

Ecological status assessment and Non-Indigenous Species in industrial and fishing harbours of the Gulf of Gabès (central Mediterranean Sea)

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Abstract

This study from January to December 2018 was the first initiative of a Port Baseline Survey (PBS) aimed at assessing the impact of biological invasions in harbours of the Gulf of Gabès (GG). A total of 12 fishing and industrial harbours were seasonally prospected during 2018. A total of 174 macrobenthos taxa were recorded, belonging to eight zoological groups, with a dominance of crustaceans (32%), molluscs (31%) and polychaetes (20%). Among these taxa, 57 were Non-Indigenous Species (NIS) for Tunisian waters, while 27 species were recorded for the first time in GG harbours and three decapods (*Dyspanopeus sayi*, *Hippolyte prideauxiana* and *Pilumnus minutus*) and one amphipod (*Hamimaera hamigera*) were newly recorded from Tunisian waters. Two main categories of harbours are distinguished according to their macrobenthic communities and environmental features (essentially edaphic factors). The industrial harbours yield higher numbers of species and abundance of NIS than the fishing ports. The ALEX metric is used to evaluate the biological invasion status of the Gulf of Gabès harbours, showing that their status ranges from unaffected in fishing harbours to extremely affected in industrial harbours. Three biotic indices (AMBI, BO2A and BENTIX) are applied to assess the ecological status of harbours, which varies from moderate to good. ALEX and the other biotic indices are significantly correlated with harbour characteristics, maritime traffic and edaphic factors (organic matter and chemical contamination). The present study gives an overview of all available benthic data, the impact of biological invasions and the ecological quality status of harbours in the Gulf of Gabès. The results could be considered as providing important baseline data for the implementation of environmental policies and management plans in the future.

Introduction

Harbours are considered as one of the most disturbed coastal ecosystems due to the increase in anthropogenic pressures (shipping activities, pollution and dredging) (Chan et al. 2016; Dauvin et al. 2017; Chatzinikolaou et al. 2018; Romanelli et al. 2019; Dimitriou et al. 2020). In addition, harbours are considered as the major pathway to Non-Indigenous Species (NIS) due to their special features (Buruaem et al., 2012; Ardura et al. 2015; Pejovic et al. 2016). In recent years, several studies have been carried out to identify the biota present in harbours to allow proper monitoring programmes of these marine environments. This strategic step needs to be preceded by a thorough and synergic study of the biological components of the ecosystems concerned (Mandal and Harkantra 2013; Lehtiniemi et al. 2015). Without baseline data on indigenous habitats, it is practically difficult to apply appropriate management protocols of marine biological invasions (Lehtiniemi et al. 2015).

In the Mediterranean Sea, harbours represent the primary recipient regions where NIS are most likely to settle, mainly because adequate habitat diversity is found in these marine ecosystems (Awad et al. 2014; Tempesti et al. 2020). The successful hosting of NIS largely depends on the compatibility between the settling of species in the new habitats and favourable conditions for establishment, including the availability of trophic resources and the absence of predators (Hayden et al. 2009). In recent years, marine bioinvasions have become one of the greatest global threats to the diversity and integrity of indigenous communities. These invasions are considered as drivers of irreversible impacts in host environments while also affecting the diversity and the stability of native habitats (Spagnolo et al. 2019). The Mediterranean Sea is one of the regions of the world ocean showing large invasive effects due to the impact of NIS; however, the number of known introductions is increasing and this may represent one of the greatest threats to Mediterranean biodiversity (i.e. Zenetos et al. 2010, 2012, 2017, 2018; Galil et al. 2018; Dragičević et al. 2019; Ballay et al. 2020). In fact, the number of NIS recorded in the Mediterranean Sea has been increasing, which affects habitats and ecosystem functioning (Katsanevakis et al. 2014, 2016).

With more than 1,400 km of coastline, Tunisia occupies a key position located at the crossroads between the western and central parts of the Mediterranean Sea. The Sicilian strait is the passageway from south to north and from east to west, and is crucial in the analysis of the spread of NIS introduced into the Mediterranean Sea. According to Ounifi-Ben Amor et al. (2016), 136 marine NIS have been recorded in Tunisian waters, with 60 records from northern coasts, and 76 from central and southern coasts. In 2019, Ben Souissi et al. (2019) reported, for the first time in Tunisia, two annelids, ten crustaceans, two bryozoans and one hydrozoan NIS. Moreover, a total of 27 non-indigenous marine macrophytes have been recorded in coastal Tunisian waters by Sghaier et al. (2016). However, the NIS inventories for Tunisian waters are not continuous over the period showing a rapid spread of NIS introductions linked to several factors: ballast water discharge and biofouling in relation to international maritime transport, aquaculture including the introduction of the Japanese oyster *Crassostrea gigas* (Thunberg, 1793), plus climate warming favouring the establishment of NIS populations. The Gulf of Gabès (southern Tunisia) includes the highest number of harbours in Tunisia; it is considered as one of the most vulnerable aquatic ecosystems in the Mediterranean Sea, mainly caused by biological invasions (Ounifi-Ben Amor, 2016). Moreover, intensive anthropogenic pressures due to trawling practices, diverse pollution (e.g. phosphogypsum inputs, industrial and urban wastes) and shipping activities have led to major environmental problems (Hattab et al., 2013; Boudaya et al. 2019; Mosbahi et al. 2019). Among the different taxonomic groups, macrobenthic invertebrates have been largely used as bio-indicators for aquatic monitoring because they respond rapidly to anthropogenic and natural stressors (Blanchet et al. 2008; Dauvin et al. 2012, 2017). Furthermore, these organisms have relatively long life spans, implying they are unable to escape from disadvantageous conditions, which means they can be used to assess accidental and chronic environmental variations (Dauvin 1993; Reiss and Kroncke 2005; Blanchet et al. 2008). In order to survey the biodiversity of harbours areas, many studies have focused on macrobenthic communities worldwide (Ingole et al. 2010; Mandal and Harkantra 2013; Dauvin et al. 2017; Chatzinikolaou et al. 2018).

Despite this research effort, biological and ecological information on harbour areas is generally scarce and seldom updated, bearing in mind that Port Biological Baseline Surveys (PBBS) can be used to provide data against which future changes in the structure and function of marine communities can be measured (Awad et al. 2014; Spagnolo et al. 2019). In Mediterranean harbours, the majority of studies have reported the prevalence of NIS on hard bottoms, including artificial structures in docks and marinas (Spagnolo et al. 2014, 2019). However, there are few quantitative studies designed to examine soft-bottom communities and the impacts of NIS in soft sediment habitats (Travizi et al. 2019). In Tunisia, the majority of benthic macrofauna studies have focused on macrobenthic communities of shallow coastal areas (i.e. Khedhri et al. 2014; 2015; Fersi et al. 2018; Boudaya et al. 2019; Mosbahi et al. 2017, 2019 and 2020). The few studies carried out on macrobenthic fauna in Tunisia harbours (Aloui-Bejaoui and Afi, 2012; Chatzinikolaou et al. 2018; Chebaane et al. 2020; Dimitriou et al. 2020) have focused on the structure of macrobenthic fauna or the identification of NIS in harbour ecosystems. Besides, the Ecological Quality

Status (EQs) of these ecosystems is not yet well studied. The aims of the present study covering the majority of the Gulf of Gabès harbours are as follows: 1) to describe their soft-bottom macrobenthic communities; 2) to assess their EQs linked to different levels of anthropogenic stress and biological invasions, so as to determine the relationship between these polluted zones and the establishment of NIS. Furthermore, this article presents baseline data for determining ecological status and bio-invasion impacts in Tunisian harbours and could serve as background information for harbour authorities and environmental managers for the design and implementation of a harmonized monitoring plan in the central Mediterranean.

