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2nd MEDITERRANEAN SYMPOSIUM ON THE NON-INDIGENOUS SPECIES

22 | 23 September 2022
Genoa, Italy

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Proceedings of the
2nd Mediterranean Symposium
on the Non-Indigenous Species

FORWARD

Dear Colleagues,

The United Nations Environment Programme Mediterranean Action Plan – Specially Protected Areas Regional Activity Centre (UNEP/MAP – SPA/RAC) – Barcelona Convention has initiated since year 2000 a series of scientific symposia, dedicated to key habitats and NIS by organising the first Mediterranean Symposium on Marine vegetation. This initiative while aiming essentially to take stock of the recently available scientific data and to promote the cooperation between specialists and key actors working in the Mediterranean, has evolved since then to cover now Coralligenous, Dark Habibtats and Non-Indigenous species as well.

These symposia have been initiated as implementation of the UNEP/MAP regional action plans related to (i) **the Conservation of Marine Vegetation in the Mediterranean Sea** (adopted by the Contracting Parties to the Barcelona Convention in 1999 and updated in 2012), (ii) **the Conservation of Coralligenous and other calcareous bio-concretions of Mediterranean** (adopted by the Contracting Parties to Barcelona Convention in 20018 and updated in 2016), (iii) **the conservation of habitats and species associated with seamounts, underwater caves and canyons, aphotic hard beds and chemo-synthetic phenomena in the Mediterranean Sea** (Action Plan for Dark Habitats adopted by the Contracting Parties to the Barcelona Convention in 2013), and (iv) the **Action Plan concerning Species Introduction and Invasive Species** (Adopted by the Contracting Parties to the Barcelona Convention in 2003 and updated in 2016).

The "Mediterranean Symposia on Marine Key Habitats and NIS" are an important output, not only of the UNEP/MAP Medium-Term Strategy for the period 2022-2027 (Decision IG.525/1), but also for NTZ/MPA project "Empowering the legacy: scaling up co-managed and financially sustainable No-Take Zones / Marine Protected Areas", financed by MAVA foundation under its Mediterranean Strategy and by the EU-funded IMAP-MPA project "Towards achieving the Good Environmental Status of the Mediterranean Sea and Coast through an Ecologically Representative and Efficiently Managed and Monitored Network of Marine Protected Areas" .

For more than two decades, the symposia have provided, within the framework of the MAP Barcelona Convention, a platform for dialogue between the scientific community, managers, and decision makers.

This year, SPA/RAC in collaboration with the Italian Institute for Environmental Protection and Research (ISPRA), the university of Genoa and its Department of Earth, the Environment and Life Sciences of the University of Genoa (DISTAV) and the association "Società Italiana di Biologia Marina" (SIBM), organized a new edition of the Mediterranean Symposia in Genoa, from 19 to 23 September 2022, as follows:

- 7th Mediterranean Symposium on Marine Vegetation (19-20 September 2022)
- 4th Mediterranean Symposium on the conservation of coralligenous and other calcareous bio-concretions (20-21 September 2022)
- 3rd Mediterranean Symposium on the conservation of dark habitats (21-22 September 2022)
- 2nd Mediterranean Symposium on the non-indigenous Species (22-23 September 2022)

This edition was also a good opportunity to discuss new topics such as monitoring and definition of Good Environmental Status (GES), monitoring and assessment scale in the Mediterranean and, in this way, contribute to enhancing Science-Policy interface and to strengthening links and cooperation between SPA/RAC and Barcelona Convention system and scientists and scientific institutions in the Mediterranean.

Khalil ATTIA
SPA/RAC Director

Tatjana Hema
UNEP/MAP Coordinator

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PROGRAMME

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14:15-14:30 **Keynote conference : "Refined and updated Non-Indigenous Species baselines for the Mediterranean Sea at the national, sub-regional and national level in the context of the Barcelona Convention's Integrated Monitoring and Assessment Programme (IMAP)"** by **Marika GALANIDI,** AISSI M., ALI M., BAKALEM A., BARICHE M., BARTOLO A., BAZAIRI H., BEQIRAJ S., BILECENOĞLU M., BUGEJA M., CARBONELL A., CASTRIOTA L., CHALABI A., ÇINAR M.E., DRAGIČEVIĆ B., DULČIĆ J., EL HAWEET A., FARRAG M., GALIL B., GUERIN L., HYAMS-KAPHZAN O., KAPEDANI R., KAMBERI E., LIVI S., MASSE C., MAVRIČ B., OUERGHI A., PETOVIC S., PNG-GONZALEZ L., RIZZO M., ROMDHANE M.S., SHENKAR N., SHAKMAN E., YAHYAOUI A., YOKEŞ M., ZENETOS A.

