146.

Boudouresque, C.F., Verlaque, M., 2010. Is global warming involved in the success of seaweed introductions in the Mediterranean Sea?. p. 31-50. In: Seaweeds and their role in globally changing environments. Cellular Origin, Life in Extreme Habitats and Astrobiology, vol 15. Seckbach, J., Einav, R., Israel, A. (Eds). Springer, Dordrecht.

Bradai, M.N., Quignard, J.P., Bouain, A., Jarboui, O., Quannes-Ghorbel, A., et al., 2004. Ichtyofaune autochtone et exotique des côtes tunisiennes: recensement et biogéographie. Cybium, 28 (4), 315-328.

Bulleri, F., Airoldi, L., 2005. Artificial marine structures facilitate the spread of a non-indigenous green alga, Codium fragile ssp. tomentosoides, in the north Adriatic Sea. Journal of Applied Ecology, 42 (6), 1063-1072.

Bulleri, F., Chapman, M.G., 2010. The introduction of coastal infrastructures as a driver of change in marine environments. Journal of Applied Ecology, 47 (1), 26–35.

Campbell, M.L., Gould, B., Hewitt, C.L., 2007. Survey evaluations to assess marine bioinvasions. Marine Pollution Bul-
recreational boating a potential vector for non-indigenous peracarid crustaceans in the Mediterranean Sea? A combined biological and social approach. *Marine Pollution Bulletin*, 140, 403-415.

Megina, C., González-Duarte, M.M., López-González, P.J., 2016. Benthic assemblages, biodiversity and invasiveness in marinas and commercial harbours: an investigation using a bioindicator group. *Biofouling*, 32, 465-475.

Mesel, I.D., Kerckhof, F., Norro, A., Rumes, B., Degraer, S., 2015. Succession and seasonal dynamics of the epifauna community on offshore wind farm foundations and their role as stepping stones for non-indigenous species. *Hydrobiologia*, 756 (1), 37-50.

Micheli, F., Halpern, B.S., Walbridge, S., Ciriaco, S., Ferretti, F. et al., 2013. Cumulative human impacts on Mediterranean and Black Sea marine ecosystems: assessing current pressures and opportunities. *PloS one*, 8 (12), e79889.

Minchin, D., Gollasch, S., Cohen, A.N., Hewitt, C.L., Olenin, S., 2009. Characterizing vectors of marine invasion. p. 109-116. In: *Biological invasions in marine ecosystems*. Springer, Berlin, Heidelberg.

Molina, S., Ros, M., Guerra-Garcia, J.M., 2017. Distribution of the Invasive Caprellid *Caprella scabra* (Crustacea: Amphipoda) in Cádiz Marina, Southern Spain: Implications for its Dispersal. *Thalassas: An International Journal of Marine Sciences*, 33(2), 81-86.

Nouira, T., Tagorti, M.A., Budzinski, H., Etcheber, H., Boussetta, H., 2013. Polycyclic aromatic hydrocarbons (PAHs) in surface sediments of Monastir Bay (Tunisia, Central Mediterranean): distribution, origin and seasonal variations. *International Journal of Environmental Analytical Chemistry*, 93 (14), 1470-1483.

Occhipinti Ambrogi, A., 2001. Transfer of marine organisms: a challenge to the conservation of coastal biocenoses. *Aquatic Conservation: marine and freshwater Ecosystems*, 11 (4), 243-251.

Occhipinti-Ambrogi, A., Savini, D., 2003. Biological invasions as a component of global change in stressed marine ecosystems. *Marine Pollution Bulletin*, 46 (5), 542-551.

Ostalé-Valriberas, E., Sempere-Valverde, J., Coppa, S., Garcia-Gómez, J.C., Espinosa, F., 2018. Creation of microhabitats (tidepools) in ripraps with climax communities as a way to mitigate negative effects of artificial substrate on marine biodiversity. *Ecological engineering*, 120, 522-531.