Materials And Methods

Study area

The Gulf of Gabès (GG) is located in the central part of the Mediterranean Sea, delimited by the south-eastern coast of Tunisia, with more than 700 km of coastline extending from Ras Kaboudia in the North to the Tunisian-Libyan border in the south (between 35°-33°N and 10-13.5°E) (Fig.1). The Gulf is a very shallow basin with waters no deeper than 50 m as far as 110 km away from the coast (200 m depth at 400 km). Its climate is dry (average annual precipitation: 210 mm year⁻¹) and sunny with strong easterly winds. The tide is semidiurnal, with a maximum range of about 2.3 m near the coast around the Kneiss Islands (Sammari et al. 2006). The GG supports one of the most productive ecosystems in the Mediterranean Sea, not only associated with significant economic importance (contributing about 65% of the national fish production in Tunisia) but also comprising 18 harbours with numerous activities (fishing, commercial, marinas), and 4 commercial harbours (Zarzis, Gabès, Skhira and Sfax) open to international shipping (Hattab et al. 2013; Ben Rejeb Jenhani et al. 2018). In spite of its importance for fisheries and high natural heritage value, the GG is considered as one of the most heavily polluted Mediterranean areas owing to the presence of well-developed coastal cities generating various sources of industrial, agricultural and domestic contaminants (El Zrelli et al. 2018; Mosbahi et al. 2019). Due to its position in the central part of the Mediterranean, the GG is subject to increasing biological invasions through the continued introduction of NIS not only from the Red Sea via the Suez Canal as well as from Atlantic waters, but also from human activities such as marine traffic and aquaculture (Ounifi-Ben Amor et al. 2016).

Sampling design and sample treatments

Four seasonal surveys were conducted from January 2018 to December 2018 [January (winter), April (spring), July (summer), and October (autumn) 2018] in 12 GG harbours (Fig. 1). During each seasonal survey, to avoid temporal effects, the sampling campaign was carried out in each harbour during the same period. Two main categories of harbours can be distinguished: three industrial harbours and nine fishing harbours (Fig.1; Table 1). For each season, three stations were sampled in each harbour to cover the entire harbour basin from its entrance to the inner part, making a total of 36 stations visited four times during 2018. The station positions were accurately determined using a GPS (Global Positioning System, WGS84). For each station, five replicate samples were taken using a Van Veen grab sampling an area of about 0.05 m² (for each replicate) and penetrating approximately 0.1m into the sediment. Four replicate samples were preserved for biological analysis covering a total surface-area of 0.2 m². The last sample was used to determine sediment characteristics. Each biological sample was sieved on a 1 mm mesh and the retained fractions were conserved in 5% formaldehyde saline solution. Some collected samples contained a hard fraction including pebbles and shells, which were preserved and considered for further studies. In the laboratory, after staining with Rose Bengal, samples were sorted; the individuals were identified to the lowest possible taxonomic level and then counted.

In addition, temperature (T°C), salinity (Sal), pH, dissolved oxygen concentration (mg.L⁻¹) and transparency (m) were measured using a thermometer (WTWLF 196), a salinometer (WTW LF 196), a pH-meter (WTW 3110), an WTW oximeter and a Secchi disc, respectively, at each station and for each seasonal campaign. The Chlorophyll a concentration (Chl a) was determined on 1 L of marine water, which was collected (at each harbour/season) and transported in the dark and at low temperature to the laboratory and then filtered on GFC filters and extracted using 100% acetone. The absorbance was measured with a spectrophotometer at 630 nm, 647 nm, 664 nm and 750 nm and the concentration was estimated according to Rodier et al. (1996).

After eliminating the coarsest fraction, the sediment characteristics of the samples were determined by sieving around 1 Kg of dry sediment on 2, 1, 0.5, 0.25, 0.125 and 0.063 mm meshes and then weighing each sediment fraction. The mud content (% mud) is expressed as dry weight of <63 µm sediment in relation to the total weight, and the organic matter content (% OM) as weight loss on ignition at 500°C for 4h, using 100 g samples of dry sediment (24h at 60°C). Contents of heavy metals (Zn, Cd, and Pb), phosphorus (P), fluorine (F) and nitrogen (N) were determined after digesting the powder sample in aqua regia at 95°C, and analysis by inductively coupled plasma-atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) (Yoshida et al. 2002).

Statistical analysis

Benthic indices

For each station, we determined the most common and appropriate macrofauna biodiversity indices defining the EQ of a given station or benthic community. In this study, some benthic species associated with hard fractions were identified but excluded from the statistical analysis. Collected data were used to calculate the species abundance (A, number of individuals estimated per m²) to determine the most commonly used biodiversity indices for each station i.e.: the taxonomic richness (S, number of taxa 0.2 m²), the Shannon-Weaver diversity index (H') in log₂ (Shannon and Weaver 1963) and Pielou's evenness (J) (Pielou 1966). Data analysis was carried out using version 6 of the PRIMER® (Plymouth Routines in Multivariate Ecological Research) software package (Clarke and Gorley, 2006).

For the assessment of environmental quality, we make use of three biotic indices: AMBI (AZTI Marine Biotic Index, Borja et al. 2000), BENTIX (Simboura and Zenetos 2002) and BO2A (Benthic Opportunistic Annelids Amphipods index; Dauvin et al. 2016; Dauvin, 2018). AMBI and BENTIX were calculated using the software from AZTI, and then applied to estimate the proportions of five ecological groups (using the species list published by the AZTI web site on 30 June 2019 <http://ambi.azti.es/>). These indices were used to qualify the ecological status on a five-class scale of pollution, which considers five ecological groups:

EGI - taxa very sensitive to organic enrichment and disturbance, which are usually only present under unpolluted conditions; EGII - taxa indifferent to enrichment or disturbance; EGIII - taxa tolerant of excess OM enrichment, which may occur under normal conditions, but which are stimulated by organic enrichment; EGIV - second-order opportunistic species and EG-V - first-order opportunistic species, able to resist strong disturbance.

For AMBI, the thresholds are <1.2: high; 1.2-3.3: good; 3.3-4.3: moderate; 4.3-6: poor; >5.5: bad. In the case of BENTIX, the thresholds are >4.5: high; 3.5-4.5: good; 2.5-3.5: moderate; 2.0-2.5: poor; <2.0: bad. The BO2A (Benthic Opportunistic Annelids Amphipods) Index was calculated as \log_{10} of the ratio of frequencies for opportunistic annelids and amphipods: i.e. the total number of opportunistic annelids and total number of amphipods +1 divided by the overall abundance counted in a sample (see Dauvin et al., 2016). For BO2A, thresholds are < 0.0245: high; 0.0246-0.1300: good; 0.1301-0.1988: moderate; 0.1989-0.2551: poor; 0.2552-0.3010: bad (Dauvin, 2018).

Furthermore, ALEX (ALien biotic indEX, Çinar and Bakir 2014) is used to evaluate the impact of NIS on indigenous assemblages (Piazzini et al. 2020). This index is based on the abundance percentages of different groups defined from the level of species establishment and invasiveness within each sample (GI: indigenous species, GII: casual NIS, GIII: established NIS, GIV: invasive NIS). Species are assigned to ALEX groups according to observations of their abundance in the area and data from the relevant literature (see Çinar and Bakir 2014). We consider cryptogenic species as NIS, in order to avoid underestimation of their impact. ALEX can vary between 0 and 5, with 0 meaning the complete absence of NIS, and 5 corresponding to an assemblage composed solely of NIS. Therefore, the ES of a benthic assemblage can be ranked in one of the following five categories of quality: high [0-1], good [1-2], moderate [2-3], poor [3-4] and bad [4-5] (see Çinar and Bakir 2014).

The identified taxa were classified into six trophic groups according to the categories used by many authors (e.g. Afligian et al. 2008; Jumars et al. 2014; Mosbahi et al. 2017, 2019 and 2020). Non-selective deposit feeders (NSDF) are burrowers that ingest the sediment from which they take their food; selective deposit feeders (SDF) ingest organic particles from the sediment surface; suspension feeders (SF) extract suspended food in the water column; carnivores (C) feed on preys and herbivores/grazers (HG) feed on macrophytes and microalgae (Appendix 1).