Session 1: **The ecology of NIS and their biological associations**
Chair: **Ernesto AZZURRO**, Rapporteur: **Mehdi AISSI**

14:30-14:45 "**Lessepsian migration and parasitism: Atypical history of introduction**" by **Wiem BOUSSELLAA**, DERBEL H., NEIFAR L.

14:45-15:00 "**A transplant experiment to test the biotic resistance in native fouling communities to bioinvasion**" by **Jasmine FERRARIO**, TAMBURINI M., LO VULLO M., OCCHIPINTI-AMBROGI A.

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16:30-16:45 "**The integration of enviromental DNA metabarcoding in the monitoring programmes for descriptor 2 of the MSFD**" by **Silvia LIVI**, VARRELLA S., CASTRIOTA L., MAGGIO T., LOMARTIRE M., VIVONA P. CORINALDESI C., DELL'ANNO A.

16:45-17:00 **"ORMEF: a new interactive geoportal on exotic fish occurrences"** by **Ernesto AZZURRO**, BIANCONE N., D'AMEN M., GRIFONI P., SMERALDO S., STRAFELLA P., DE MARCO R., FERRI F.

17:00-17:15 "**A local ecological knowledge approach for a collaborative NIS mapping in Sardinia (Italy)**" by **Daniele GRECH**, PILLONI Z., BURTON M., SERRA E., BRUNDU G., BAROLI M., PORPORATO E.M.D., MASSARO G., CECCHERELLI G., CERRI J., AZZURRO E.

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Friday 23 September 2022

Session 3: **Risks, impacts and management options**
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8:45-9:00 "**Invasion ecology through risk assessment questions: a multivariate comparison among Non-Indigenous Species traits in the marinas of Tunisia** " by Juan SEMPERE-VALVERDE, BOUHLEL R., BENZARTI O., **CHEBAANE S.**

9:00-9:15 "**The role of recreational boating in shaping the structure of fouling communities: comparison among Ligurian marinas with different boating traffic**" by **Federica GAZZOLA**, FERRARIO J., GROSSO B., QUARONI D., TAMBURINI M., OCCHIPINTI-AMBROGI A.

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9:45-10:00 ***Coffee break***

10:00-11:45 **Poster Session**

11:45-12:00 **Awards for best oral communication and poster**

12:00-12:15 **Closure of the Symposium**

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KEYNOTE CONFERENCE

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Marika GALANIDI, AISSI M., ALI M., BAKALEM A., BARICHE M., BARTOLO A., BAZAIRI H., BEQIRAJ S., BILECENOĞLU M., BUGEJA M., CARBONELL A., CASTRIOTA L., CHALABI A., ÇINAR M.E., DRAGIČEVIĆ B., DULČIĆ J., EL HAWEET A., FARRAG M., GALIL B., GUERIN L., HYAMS-KAPHZAN O., KAPEDANI R., KAMBERI E., LIVI S., MASSE C., MAVRIČ B., OUERGHI A., PETOVIC S., PNG-GONZALEZ L., RIZZO M., ROMDHANE M.S., SHENKAR N., SHAKMAN E., YAHYAOUI A., YOKEŞ M., ZENETOS A.
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REFINED AND UPDATED NON-INDIGENOUS SPECIES BASELINES FOR THE MEDITERRANEAN SEA AT THE NATIONAL, SUB-REGIONAL AND NATIONAL LEVEL IN THE CONTEXT OF THE BARCELONA CONVENTION'S INTEGRATED MONITORING AND ASSESSMENT PROGRAMME (IMAP)

Abstract
This work aimed to collect the available material on the presence of marine Non-Indigenous Species (NIS) in the Mediterranean countries starting with existing national inventories and updating with new species records observed until December 2020 and other taxonomic and status amendments as appropriate. The final outcome is the result of a collaborative process between national and regional experts, involving detailed exchange of information and the building of consensus on the final lists to be used as the baselines for the implementation of the Integrated Monitoring and Assessment Programme for the Mediterranean. (IMAP). In total, 1011 non-indigenous species have been found in Mediterranean marine waters, of which 748 are currently considered established. The highest number of species is observed in Israel, Turkey, Lebanon and Italy. Approximately 50 species were categorized as Data Deficient, either due to lack of consensus on their alien status or the validity of their identification. Polychaeta and Foraminifera were the taxonomic groups with the highest number of controversial species. There was a general increase in the yearly rate of new NIS introductions after the late 1990's, which appears to be slowing down in the last decade. The refined NIS inventories are an important step for the full operationalization of the Common Indicator 6 for alien species in the context of IMAP.