Ounifi-Amor, K.O.B., Rifi, M., Ghaneem, R., Draif, I., Zaouali, J. et al., 2016. Update of alien fauna and new records from Tunisian marine waters. *Mediterranean Marine Science*, 17 (1), 124-143.

Piola, R.F., Johnston, E.L., 2008. Pollution reduces native diversity and increases invader dominance in marine hard-substrate communities. *Diversity and Distributions*, 14 (2), 329-342.

Piola, R.F., Johnston, E.L., 2009. Comparing differential tolerance of native and non-indigenous marine species to metal pollution using novel assay techniques. *Environmental Pollution*, 157 (10), 2853-2864.

PNUE-PAM-CAR/ASP, 2017. *Programme National de surveillance de la biodiversité marine et des espèces non indigènes en Tunisie*. CAR/ASP – Projet EcApMEDII. Ben Haj S. (Ed.), Tunis, 49 pp.

Ramalhosa, P., Gestoso, I., Duarte, B., Caçador, I., Cannings-Clode, I., 2019. Metal pollution affects both native and non-indigenous biofouling recruitment in a subtropical island system. *Marine Pollution Bulletin*, 141, 373-386.

Ramos-Espla, A.A., Ben Mustapha, K., 2010. *Rapport de la mission d’tude des habitats marins et des principales espèces des Îles Kuriat (Tunisie) (octobre 2008)*. APAL-CAR/ASP-INSTM Salammbo-UA/UBM, 86 pp.

Rech, S., Salmina, S., Pichs, Y.J.B., García-Vázquez, E., 2018. Dispersal of alien invasive species on anthropogenic litter from European mariculture areas. *Marine pollution bulletin*, 131 (A), 10-16.

Rilov, G., Crooks, J.A., 2009. Marine bioinvasions: conservation hazards and vehicles for ecological understanding. p. 3-11. In: *Biological invasions in marine ecosystems*. Rilov, G., Crooks, J.A. (Eds). Springer, Berlin.

Ros, M., Vázquez-Luis, M., Guerra-García, J.M., 2013. The role of marinas and recreational boating in the occurrence and distribution of exotic caprellids (Crustacea: Amphipoda) in the Western Mediterranean: Mallorca Island as a case study. *Journal of Sea Research*, 83, 94-103.

Ros, M., Vázquez-Luis, M., Guerra-García, J.M., 2015. Environmental factors modulating the extent of impact in coastal invasions: the case of a widespread invasive caprellid (Crustacea: Amphipoda) in the Iberian Peninsula. *Marine pollution bulletin*, 98 (1-2), 247-258.

Ruiz, G.M., Freestone, A.L., Fonfonoff, P.W., Simkanin, C., 2009. Habitat distribution and heterogeneity in marine invasion dynamics: the importance of hard substrate and artificial structure. p. 321-332. In: *Marine hard bottom communities*. Springer, Berlin, Heidelberg.

Sempere-Valverde, J., Ostalé-Valriberas, E., Farfán, G.M., Espinosa, F., 2018. Substratum type affects recruitment and development of marine assemblages over artificial substrata: A case study in the Alboran Sea. *Estuarine, Coastal and Shelf Science*, 204, 56-65.

Sghaier, Y.R., Zakkhama-Sraieb, R., Benamer, I., Charfi- Cheikhrouha, F., 2011a. Occurrence of the seagrass *Halophila stipulacea* (Hydrocharitaceae) in the southern Mediterranean Sea. *Botanica Marina*, 54 (6), 575582.

Sghaier, Y.R., Zakkhama-Sraieb, R., Charfi-Cheikhrouha, F., 2011b. On the distribution of *Percnon gibbest* (H. Milne Edwards, 1853) (Crustacea, Decapoda, Plagusiidae) along the Tunisian coast. *Mediterranean Marine Science*, 12 (1), 233-237.