One-factor analyses of variance (ANOVA) were performed to test the differences in physicochemical parameters, taxonomic richness, abundance (total abundance), diversity index, evenness, trophic groups and biotic indices between the studied stations and harbours as well as between sampling campaigns (four seasons). A *post hoc* Tukey test ($p < 0.05$) was used for *a posteriori* multiple comparisons. Prior to the ANOVAs, analyses were carried out to test the normality (*Kolmogorov-Smirnov*) and verify the homogeneity of variances (*Barlett*). The structure of benthic communities between seasons (Se; 4 levels) and harbours sites (Si; 12 levels) was compared by a two-way permutational multivariate analysis of variance (PERMANOVA). These statistical procedures were performed using R software.

Multivariate analysis

The spatial distribution of the harbour macrofauna community was analysed by group-average sorting classification. This was followed by a cluster analysis and Non-metric Multidimensional Scaling (n-MDS) ordination of stations or groups of stations, based on the Bray-Curtis similarity measure and calculated from square-root transformed data. SIMilarity PERcentages (SIMPER) tests were performed to determine which species contributed most to within-group similarity. The significance of differences between the groups of samples so obtained was assessed by an Analysis of similarities (ANOSIM). The BIOENV procedure was used to identify which combination of environmental variables best explains the differences in macrofauna distribution. These multivariate analyses were applied by PRIMER^{®-v6} software (Clarke and Gorley, 2006). Spearman's correlation coefficients were used to determine relationships between the biotic indicators and the environmental factors of the GG harbours. Spearman's correlation coefficients were calculated by the SPSS Statistics 20 software.

Results

Environmental characteristics

Some samples contained hard substrate material such as pebbles; samples taken from rocky pools or shingle substrates were excluded from the granulometric analyses. Sediment analyses show that the industrial harbours contain muddy sediment, while fishing harbours are dominated by fine and medium sand (Table 1). The organic matter (OM) and elemental contents show significant variation between the studied harbours ($F = 12.03$; $p < 0.01$) for OM; for trace metals $p < 0.01$ in all cases). The higher values of OM and metal contamination are recorded in the industrial harbours (GAI, SKI and SFI) (Table 2).

The chemical and physical parameters show seasonal variations. The temperature is maximal in summer ($33\text{ C}^{\circ} \pm 0.1$ in SKI and HSF), while minimum values are recorded in winter ($11\text{ C}^{\circ} \pm 0.02$ in HSF and SFF). Salinity is higher in summer and autumn, with high values being recorded in SFF (41.5 ± 0.05) and GAF (41.2 ± 0.01). pH shows a spatial variation during the sampling period, with low values in industrial harbours ($F = 12.03$; $p < 0.01$). Dissolved oxygen shows a significant spatial ($F = 11.12$; $p < 0.01$) and seasonal variability ($F = 28.13$; $p < 0.01$). Transparency only displays spatial changes ($F = 112.2$; $p < 0.05$), with low values being recorded in industrial harbours (SFI, SKI and GAI) during the all-sampling campaign (Appendix 1).

Macrobenthic community composition

A total of 16,102 individuals were identified, belonging to 174 macrozoobenthic taxa, 109 families and eight zoological groups unequally distributed among the sampling sites. Crustaceans are the dominant group in terms of number of taxa (55 taxa; 32 % of total number of taxa), followed by molluscs (53 taxa; 31%) and annelid polychaetes (35 taxa; 20%). The other five phyla taken together account for 17 % of the total number of taxa (Appendix 2). As regards the abundance, crustaceans (34% of the total abundance) and polychaetes (30%) are the most abundant groups in the all prospected harbours.

Among the species listed in this study, 57 are NIS (representing 33% of total taxonomic richness), including 23 that are recorded here for the first time in the GG. Four macrobenthic species are new for Tunisian waters: the decapods *Dyspanopeus sayi* (Smith, 1869), *Hippolyte prideauxiana* Leach, 1817 and *Pilumnus minutus* De Haan, 1835, and the amphipod *Hamimaera hamigera* (Haswell, 1879) (Appendix 3). The most abundant NIS are *Cerithium scabridium* Philippi, 1848 (58% of total individuals) *Pinctada imbricata radiata* Leach, 1814, (46%), *Cymadusa filosa* Savigny, 1816, (36%) and *Bursatella leachii* Blainville, 1817 (22%).

In each harbour, taxonomic richness and abundance varies significantly between the three sampled stations (ANOVA; for all harbours $p < 0.001$). The two stations located inside the harbour contain more taxa and numbers of individuals compared to the entrance station. Likewise, the inner stations are dominated by tolerant and opportunistic species such as *Capitella capitata* (Fabricius, 1780), *Hediste diversicolor* (O.F. Müller, 1776), *Cirratulus cirratus* (O. F. Müller, 1776), *Paracerceis sculpta* (Holmes, 1904) and *Dyspanopeus sayi*. Contrariwise, the harbour entrance stations are dominated by sensitive and indifferent species such as *Scrobicularia plana* (Linnaeus, 1758), *Anadara transversa* (Say, 1822), *Bursatella leachii* Blainville, 1817, *Eunice tubifex* (Crossland, 1904) and *Drilonereis filum* (Claparède, 1868).

The taxonomic richness differs significantly between harbour sites (ANOVA; $F = 80.12$; $p < 0.001$) (Fig.2). The mean taxonomic richness varies from 26.5 in the Zabboussa fishing harbour (ZBF) to 55 taxa in the Skhira industrial harbour (SKI). As well, the abundance of macrobenthic taxa shows a significant spatial difference between the studied harbours (ANOVA; $F = 127.2$; $p < 0.001$). The mean abundance varies from 6,157 ind.m⁻² in the fishing harbour of Skhira (SKF) to 9,968 ind.m⁻² in ZBF.

The species number and abundance of the NIS shows significant differences between the GG harbours. The higher number of NIS (ANOVA; $F = 181.9$; $p < 0.001$ for number of species) and abundance (ANOVA; $F = 92.6$; $p < 0.001$ for abundance of NIS) are recorded in the industrial harbours (Gabès, Skhira and Sfax) compared to the fishing harbours (Fig.2). The two-way PERMANOVA highlights a significant influence of site factors ($F = 1.412$; $p < 0.001$) on the structure of benthic communities (number of taxa and abundance) between harbours. The harbour macrofauna communities are strongly dominated by carnivores (43%), detritus-feeders (32%) and selective deposit feeders (22%). The diversity and abundance of the trophic groups varies significantly between harbour sites ($p < 0.05$). During the whole year, the benthic communities in industrial harbours are dominated by carnivores groups. On contrary, the trophic groups show a seasonal variation in the fishing harbours ($F = 84.12$; $p < 0.05$).

As regards seasonal changes, higher seasonal mean values of taxonomic richness are recorded in spring (88 taxa in SKI; 80 taxa in GAI), and lower values in winter (14 taxa) in the Zabboussa fishing harbour. Highest abundance values are obtained in spring (SKI; $A = 12,462 \pm 614$) and summer (GAI; $12,526 \pm 328$), while lower abundances are recorded in winter (HSF and ZBF; $A = 2,250 \pm 415$ and $3,050 \pm 220$ indm⁻², respectively). The taxonomic richness and abundances show significant seasonal variations in the studied harbours (PERMANOVA; $F = 3.104$; $p < 0.05$ for taxa number; $F = 122.12$; $p < 0.05$ for abundance). The number and abundance of NIS show seasonal variability (PERMANOVA; $F = 12.04$; $p < 0.01$; $F = 112.2$; $p < 0.05$ respectively), with higher values for NIS being recorded in spring (SFI; $N_{NIS} = 42 \pm 2.5$ species; $A_{NIS} = 5,206 \pm 118$ ind. m⁻²) and lower values in winter (ZBF; $N_{NIS} = 5 \pm 1.1$ species; $A_{NIS} = 865 \pm 84$ ind.m⁻²) (Appendix 1).