Key-words: Barcelona Convention, IMAP, NIS Baseline; Mediterranean; trends indicator

Introduction

Non-indigenous, invasive species are globally acknowledged as one of the major threats to biodiversity, ecosystems and the services they provide (CBD, 2010; EU, 2011). As a consequence, they constitute one of the elements that are taken into consideration when assessing the health of the environment and formulating management strategies in order to achieve and sustain good ecological status (EU, 2008; UNEP/MAP, 2016). In the framework of the Integrated Monitoring and Assessment Programme for the Mediterranean Sea (IMAP), NIS are addressed with Common Indicator 6 (CI6), which assesses "Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species". The national implementation and harmonization of IMAP across all Mediterranean countries requires the elaboration of a number of parameters, among which the establishment of a refined baseline of the NIS present at the national and regional level is fundamental as a starting point for any further evaluations. The current work aimed to fulfill this target by bringing together the available information from a variety of sources and employed a large pool of expertise in order to resolve discrepancies in a collaborative and consultative manner.

Materials and methods

The national inventories of EU Mediterranean countries submitted to JRC (Joint Research Centre of the European Commission) for the purposes of the 2012-2017 assessment cycle (Tsiamis *et al.*. 2021), were made available and formed the starting point for the revision process of 8 EU countries~~.~~ For the rest of the Mediterranean countries, national NIS inventories were provided by designated national experts. For a small number of countries that had not submitted national inventories initial national baselines were created with data retrieved from the Hellenic Centre for Marine Research (HCMR) offline database.

Besides NIS, cryptogenic and data deficient species were reported along with their year of first detection, establishment success and most likely pathway(s) of introduction. With regards to taxonomic groups, Foraminifera were treated inconsistently, with most but not all countries including them in their inventories, and a strong divergence of opinion among national experts on the status of many species.

The workflow of the elaboration process included 3 major steps: a) the critical revision and update of the existing national inventories by SPA/RAC regional experts based on recently published literature and major taxonomic/national/regional reviews, b) the subsequent validation of the revised lists with the contribution of national experts, invited/consulted taxonomic experts and the NIS Online Working Group, leading to the refined and finalized inventories. The validation process included several rounds of communication with countries' experts whereby many discrepancies were resolved, and a number of controversial species were agreed upon, c) the compilation of the regional and subregional baselines, where national data were aggregated at two levels, the EcAp subregion level and the pan-Mediterranean level. Once all national inventories were completed, year of first detection and establishment success of each species were adjusted accordingly.

Results

Results are reported based on data as collated until February 2022. In total, 1011 non-indigenous species were found in Mediterranean marine waters, of which 748 are currently considered established (Tab.1), which makes the overall establishment rate almost 74%. This value varies in the different subregions, with the lowest establishment rate in CMED and the highest in EMED. When it comes to actual numbers, as expected, the eastern Mediterranean has the highest number of NIS with 786 species, followed by WMED, CMED and ADRIA.

Regarding unicellular phytoplankton species, following the review of the Tsiamis *et al.*. (2019) inventory by Gomez (2019), only a handful were included as NIS in the national inventories, namely: *Chaetoceros bacteriastroides* G.H.H.Karsten, 1907, *Chaetoceros pseudosymmetricus* Nielsen, 1931, *Photobacterium damsela* Love, Teebken-Fisher, Hose, Farmer III, Hickman & Fanning, 1981, *Pseudo-nitzschia australis* Frenguelli, 1939, *Pseudo-nitzschia multistriata* (Takano) Takano, 1995.

Table 1: Number of NIS at the regional and subregional level and their establishment success

(Sub)region	NIS	Established	Casual	Unknown
EMED	786	570	175	41
CMED	286	180	73	33
ADRIA	207	140	43	24
WMED	322	221	74	27
MED	**1011**	**748**	**224**	**39**

The total number of NIS together with the cryptogenic and data-deficient species per Mediterranean country is depicted in Figure 1. The highest number of species is observed in Israel and Turkey, followed by Italy, Lebanon and Egypt, with values generally decreasing as we move towards the Adriatic and west Mediterranean countries. It is worth noting that the relatively high numbers of cryptogenic and data deficient species in Italy and Spain is related to the more comprehensive reporting by these countries.