Sghaier, Y.R., Zakkhama-Sraieb, R., Mouelhi, S., Vazquez, M., Valle-Pérez, C. et al., 2016. Review of alien marine macrophytes in Tunisia. *Mediterranean Marine Science*, 17 (1), 109-123.

Sghaier, Y.R., Zakkhama-Sraieb, Ben Hmida, A., Charfi-Cheikhrouha, F., 2019. An inventory of non-indigenous species (NIS) inside and outside three tourist marinas from the southern Mediterranean coast. *Journal of the Black Sea / Mediterranean Environment*, 25 (1), 29-48.

Streftaris, N., Zenetos, A., 2006. Alien marine species in the Mediterranean – the 100 ‘worst invasive’ and their impact. *Mediterranean Marine Science*, 7 (1), 87-118.

Tilman, D., 1997. Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology*, 78 (1), 81–92.

Tlig-Zouari, S., Raboui, L., Irahtni, I., Ben Hassine, OK., 2013. Cumulative human impacts on Mediterranean waters. *Mediterranean Marine Science*, 14 (1), 65-70.
2009. Distribution, habitat and population densities of the invasive species *Pinctada radiata* (Molluca: Bivalvia) along the Northern and Eastern coasts of Tunisia. *Cahiers de Biologie Marine*. 50 (2), 131142.

Ulman, A., Ferrario, J., Occhpinti-Ambrogi, A., Arvanitidis, C., Bandi, A. *et al*., 2017. A massive update of non-indigenous species records in Mediterranean marinas. *PeerJ*, 5, e3954.

Ulman, A., Ferrario, J., Forcada, A., Arvanitidis, C., Occhipinti, A. *et al*., 2019. A Hitchhiker’s guide to Mediterranean marina travel for alien species. *Journal of Environmental Management*. 241, 328-339.

UNEP/MAP, 2016a. Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria. *United Nations Environment Programme Mediterranean Action Plan. Athens, Greece*. 22 (7), 1-162.

UNEP/MAP, 2016b. Integrated Monitoring and Assessment Guidance. *United Nations Environment Programme Mediterranean Action Plan. Athens, Greece*.

UNEP/MAP, 2018. Agenda items 3: Progress Report for April - September 2018. Compilation of Project Fiches. *United Nations Environment Programme Mediterranean Action Plan. Athens, Greece*. 87 (5), 53-56.

Verlaque, M., 2001. Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. *Oceanologica acta*, 24 (1), 29-49.

Vitousek, P.M., D’Antonio, C.M., Loope, L.L., Rejmanek, M., Westbrooks, R., 1997. Introduced species: a significant component of human caused global change. *New Zealand Journal of Ecology*, 21 (1), 1-16.

Woods, C.M., Floerl, O., Jones, L., 2012. Biosecurity risks associated with in-water and shore-based marine vessel hull cleaning operations. *Marine pollution bulletin*, 64 (7), 1392-1401.

Zaafrane, S., Maatouk, K., 2016. Note sur la présence de l’espèce *Siganus rivulatus* (Forsskål, 1775) au niveau des côtes–Est de la Tunisie. *Bulletin de l’Institut National des Sciences et Technologies de la Mer de Salammbô*, 43, 169177.

Zenetos, A., Gofas, S., Verlaque, M., Çinar, M. E., García Raso, E. *et al*., 2010. Alien species in the Mediterranean by 2010. A contribution to the application of European Union’s Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution. *Mediterranean Marine Science*, 11 (2), 381-493.

Zenetos, A., Gofas, S., Morri, C., Rosso, A., Violanti, D. *et al*., 2012. Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union’s Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. *Mediterranean Marine Science*, 13 (2), 328-352.

Zenetos, A., Çinar, M., Crocetta, F., Golani, D., Rosso, A. *et al*., 2017. Uncertainties and validation of alien species catalogues: The Mediterranean as an example. *Estuarine, Coastal and Shelf Science*, 191, 171-187.

Supplementary information is available for the article on-line.