Spatial patterns of the macrofauna assemblage

Cluster analysis based on four replicates from the three stations at each harbour highlights a clear separation between internal and external stations (not shown here). Moreover, the cluster analysis and MDS ordination allows us to separate the 36 stations (three stations in 12 harbours) into two main groups at a similarity level of 30 %: the first group includes the nine stations sampled in industrial harbours (Gabès, Skhira and Sfax), while the second group corresponds to stations sampled in fishing harbours (Fig.3). SIMPER analysis shows that the second group (61.50% contribution to total similarity) is characterized by numerous representative indigenous species such as the amphipods *Gammarus insensibilis* (Stock, 1966), *Maera hironellei* Chevreux, 1900, *Dexamine spiniventris* (Costa, 1853), the polychaetes *Hediste diversicolor* (O.F. Müller, 1776), *Perinereis cultifera* (Grube, 1840), *Glycera tridactyla* Schmarda, 1861, *Heteromastus filiformis* (Claparède, 1864) and *Nephtys hombergii* Savigny in Lamarck, 1818 as well as the gastropod *Hexaplex trunculus* (Linnaeus, 1758) and the isopods *Cymodoce truncata* Leach, 1814 and *Paracerceis sculpta* (Holmes, 1904). However, the first group is largely represented by NIS such as *Pinctada imbricata radiata* (Leach, 1814), *Bursatella leachii*, *Cerithium scabridium* Philippi, 1848, *Eunice floridana* (Pourtalès, 1867), *Eunice tubifex*, *Cymadusa filosa* Savigny, 1816, *Portunus segnis* (Forskål, 1775) and also indigenous species such as the polychaetes *Euclymene lombricoides* (Quatrefages, 1866) and *Lumbrineris tetraura* (Schmarda, 1861) (Table 3). ANOSIM analysis reveals a significant difference between the two harbours groups ($R_{ANOSIM} = 0.38$; $p < 0.01$).

The BIOENV procedure indicates that the variations in macrofaunal distribution in harbours of the Gulf of Gabès can be explained by a combination of several variables (Table 4, correlation= 0.60). These correspond to sediment characteristics (OM, mud content and pollutant contamination) and harbour characteristics (depth and surface-area). Organic matter content individually shows the highest correlation with species distribution (correlation= 0.24).

Ecological Quality Status

The average biotic indices H' , J' , AMBI, BO2A and BENTIX differ significantly between sampling sites (ANOVA; $p < 0.05$), with H' values ranging from 2.02 (at SKI) to 3.88 bits.ind⁻¹ (at HSF), J' from 0.48 (at SKI) to 0.82 (at HSF and MAF), AMBI from 2.24 to 4.83, BO2A from 0.011 (at MAF) to 0.182 (at SKI) and BENTIX from 2.40 to 4.22. In the same way, ALEX values are statistically different between harbours (ANOVA; $F = 24.6$; $p < 0.05$), with values varying from 0.86 (MAF) to 4.20 (SKI). The three industrial harbours (Sfax, Skhira and Gabès) appear to be have moderate ES and are heavily to extremely affected by biological invasion, being strongly dominated by tolerant (EGIII) and opportunistic polychaete species. Contrariwise, biotic indices classify the majority of fishing harbours in good ecological status, with low ALEX values indicating these harbours are unaffected to moderately affected by biological invasion (Table 5).

The Spearman's rank correlation coefficients between environmental variables and ALEX, AMBI, BENTIX and Shannon-Weaver diversity show that ALEX is positively and significantly correlated with harbour surface-area ($\rho_r = 0.62$), temperature ($\rho_r = 0.46$), salinity ($\rho_r = 0.03$), organic matter ($\rho_r = 0.08$), silt percentage

in sediment ($p_r = 0.30$) and mud percentage ($p_r = 0.38$) as well as other environmental factors such as chemical contamination involving Pb, Cd, Zn, phosphorus, fluorine and nitrogen; on the other hand, the results show that ALEX is negatively and significantly correlated with depth ($p_r = -0.62$), transparency ($p_r = -0.25$) and dissolved oxygen ($p_r = -0.11$). The ALEX values ($p_r = 0.42$), number of NIS ($p_r = 0.26$), the number of individuals of NIS ($p_r = 0.36$) and the ratio between the number of individuals of NIS and indigenous species ($p_r = 0.46$) significantly increase with harbour surface-area. AMBI and BENTIX are negatively correlated with harbour surface ($p_r = -0.54$), organic matter content ($p_r = -0.33$), mud ($p_r = -0.32$), phosphorus ($p_r = -0.76$) and cadmium ($p_r = -0.12$). BO2A is positively correlated with organic matter ($p_r = 0.42$) and mud ($p_r = 0.58$) and negatively correlated with trace metals such as Cd ($p_r = -0.14$) and Pb ($p_r = -0.05$) (Table 6).

Discussion

Due to their special features, harbours are considered as disturbed marine environments where different sources of pollution, combined with global changes, result in complex ecological relations and different processes which often mask the distinction between environmental and natural impacts (Kapsimalis et al. 2014; Chan et al. 2016; Chatzinikolaou et al. 2018; Tempesti et al. 2020). The present study investigates the benthic macrofauna structure, the distribution of benthic assemblages and EQ assessment by the application of some functional and biotic indices in response to multiple stressors in the GG harbour ecosystems.

In this study, the species inventory comprises 29 macrobenthic taxa recorded for the first time in the GG, including four species new to Tunisian waters that have already been described in the Mediterranean Sea. Therefore, taking into account the 136 species listed by Ounifi-Ben Amor et al. (2016) and the latest inventory added by Ben Souissi et al. (2017; 2019), the number of NIS recorded in Tunisian waters now reaches a total of 157. The number of NIS recorded in Tunisia has been increasing during the last few decades. The massive introduction of new NIS could be related to certain economic activities such as marine traffic, fisheries, aquaculture and tourism (Streftaris and Zenetos 2006; Ben Souissi et al. 2014; Ounifi-Ben Amor et al. 2016).

In terms of taxonomic composition, the structure of communities in GG harbours is similar to that observed in other harbours of the Mediterranean ecosystem, being dominated mainly by molluscs, crustaceans and polychaetes (Dauvin et al. 2017; Chatzinikolaou et al. 2018; Travizi et al. 2019; Dimitriou et al. 2020). The number of taxa (174) and abundance of macrobenthic communities observed in the GG harbours are relatively high compared to other Mediterranean harbours. For example, Chatzinikolaou et al (2018) indicated 33 species in Cagliari harbour (Italy) and 23 species at Heraklion (Greece). Similarly, Travizi et al. (2019) identified 43 species in Rijeka harbour (Croatia), 112 species in Ancona harbour (Italy) and 121 species in Koper harbour (Slovenia). However, this diversity in Gabès harbours appears low compared to the biodiversity recorded recently by Chatzinikolaou et al. (2018) in the El Kantaoui harbour (Tunisia) (211 taxa), as well as by Travizi et al. (2019) in the Bari harbour (Italy) (224 taxa) and by Dauvin et al. (2017) for 10 Algerian harbours (847 taxa). This difference can be attributed to the sampling strategy adopted for each zone (sampling effort, time and methods of sampling), the number of sampled harbours and the specificity of the harbour environment (e.g environmental conditions, anthropogenic stressors) (Table 7).