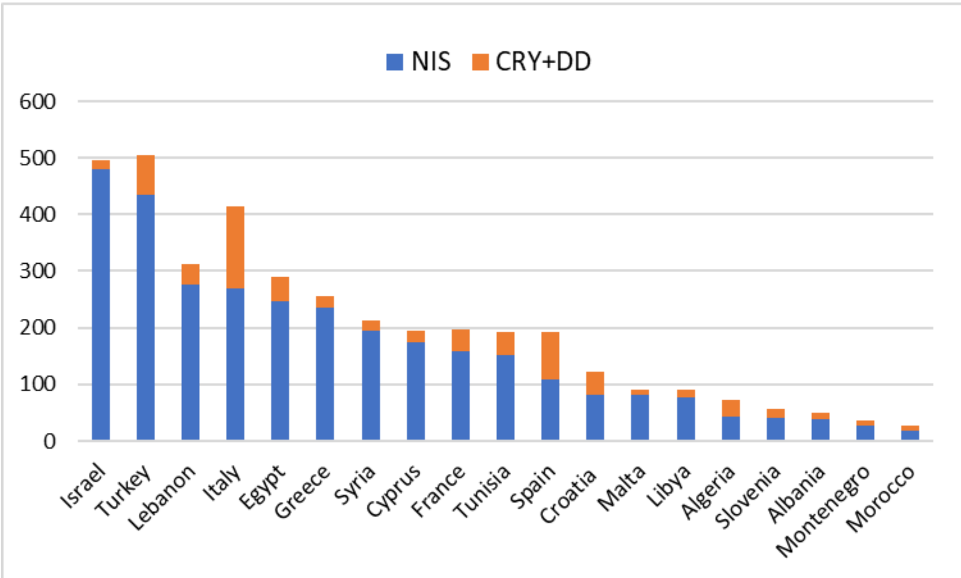

Fig. 1: Total number of validated NIS per Mediterranean country (blue) with cryptogenic (CRY) and data deficient (DD) species displayed in orange

The rate of new NIS introductions displays a generally upwards trend with a noticeable increase in the slope after the 1990's in most Mediterranean subregions with the exception of WMED (Fig. 2). In the last decade this trend appears to be levelling off, although values for 2010-2020 may still change slightly, given the lag between introduction, observation as well as reporting of new NIS sightings. The CMED is the only subregion where new NIS continue to appear at an increasing rate. The highest yearly rate is observed in the EMED, with 15-16 new species/year after 2000.

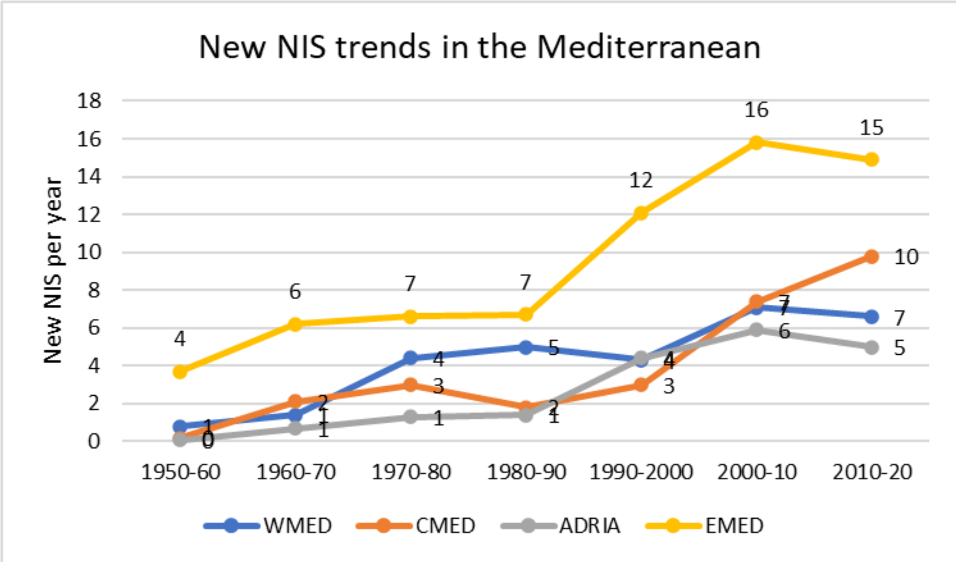

Fig. 2: Yearly rates of new introduction of validated NIS per decade in the Mediterranean subregions