The macrobenthic fauna composition and benthic diversity in GG harbours show spatial variations between the inner basins and the entrances. These differences could be explained by changes in the levels of organic and metal contamination, which vary according to the hydrodynamic regime corresponding to the water exchange between the inner basins and the entrances of the harbours (Grimes 2010; Dauvin et al. 2017). Equally, the spatial distribution of macrobenthic communities in GG harbours reflects the existence of two distinct macrofaunal assemblages. The first assemblage corresponds to the stations sampled in industrial harbours, which appear severely and extremely affected by NIS such as *Pinctada imbricata radiata*, *Cerithium scabridium*, *Bursatella leachii*, *Paracerceis sculpta*, *Cymadusa filosa*, *Portunus segnis* and *Libinia dubia*. The second assemblage regroups stations sampled in fishing harbours dominated by indigenous opportunistic species such as *Gammarus insensibilis*, *Maera hironellei*, *Dexamine spiniventris*, *Glycera tridactyla*, *Perinereis cultifera*, *Nephtys hombergii* and *Heteromastus filiformis*; this assemblage is unaffected or only slightly affected by biological invasions. The difference between the two assemblages could be explained by the difference of environmental parameters between the GG harbours and especially their sediment type, organic matter content and contamination by pollutants. In fact, industrial harbours are characterized by muddy sediment richer in organic matter and heavy metals. Several previous studies in harbours have indicated that sediment features, water depth, hydrodynamics and pollution (i.e. hydrocarbons) are significant environmental factors which are associated with the clustering of samples from harbours in terms of abundance (Blanchard et al. 2002; Dauvin et al. 2017; Chatzinikolaou et al. 2018).

The effect of the frequency of marine traffic and environmental disturbances is highlighted by the high number of NIS recorded in the three industrial harbours of the GG (Gabès, Skhira and Sfax). In the Mediterranean Sea, maritime traffic in commercial and industrial harbours is the main pathway evoked for the introduction of NIS (Molnar et al. 2008; Nunes et al. 2014). The introduced species are attached to the submerged parts of vessel hulls, while organisms transported by water or sediment are contained in ballast water discharges (Simkanin et al. 2009; David and Gollasch 2015; Travizi et al. 2019). For these reasons, industrial harbours (marinas as well) are considered as coastal environments especially susceptible and vulnerable to the establishment of NIS due to their favourable abiotic and biotic conditions (i.e. trophic availability, less competition and predation) (Çinar et al. 2012; Awad et al. 2014). Several authors have shown that harbours do not represent good habitats for indigenous communities and are instead populated by highly tolerant and opportunistic introduced species. Likewise, shipping is considered as the major pathway for NIS introduction in Mediterranean harbours (López-Legentil et al. 2015; Ulman et al. 2019; Tempesti et al. 2020). These authors also found a significant relationship between harbour size and species richness, indicating that larger harbours tend to contain higher numbers of species associated with higher abundances. This is in agreement with the results of our study, which show that the major harbours of the GG (industrial harbours) are those which shelter more species, especially the NIS. Recent studies also show that recreational boating and fishing vessels might play an important role, especially in the spreading of NIS at the small to medium spatial scale (Ferrario et al. 2015; López-Legentil et al. 2015; Ulman et al. 2017, 2019; Tempesti et al. 2020).

The GG harbours are strongly dominated by carnivores, detritus-feeders and selective deposit feeders related to the availability of trophic resources. Several authors have mentioned a significant relationship between benthic trophic structure, sediment contaminants and environmental variables (Guerra-García and

García-Gómez 2005; Chatzinikolaou et al. 2018). Therefore, changes in trophic structure could be used as an indicator of disturbance. The reduction in trophic complexity is associated with organic-rich and chemically-contaminated sediments in polluted environments (i.e. harbours), where the benthic assemblages are dominated by opportunistic species (Rakocinski et al. 2000; Putro et al. 2009; Dauvin et al. 2017).

In Mediterranean harbours, several multivariate biotic indices such as AMBI (Borja et al. 2000), BENTIX (Simboura and Zenetos 2000), BO2A (Dauvin et al. 2016) and BQI (Leonardsson et al. 2015) have been recommended and extensively used to assess and monitor EQs (Riera et al. 2011; Sany et al. 2015; Tomassetti et al. 2016; Dimitriou et al. 2020). In the present study, three biotic indices (AMBI, BO2A and BENTIX) are used to assess EQs, allowing us to classify the GG harbours as having poor to good ecological status. The first group includes stations sampled in fishing harbours, which appear to be in good ecological status. On the other hand, the second group is made up of industrial harbours showing a moderate ecological status, dominated by pollution-tolerant species and opportunistic polychaetes species indicative of stressed environments, such as *C. capitata*; *S. (Scolelepis) squamata*, *M. fuliginous* and *C. cirratus*. These latter species form part of normal benthic communities found in the open sea, colonizing fine sediment habitats, which could be associated with pollution indicator species showing a slightly polluted system. These opportunist species are also observed in other polluted harbours such as Marseilles along the French Mediterranean coast (Bellan et al. 1980) and the Bethioua and Djendjen harbours in Algeria (Dauvin et al. 2017). Both of these south Mediterranean harbours are strongly affected by diverse anthropogenic pressures which mean these environments are subject to heavy metal and hydrocarbon pollution, as well as accumulation of excessive OM, the release of warm waters from power plants and the input of nutrients (Bellan et al. 1980; Dauvin et al. 2017). The relationships between biotic indices and environmental stressors of harbours ecosystems have been studied by many ecologists, who show a highly significant negative correlation between functional diversity, biotic indices and pollution proxies (i.e. chemical pollutants, sediment contamination and plastic litter) (Cole et al. 2011; D'Alessandro et al. 2018, 2020; Dimitriou et al. 2020). In fact, the lower index values recorded by D'Alessandro et al. (2020) in Maltese harbours suggests a highly disturbed community, by virtue of a heterogeneously occupied functional space with a high level of pollution.

The ALEX index is used here to describe the bio-invasion status in GG harbours, showing that the environmental status of benthic habitats varies from unaffected to extremely affected. The high ALEX score recorded in the industrial harbours shows that some areas in the GG have been modified by biological invasion, with a predominance of established and invasive species. Lower scores are obtained in fishing harbours, indicating unaffected areas, which confirms the suitability of ALEX for discriminating between the sampled harbours. The results obtained by ALEX are in agreement with other studies showing that the GG harbours are hotspots of biological invasions and presented highest values of ALEX (Piazzi et al. 2018, 2020; Tempesti et al. 2020). The correlations between ALEX values and environmental variables indicates that NIS largely prefers shallow-water habitats with a high content of organic matter, mud and silt, along with enrichment in heavy metals (Pb, Cd and Zn) and certain other elements such as phosphorus, fluorine and nitrogen. This pattern of enrichment had been observed by Çinar and Bakir (2014) on the coasts of Turkey, where NIS are found coexisting in disturbed environments. It is generally accepted that the majority of Lessepsian migrants have managed to colonize the shallow-water habitats of the eastern Mediterranean, while the shallowness of the canal has acted as a filter for the deep-water biota of the Red Sea (Por 1978; Çinar et al. 2011; 2014). ALEX is positively correlated with the surface-area of GG harbours. In fact, the high number of NIS recorded in the industrial GG harbours seems to be related to shipping activities and associated biosecurity risks, which include hull biofouling, standing waters from commercial vessels (e.g. in ballast tanks, bilges, anchor chains and engine cooling systems) (Minchin et al. 2009; Lawrence and Cordell 2010; Ferrario et al. 2017; Ulman et al. 2019). The industrial harbours of the GG represent one of the most important economic activities of Tunisia; in 2017, more than 1,400 international vessels from several destinations around the world visit the three industrial harbours in the GG (OMMP 2018). These results confirm that marine traffic is a main vector responsible for the introduction of NIS into central Mediterranean harbours.

Conclusion And Future Perspectives

The present overview provides a valuable baseline database of benthic diversity in the GG harbours, covering a part of the central Mediterranean Sea that is still insufficiently studied. Different environmental parameters affect the distribution of benthic species. Many NIS are newly reported for the GG and have become established in the industrial ports owing to the continual increase of harbour activities. The ALEX metric shows that the ecological status of the GG harbours varies from unaffected to extremely affected by the rapid increase of NIS introduction. Changes in the metric according to the different sites show that AMBI, BENTIX, BO2A and ALEX respond to the range of environmental conditions found in each studied harbour (sediment type, harbour activity, pollution level). Finally, the results of this study suggest that indigenous biota require some protection against biological invasions in harbour ecosystems. Since these harbours offer hubs for invaders (Floerl et al. 2009), they should be priority targets for control actions through the setting up of '*Biological Invasions Risk Management Programmes*' in the GG harbours, considering that these areas are very sensitive to anthropogenic pressures and biological invasions. Such programmes should be operated by local port management authorities, local fishermen and research committees to file and implement a monitoring strategy for future management actions. For future research, it appears very important to supplement this work by studies on the macrozoobenthic species of harbour hard substrates since these habitats are known to be colonized by numerous NIS (López-Legentil et al. 2015; Piazzi et al. 2020).

Declarations

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Authors' contributions

This collaboration work was carried out among all the authors. MN, JC-D and LN contributed to the conception and design of the study. MN wrote the first draft of the manuscript, JP-P performed some statistical analyses. All authors contributed to manuscript revision, read, and approved the submitted version. Dr, M.S.N. Carpenter post-edited the English style and grammar.

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All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Ethical approval

Not applicable.

Consent to participate

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Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1 List of the 12 harbours surveyed in this study, with code (abbreviation); type (F: fishing; I: industrial); Exp date: start of exploitation; Surf: surface-area (ha); Dep: Depth (m); Sed type: sediment type (M: mud; Fs: fine sand; Ms: medium sand); *hard substrate: large empty shells, pebbles and rocky pools and shingle; (-) absence, (+) presence.

| Harbour | Code | Geographical position | | Type | Exp date | Surf (ha) | Dep (m) | Sed type | Presence |
|------------|------|-----------------------|------------|------|----------|-----------|---------|----------|----------------|
| | | Latitude | Longitude | | | | | | hard substrate |
| Ajim | AJF | 33.71773°N | 10.74244°E | F | 1985 | 2.6 | 2 | Ms | - |
| Houmt Souk | HSF | 33.88936°N | 10.85677°E | F | 1948 | 5.15 | 6 | Fs | - |
| Zarzis | ZAF | 33.51500°N | 11.27678°E | F | 1976 | 28 | 6 | M | + |
| Gabes | GAI | 33.90724°N | 10.10169°E | I | 1885 | 30 | 10 | M | - |
| | GAF | 33.97524°N | 10.18381°E | F | 1985 | 4.5 | 5 | Fs | - |
| Skhira | SKI | 34.29949°N | 10.11677°E | I | 1985 | 22 | 15 | M | + |
| | SKF | 34.26743°N | 10.17523°E | F | 1975 | 2.6 | 2 | M | - |
| Zabboussa | ZBF | 34.34790°N | 10.21108°E | F | 1998 | 4 | 3 | Ms | + |
| Mahres | MAF | 34.51738°N | 10.49932°E | F | 1987 | 5 | 4.5 | Ms | - |
| Sfax | SFI | 34.72957°N | 10.77660°E | I | 1905 | 24 | 10 | M | - |
| | SFF | 34.66412°N | 10.86530°E | F | 1981 | 37.5 | 4.5 | Fs | + |
| Kerkennah | KEF | 34.65685°N | 10.96867°E | F | 1992 | 10 | 5 | Ms | + |

Table 2 Organic matter content and pollutant concentrations in surface sediments of the Gulf of Gabès harbours (\pm SD average of the four seasons).

| | AJF | HSF | ZAF | GAI | GAF | SKI | SKF | ZBF | MAF | SFI | SFF |
|-----------------|-----------|-----------|------------|-------------|-------------|------------|----------|-----------|-----------|------------|----------|
| OM (%) | 18.75±2.4 | 16.18±2.8 | 19.20 ±1.9 | 33.82 ±3.2 | 24.60±3.4 | 42.20 ±2.8 | 20.4±3.1 | 15.6±1.2 | 18.14±1.8 | 40.22±3.0 | 32.4±2.7 |
| F (%) | 0.2±0.04 | 0.1±0.2 | 1.5±0.3 | 1.8±0.05 | 1.4±0.2 | 1.8±0.2 | 2.1±0.2 | 1.21±0.04 | 2.0±0.1 | 1.4±0.01 | 1.8±0.04 |
| N (%) | 0.9±0.2 | 1.2±0.1 | 0.3±0.02 | 2.8±0.1 | 2.2±0.01 | 2.8±0.05 | 2.2±0.1 | 2.01±0.02 | 0.4±0.01 | 2.6±0.02 | 2.6±0.02 |
| P (ppm) | 248±88.2 | 118± 64.5 | 202± 74.2 | 2.214± 116 | 1.220 ± 202 | 2.442±218 | 842±312 | 442±114 | 312± 104 | 3.442±218 | 1.824±12 |
| Pb (ppm) | 3.8± 0.6 | 2.7±0.8 | 1.4± 0.5 | 14.2± 1.4 | 3.2± 0.8 | 10.24± 1.6 | 6.7± 1.1 | 2.5± 0.4 | 3.9± 0.6 | 12.24±1.2 | 8.6±0.8 |
| Zn (ppm) | 645±98 | 605±101 | 304±102 | 2.612±212 | 980±108 | 2.520±140 | 988±104 | 402±98 | 366±140 | 3.422±162 | 1.042±10 |
| Cd (ppm) | 120± 86 | 118± 94 | 204± 66 | 1.030 ± 101 | 402± 92 | 930 ±126 | 502±78 | 126±88 | 241±74 | 1.210 ±112 | 860±102 |

Table 3 MDS groups, with indication of similarities within each group (%) and the most representative species (RS) contributing to the similarity (Cs in %) within the group, determined by SIMPER analysis, (*) Non Indigenous Species.

| Group | Industrial harbours | | Fishing harbours | |
|----------------------|-------------------------------------|-------|----------------------------------|-------|
| | RS | Cs | RS | Cs |
| Similarity (%) | 40.20 | | 61.50 | |
| Dominant species (%) | <i>Cerithium scabridium</i> * | 56.75 | <i>Gammarus insensibilis</i> | 62.40 |
| | <i>Pinctada imbricata radiata</i> * | 52.42 | <i>Maera hirondellei</i> | 57.16 |
| | <i>Bursatella leachii</i> * | 48.10 | <i>Dexamine spiniventris</i> | 53.10 |
| | <i>Paracerceis sculpta</i> * | 44.96 | <i>Hediste diversicolor</i> | 47.30 |
| | <i>Cymadusa filosa</i> * | 43.42 | <i>Perinereis cultifera</i> | 43.62 |
| | <i>Eunice tubifex</i> | 39.19 | <i>Glycera tridactyla</i> | 40.23 |
| | <i>Portunus segnis</i> * | 35.25 | <i>Heteromastus filiformis</i> | 36.80 |
| | <i>Paracaprella pusilla</i> * | 31.05 | <i>Nephtys hombergii</i> | 34.42 |
| | <i>Diopatra hupferiana monroi</i> | 27.05 | <i>Scrobicularia plana</i> | 29.10 |
| | <i>Hydroides elegans</i> * | 24.15 | <i>Hexaplex trunculus</i> | 28.16 |
| | <i>Dexamine spiniventris</i> | 22.30 | <i>Cymodoce truncata</i> | 26.50 |
| | <i>Libinia dubia</i> * | 20.12 | <i>Paracerceis sculpta</i> | 24.40 |
| | <i>Dexamine spinosa</i> | 17.20 | <i>Eunice vittata</i> | 21.80 |
| | <i>Capitella capitata</i> | 16.10 | <i>Microdeutopus gryllotalpa</i> | 17.45 |
| | <i>Mitrella psilla</i> * | 11.02 | <i>Lysianassa costae</i> | 12.50 |

Table 4 Correlations obtained by associating different numbers of environmental variables with macrofaunal distribution in the Gulf of Gabès harbours, as revealed by the BIOENV procedure. The best correlation (0.60) is obtained by associating four variables. With OM: organic matter, Do: dissolved oxygen, Trp: transparency, MT: marine traffic, HS: harbour surface.