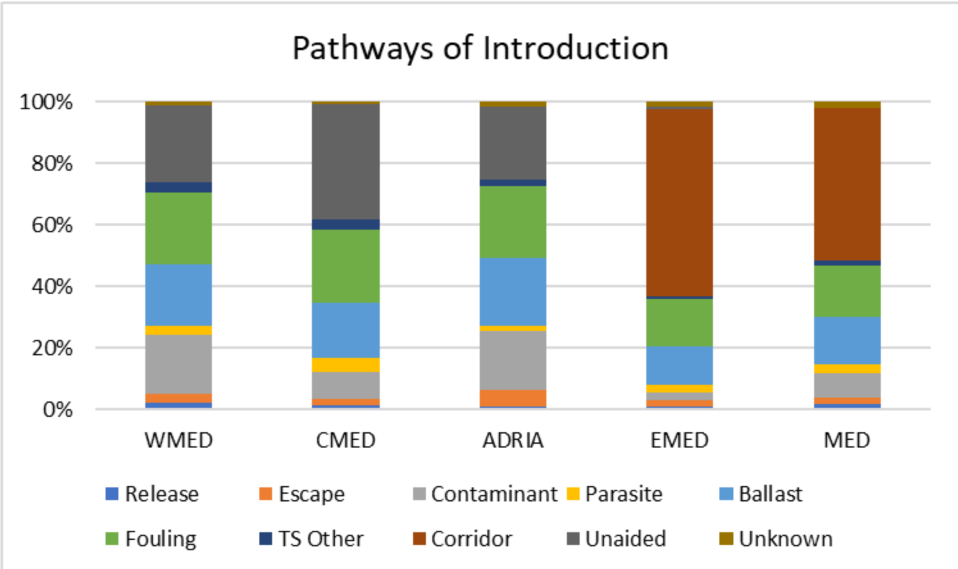

Fig. 3: Primary pathways of introduction of marine NIS per Mediterranean subregion and regionally

Roughly half the non-indigenous species present in the Mediterranean have Corridor as their primary pathway of introduction (Fig. 3), i.e., have most likely entered through the Suez Canal. This number reaches 60% in the Eastern Mediterranean, but this pathway is not applicable as we move westwards and northwards to the other subregions, where Lessepsian species migrate to a large extent by natural dispersal (pathway Unaided). CMED has the largest proportion of Unaided species, as it accepts naturally dispersing NIS propagules from all other subregions. Noteworthy also is the higher percentage of Contaminant species in ADRIA (19%) and the WMED (19.4%), which are linked to aquaculture activities, while escapees have their largest representation in ADRIA with close to 6% of the species believed to be accidental escapes from aquaculture/mariculture. The two main shipping vectors together (i.e., Ballast water and Hull fouling) constitute the primary pathway for almost one third of the NIS entering the Mediterranean but as high as 45% of the NIS present in ADRIA.

Discussion

The current reflection served to deliver refined baselines for NIS in the Mediterranean and its four subregions separately. The baselines include only validated species records observed until the end of 2020 and are the result of much deliberation and collaboration among a large number of national and taxonomic experts. As a result, both the alien status but also the validity of records of a large number of species have been clarified and agreed upon. Where knowledge was incomplete or consensus could not be reached, species/records were flagged as Data Deficient but are still reported in the final inventories for future reference. This was particularly the case for two major taxonomic groups, the Polychaeta and the Foraminifera. In the first case, the status of a number of species recently postulated as questionable aliens by Langeneck *et al.*. (2020) still remains unresolved among taxonomic experts and it is recommended that specimens collected throughout the Mediterranean are compared with type material before final conclusions are reached. In the case of the Foraminifera, even though consensus was not reached for many species, those that were agreed upon as valid NIS were included while cryptogenic and Data Deficient were not considered in our analyses.
This is an important milestone for the implementation of IMAP and sets the foundation for further steps, i.e., the elaboration of threshold values for new NIS introductions and work towards numerically defining and assessing GES (Tsiamis *et al.*., 2019; 2021). Thus far, assessment of GES for NIS temporal trends has been qualitative, based on directional trends (e.g. UNEP/MAP, 2017). Even though threshold values for trends indicators have not been elaborated yet (UNEP/MED, 2022; Vasilakopoulos *et al.*., 2022), refined baselines are a prerequisite for any potential approaches (e.g., Galanidi & Zenetos, 2022; Tsiamis *et al.*., 2021). Furthermore, they constitute an important tool for all other aspects of CI6, as a basis for species selection for the monitoring of species distribution and abundance and the assessment of impacts. Nevertheless, only with standardized and consistent monitoring schemes in place can CI6 become fully operational.

Acknowledgments

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VASILAKOPOULOS P., PALIALEXIS, A., BOSCHETTI, S.T., CARDOSO, A.C., DRUON, J.-N., KONRAD, C., KOTTA, M., MAGLIOZZI, C., PALMA, M., PIRODDI, C., RUIZ-OREJÓN, L.F., SALAS-HERRERO, F., STIPS, A., TORNERO, V., HANKE, G. (2022) - *Marine Strategy Framework Directive, Thresholds for MSFD Criteria: state of play and next steps*, EUR 31131 EN, Publications Office of the European Union.

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ORAL COMMUNICATIONS

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ORMEF: A NEW INTERACTIVE GEOPORTAL ON EXOTIC FISH OCCURRENCES

Abstract
Increasing efforts are dedicated to monitor the occurrence, progressive expansion and ecological impacts of exotic species in the Mediterranean Sea but scientific information often remains scattered in the scientific literature with limited access by the general public and policy makers. The ORMEF Geoportal is the first interactive website on Mediterranean exotic fishes, which capitalizes the homonymous database, a comprehensive and robust compilation of observations recorded in the entire Mediterranean Region from 1896 to present days. Today, the ORMEF geoportal allows searching among 5017 georeferenced occurrences distributed in the territorial waters of 20 Mediterranean Countries and extracted from almost 700 scientific papers. The ORMEF Geoportal provides spatio-temporal data on a total of 199 fish taxa, introduced by different vectors. ORMEF records were submitted to severe quality controls, checked for geographical and taxonomic biases and the entire dataset was peer reviewed two times by anonymous referees to be finally stored to the SEANOE repository and published by the journal Scientific data. The ORMEF Geoportal provides a new authoritative reference for Mediterranean bioinvasion research, informed management and policy making.

Keywords: *Mediterranean*, exotic fishes, invasions, georeferenced records, geoportal

Introduction
Exotic species introduced in the Mediterranean Sea provide one of the best documented and impressive examples of biological invasions in the marine environments, especially fish species, due to their relative easiness of detection and increasing monitoring efforts (Golani *et al*., 2021). Related spatial information is currently handled by many online databases, but updated archives of georeferenced point records are seldom displayed and interactive interplay is generally limited: see Aqua NIS (www.corpi.ku.lt/databases/aquanis), DAISIE (https://www.gbif.org/dataset/39f36f10-559b-427f-8c86- 2d28afff68ca), EASIN (https://easin.jrc.ec.europa.eu/)13, ESENIAS (http://www.esenias.org/), ELNAIS (https://www.eea.europa.eu/data-and-maps/data/external/elnais-invasive-alien-species-data), NOBANIS (https://www.nobanis.org/), MAMIAS (http://www.mamias.org/), (MedMIS. http://www.iucn-medmis. org).

Here we introduce the ORMEF geoportal, an interactive website, which capitalizes a comprehensive repository of georeferenced data published in the scientific literature (Azzurro *et al.*, 2021; Azzurro *et al.*, 2022a). The aim of the ORMEF Geoportal is to provide an easy to access system to explore exotic fish occurrences in the Mediterranean Sea.

The ORMEF Database

The ORMEF geoportal capitalizes the ORMEF database (Azzurro *et al.*, 2021; Azzurro *et al.*, 2022a), i.e. a comprehensive compilation of exotic fish records gathered through an extensive literature search, which already served several basin scale investigations on fish invasions (Parravicini *et al.*, 2015; D'Amen & Azzurro, 2020a; 2020b; Azzurro & D'Amen, 2022, Azzurro *et al.*, 2022b). All records were submitted to a rigorous validation procedure and checked against the information provided by the original source. All the historical observations of species included in the ORMEF database were included, spanning from the earliest documented observation of *Pampus argenteus* in 1896, to the most recent ones included in the latest version of the database which extracted data from 670 papers published between 1902–2020.

Today the ORMEF geoportal includes 5015 georeferenced records of occurrence on 188 accepted species of fish, and 83 families, plus the taxon of uncertain identification *Abudefduf* cf. *saxatilis/vaigiensis/troschelii* (Dragičević *et al.*, 2021).

The list of species included in the ORMEF database follows the authoritative CIESM Atlas of exotic species (Golani *et al.*, 2021) adopting the same terminology. In agreement with this atlas, we grouped the species according to their presumed introduction path: EXOTIC CAN = fishes introduced through the Suez Canal; EXOTIC HM = fishes introduced by other human vectors, such as shipping, mariculture or aquarium release; NRE (natural range expansion) = fishes of Atlantic origin, which are supposed to have entered into the Mediterranean through Gibraltar, without direct assistance of human agency. The ORMEF database therefore includes observations of Non Indigenous Fishes but also of Atlantic fishes that presumably arrived through the straits of Gibraltar without the direct assistance of human agency. In agreement with Golani *et al.* (2021), we excluded cryptogenic, brackish, and vagrant species from our list of taxa. Species names were checked with the Eschmeyer's Catalog of Fishes (Fricke *et al.*, 2021) taking into account recent taxonomic changes and documented misidentifications.