| N° of variables | Correlation | Selections |
|-----------------|-------------|------------------------------------|
| 1 | 0.24 | OM |
| 1 | 0.15 | Depth |
| 1 | 0.21 | Mud |
| 2 | 0.26 | OM, mud |
| 2 | 0.21 | OM, silt |
| 3 | 0.30 | OM, mud, Trp |
| 3 | 0.42 | OM, mud, depth |
| 3 | 0.38 | OM, mud, P |
| 4 | 0.44 | OM, mud, Trp, silt, |
| 4 | 0.60 | Mud, Pb, Zn, OM |
| 5 | 0.56 | HS, mud, OM, Cd, Pb |
| 5 | 0.45 | OM, depth, MT, P, Trp |
| 6 | 0.54 | Mud, OM, depth, Pb, Zn, MT |
| 7 | 0.60 | OM, Pb, P, mud, Cd, depth, HS |
| 8 | 0.48 | OM, mud, P, Zn, Pb, Cd, Trp, depth |

Table 5 Average values of the biotic indices and Alien Index for each Gulf of Gabès harbour (mean of three sampled stations sampling during the four seasons; \pm SD). Colours indicate status as follows: red: Bad; orange: Poor; Yellow: Moderate; Green: Good; Blue: High. Codes see Table 1.

| | AJF | HSF | ZAF | GAI | GAF | SKI | SKF | ZBF | MAF | SFI |
|---------------|------------------|------------------|------------------|------------------|-----------------|------------------|------------------|------------------|------------------|----------------|
| H' | 3.14 \pm 0.41 | 3.85 \pm 0.38 | 3.60 \pm 0.28 | 2.32 \pm 0.46 | 3.35 \pm 0.38 | 2.02 \pm 0.41 | 3.65 \pm 0.32 | 3.68 \pm 0.52 | 3.40 \pm 0.61 | 2.12 \pm 0.2 |
| J' | 0.78 \pm 0.11 | 0.82 \pm 0.05 | 0.79 \pm 0.02 | 0.59 \pm 0.09 | 0.81 \pm 0.05 | 0.48 \pm 0.10 | 0.78 \pm 0.08 | 0.80 \pm 0.05 | 0.82 \pm 0.06 | 0.52 \pm 0.1 |
| AMBI | 3.24 \pm 0.04 | 2.64 \pm 0.08 | 2.88 \pm 0.04 | 4.13 \pm 0.50 | 3.80 \pm 0.48 | 4.32 \pm 0.25 | 3.84 \pm 0.62 | 2.62 \pm 0.44 | 2.24 \pm 0.36 | 4.83 \pm 0.5 |
| BO2A | 0.052 \pm 0.01 | 0.071 \pm 0.02 | 0.012 \pm 0.01 | 0.142 \pm 0.01 | 0.09 \pm 0.02 | 0.182 \pm 0.04 | 0.078 \pm 0.02 | 0.051 \pm 0.01 | 0.011 \pm 0.02 | 0.124 \pm 0. |
| BENTIX | 3.80 \pm 0.60 | 4.10 \pm 0.51 | 4.22 \pm 0.05 | 2.61 \pm 0.08 | 4.12 \pm 0.62 | 2.40 \pm 0.41 | 4.25 \pm 0.22 | 3.74 \pm 0.42 | 3.75 \pm 0.28 | 2.20 \pm 0.1 |
| ALEX | 1.12 \pm 0.22 | 1.9 \pm 0.08 | 2.1 \pm 0.16 | 3.44 \pm 0.28 | 1.2 \pm 0.05 | 4.20 \pm 0.04 | 1.34 \pm 0.06 | 0.88 \pm 0.02 | 0.86 \pm 0.05 | 4.16 \pm 0.1 |

Table 6 Spearman's rank correlation coefficients between the means of environmental variables and the mean of ALEX values, benthic and biotic indices and other community parameters. AS: Number NIS; AN: Number of NIS individuals; NIS/IS: ratio between NIS and indigenous species; NIS/IN: ratio between NIS individuals and indigenous individuals; marine traffic: number of vessels recorded in 2018 by APIP and OMMP. Values in bold are statistically significant ($p < 0.05$).

| | ALEX | AMBI | BENTIX | BO2A | H' | AS | AN | NIS/NS | NIS/NN |
|------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Temperature | 0.930 | 0.029 | 0.12 | 0.22 | 0.158 | 0.624 | 0.011 | 0.973 | 0.136 |
| Salinity | 0.03 | 0.028 | 0.32 | 0.12 | 0.15 | 0.62 | 0.011 | 0.97 | 0.13 |
| pH | 0.41 | -0.21 | 0.11 | 0.01 | 0.01 | 0.11 | 0.03 | 0.12 | 0.15 |
| Dissolved oxygen | -0.11 | 0.18 | 0.05 | 0.001 | 0.20 | 0.16 | 0.21 | -0.16 | -0.18 |
| Transparency | -0.25 | 0.36 | -0.06 | 0.10 | -0.13 | 0.18 | 0.36 | -0.28 | -0.52 |
| Organic matter | 0.08 | -0.33 | -0.40 | 0.42 | -0.12 | 0.02 | 0.40 | 0.22 | -0.32 |
| Harbour surface | 0.42 | -0.54 | 0.22 | 0.02 | 0.03 | 0.26 | 0.38 | 0.56 | 0.46 |
| Depth | -0.62 | 0.036 | -0.16 | 0.01 | -0.43 | -0.28 | -0.56 | -0.48 | -0.19 |
| Marine traffic | 0.46 | -0.29 | -0.26 | 0.12 | -0.12 | 0.30 | 0.34 | 0.22 | 0.38 |
| Chl a | 0.09 | 0.0001 | 0.22 | 0.12 | 0.93 | 0.0001 | 0.09 | 0.001 | 0.19 |
| Sand | -0.22 | -0.26 | 0.25 | 0.21 | 0.10 | 0.03 | 0.06 | -0.42 | -0.28 |
| Silt | 0.30 | 0.18 | 0.49 | 0.06 | 0.22 | 0.01 | 0.11 | 0.03 | 0.16 |
| mud | 0.38 | -0.32 | 0.41 | 0.58 | 0.21 | 0.016 | 0.34 | 0.16 | 0.35 |
| Phosphorus | 0.54 | 0.76 | 0.05 | 0.2 | 0.62 | 0.30 | -0.65 | 0.58 | 0.83 |
| Nitrogen | 0.15 | 0.63 | 0.02 | 0.10 | 0.18 | 0.52 | -0.23 | 0.46 | -0.16 |
| Fluorine | 0.865 | 0.12 | 0.04 | 0.01 | -0.92 | 0.01 | 0.28 | 0.001 | 0.91 |
| Pb | 0.029 | 0.30 | -0.16 | -0.05 | 0.58 | 0.24 | 0.11 | 0.17 | 0.36 |
| Zn | 0.057 | 0.02 | 0.22 | 0.001 | 0.67 | 0.30 | -0.56 | 0.058 | 0.58 |
| Cd | 0.473 | 0.120 | -0.18 | -0.14 | -0.51 | 0.093 | 0.10 | 0.18 | 0.08 |

Table 7 Previous studies on macrobenthic fauna in other harbour ecosystems, with sampling technique used (VV: VV Van Veen grab; b cor: box corer; h cor: hand corer), number of stations and replicates sampled by harbour (S (R)), sampled surface-area by harbour (SS), total number of taxa (TS) and mean abundance (ind.m⁻²) by harbour.