The ORMEF GeoPortal

The ORMEF Geoportal, available at https://www.ormef.eu., is a new interactive tool, which allows exploring the historical occurrences of exotic fishes in the Mediterranean Sea. It serves the needs of researchers, scholars and MPA managers, but it can be also accessed by the general public and be used to provide information at the policy level. The Geoportal has been developed in Java language, using the Spring Boot Framework (https://spring.io/projects/spring-boot) and following the MVC architectural pattern (https://martinfowler.com/eaaCatalog/modelViewController.html). A series of visualization tools and interactive functions are available from the *homepage* (Fig. 1), which provides a first Map of Mediterranean occurrences. Each point record displayed in the map brings a series of information on the data source, bibliographic reference and data on the capture or observation. Users could search for species in two different ways: by keyword or through the map. Searching through keywords, users can land on the species page where they can find all the related information, e.g. year of the first record in the Mediterranean, description of the species distribution, records location, etc. Searching through the map, users can visualize species' records on the map and filter the dataset according to different fields:

- *Year of first record only* – for each species, users can view information related to its first sighting in the Mediterranean area.
- *Most probable path* – according to the most probable path of access to the Mediterranean for the species as reported in the literature;
- *Category of path* – i.e. EXOTIC CAN, EXOTIC HM, NRE;
- *Species, Family*
- *Author of the record*

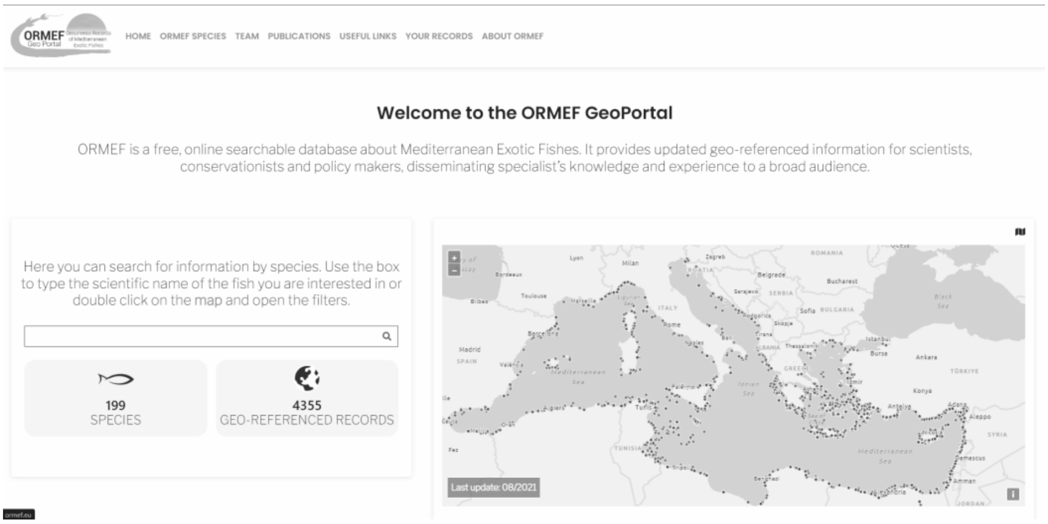

Fig. 1. Screenshot of the ORMEF homepage (https://www.ormef.eu **– accessed July 24 2022)**

The system also allows producing animations of point records according to the selected filters and along the chosen temporal frame.
Information related to each species is accessible through a dedicated page, containing pictures and interactive links to the entire list of Mediterranean Exotic Fishes. Clicking on each of these species, users can access to the related species summary (Fig. 2) which contains basic information about the first Mediterranean record, most probable path, the distribution, the time series of occurrences and a map showing the interactive point records.

Fig. 2. Species summary page (an example is given using *Fistularia commersonii*)

The Geoportal also includes other pages such as: The *Team* page, presenting the working group related to the geoportal; The *Publications and Useful links* pages: containing information regarding publications and useful links on the topic; *Your records* page, to allow users to check/add published records themselves, in order to enrich and maintain the database updated; the *About ORMEF* page, synthetically describing the scope of the project.

The ORMEF Geoportal is currently being enriched with the most recent information on new arrivals, range expansions, changes in abundances, changes in identification/nomenclature/taxonomy.

Usage Notes

The ORMEF Geoportal is presented here as the most accurate and interactive source of information on the distribution of non-indigenous and *neonative* fishes in the Mediterranean Sea. It represents an authoritative benchmark for visualizing the most complete geo-referenced data on invasive species in the Mediterranean and for getting updated on the progress of each invasion. It may serve various needs of bioinvasion research and regional monitoring programs, mainly the Marine Strategy Framework Directive of the European Union, and the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and related Assessment Criteria. Data can be also used to highlight changes in the monitoring effort through time and among the different Mediterranean countries. It should be noted that both the ORMEF database and the ORMEF geoportal did not consider non-georeferenced checklists and thus it is advisable to integrate such information, when compiling or updating inventories at the level of countries or Mediterranean subregions.

In the future, the ORMEF geoportal will be subjected to periodical updates and implemented with new fields of information, which may further expand the applications of this dataset to predict and to map future species distribution according to climate change scenarios.