| Study area | Sampling technique | Sampled period | S (R) | SS | TS | A | Reference |
|---------------------------|---------------------------------|----------------------------------|---------|-------|-----|--------|------------------------------|
| harbours (Tunisia) | VV (0.05m ²) | Four seasons (2018) | | 0.6 | 174 | 7, 936 | This study |
| | | | 3 (4) | | | | |
| | | | | 0.12 | 211 | 412.7 | |
| antaoui harbour (Tunisia) | b cor (13.5 × 13.5 × 16 cm). | winter, spring and summer (2012) | 3 (5) | | | | Chatzinikolaou et al. (2018) |
| | | | | 0.12 | 33 | 207.3 | |
| gliari harbour (Italy) | | winter, spring and summer (2012) | 5(5) | | | | |
| | | | | 0.12 | 23 | 193 | |
| aklion harbour (Greece) | | winter, spring and summer (2012) | 4 (5) | | | | |
| | h corer | | | 0.225 | 112 | 532 | |
| ona harbour (Italy) | (0.025m ²) | spring and autumn 2014 | 3 (3) | | | | |
| | | | | 0.75 | 224 | 1, 076 | Travizi et al. (2019) |
| i harbour (Italy) | | spring and autumn 2014 | 3 (1) | | | | |
| | | | | 0.75 | 121 | 555.7 | |
| er harbour (Slovenia) | | spring and summer 2013 | 3 (1) | | | | |
| a harbour (Croatia) | | autumn 2014 and spring 2015 | | 0.75 | 127 | 172 | |
| | | | 3 (1) | | | | |
| tijeka harbour (Croatia) | | autumn 2014 and spring 2015 | 3 (1) | 0.75 | 43 | 68.3 | |
| erian harbour (Algeria) | VV (0.05m ²) | Spring and summer (1983-2001) | 571 (2) | 0.25 | 847 | 643.5 | Dauvin et al. (2017) |

Figures

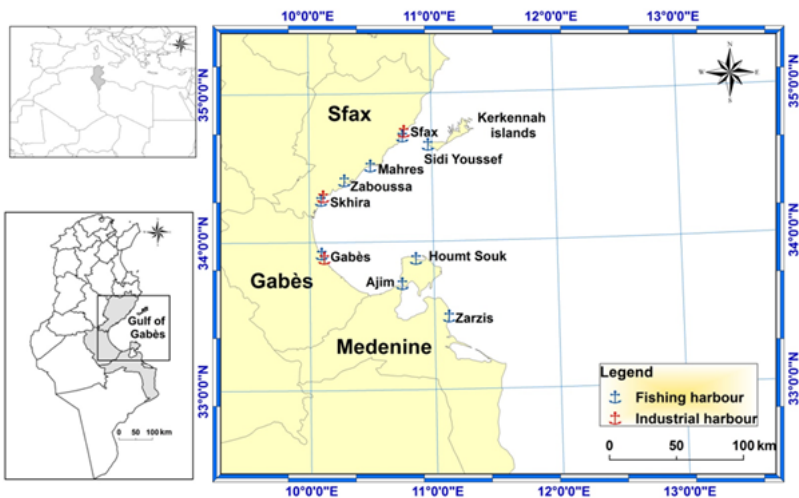


Figure 1

Map of the study area indicating the surveyed harbours. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

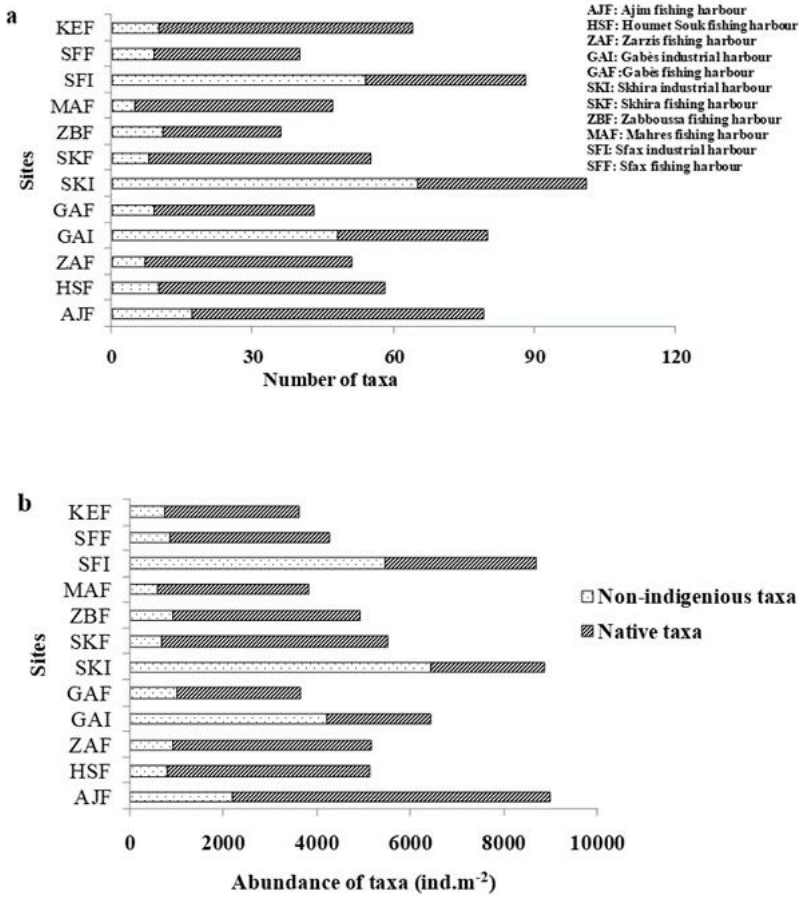


Figure 2

Distribution of Indigenous and Non-Indigenous taxa in the 12 studied Gulf of Gabès harbours, with (a) Number of taxa; (b) Abundance of taxa (number of individuals per m²).

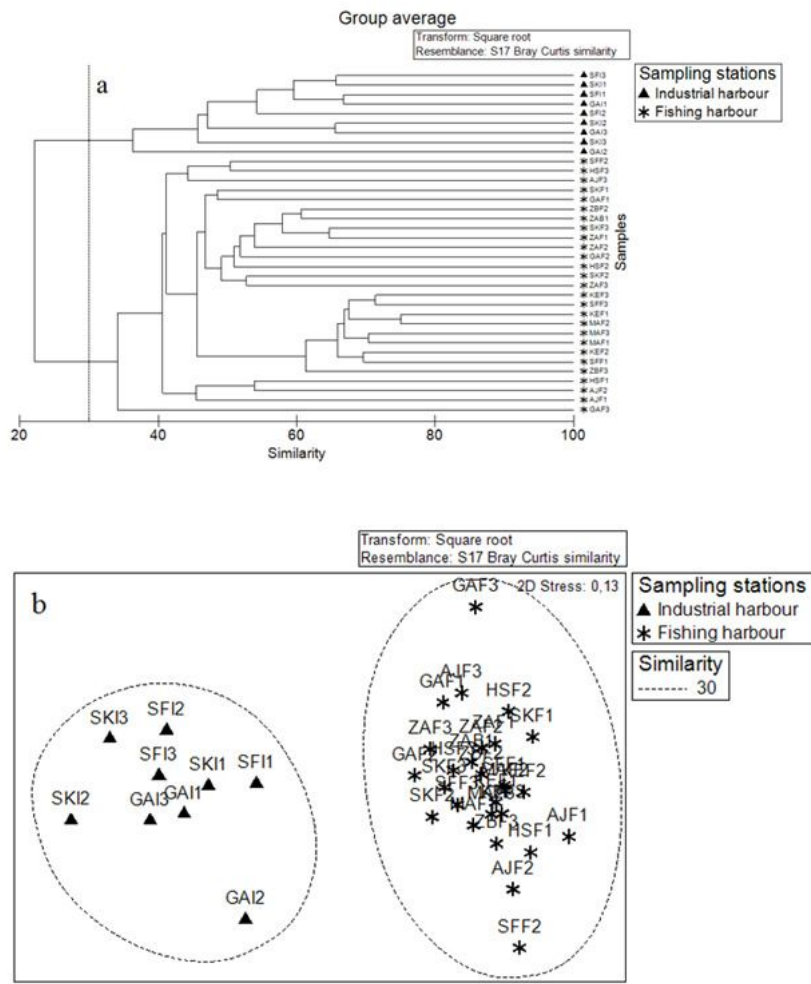


Figure 3
 Cluster (a) and non-metric multidimensional scaling (n-MDS) (b) of stations sampled in 12 Gulf of Gabès harbours based on Bray-Curtis similarity matrix after square root-transformation of abundance data. Two groups of stations are identified by Cluster Analysis at a 30% similarity level.

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