Acknowledgments

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STATUS OF THE ATLANTIC BLUE CRAB CALLINECTES SAPIDUS RATHBUN, 1896 IN TUNISIAN WATERS (CENTRAL MEDITERRANEAN)

Abstract
The Atlantic blue crab Callinectes sapidus is listed among the "worst invasive" species in the Mediterranean, with reported impacts on both biodiversity and socioeconomics. This decapod was recently recorded in the northern Tunisian coasts, when few specimens were caught by nets in the Gulf of Tunis, then in Ariana and Bizerte. In spring 2020, the abundance of this invasive species increased, especially in the coastal areas in northern Tunisia. Very recently, C. sapidus was recorded in the Gulf of Gabès in April 2020 and in the Bay of Monastir in August 2020.
The abundance of C. sapidus is resulted to be higher on the northern coasts, and a little less on the eastern coasts. In these two areas, a fishing activity targeting the species seems to be taking hold. The quantities caught are more abundant during the hot season and are sold on local markets. In southern Tunisia, the presence of C. sapidus is still sporadic.

Key words: *Callinectes sapidus,* Central Mediterranean, Tunisia, fisheries, status.

Introduction

The blue crab *Callinectes sapidus* Rathbun, 1896 (Crustacea: Decapoda: Portunidae) is a commercially important species, widely distributed in estuaries and lagoons along the entire Western Atlantic coast from southern Nova Scotia, Canada, to Argentina (Nehring 2011). It is an euryhaline crustacean commonly found on muddy and sandy bottoms, generally at depths < 35 m (Hill *et al.*, 1989). The earliest confirmed record in the Mediterranean was in 1948, when two specimens were found in the Northern Adriatic (Giordani, 1951), although its presence in the Aegean Sea was suspected as early as 1935 (Nehring 2011). Since then, *C. sapidus* has been widely recorded in Mediterranean coastal waters (Galil *et al.*, 2008; Galil 2011; Daban *et al.*, 2016). This American blue crab was recently reported in the northern Tunisian coasts (Bdioui *et al.*, 2019; Ben Abdallah-Ben Hadj Hamida *et al.*, 2019), when few specimens were caught in the Gulf of Tunis, then in Ariana and Bizerte and by nets. Very recently, *C. sapidus* was recorded in the Gulf of Gabès in July 2020 and in the Bay of Monastir in August 2020.
The present study aims to detect the status of this invasive species in Tunisian waters and to assess trends in its abundance to predict its impact on local resources and fisheries.

Material and methods

In order to collect information on the spread and the status of the American blue crab *C. sapidus* in Tunisia, surveys based on the Local Ecological Knowledge (LEK) were conducted in the main ports and landing points of fishery products in the area.

Moreover, information was also collected during 43 observations on board of fishing professional units targeting demersal species, particularly the lesseptian blue swimming crab *Portunus segnis* (Forskål, 1775) mainly in the Gulf of Gabès (Southern Tunisia).
A total of 173 interviews were conducted with fishermen from the three fishing areas (59 in GSA 12, 41 in GSA 13 and 73 in GSA 14), collecting data on fishing fleet, awareness about the appearance of *C. sapidus* in the area, catches of *the species* (quantities, number of individuals, sex, size, area, gear, …) and their destinations and perceptions on the potential impacts of the American blue crab on local ecosystems and fishery resources.

Results

Interviews based on LEK were conducted with fishermen older than 30 years and having more than 20 years' experience in the fishing activity. In the three GSAs (12, 13 and 14), almost all the fishermen (97%) resultred able to reconize and identify *C. sapidus.*
According to most of the fishermen interviewed, *C. sapidus* has appeared since 2019-2020 in the North (GSA 12) and East (GSA 13) regions and in 2020 in the Gulf of Gabès (GSA 14). Respondents believe that the species was introduced into Tunisian waters mainly through ballast water. Intentional introduction, natural migration and egg transport by fishing gear/nets were reported by 16%, 13% and 2% of local fishers.
According to data collected through interviews and observations on board fishing units, the abundance of the species seems to have increased over the years (figure 1). Indeed, American blue crab catches have increased over time, particularly in the North and East regions. Thus, a fishery for this new invader, still on a small scale, seems to be settling in these two regions, where the species is then exploited by fishing nets (particularly damaged fishing nets). The catches are then intended for sale to local markets and to private consumers at a price of 2 to 8 DT per kilo (i.e. 0.64-2.5€/kg). However, the increasing abundance of this crab in these regions seems to pose problems, particularly for artisanal fisheries in the sense that the species has negative interactions with fishing gears. Damage is reported by fishermen affecting fishing gear and catches (e.g., negative interactions with the European eel fishery in the lagoon of Ghar el Melh and the lagoon of Tunis).

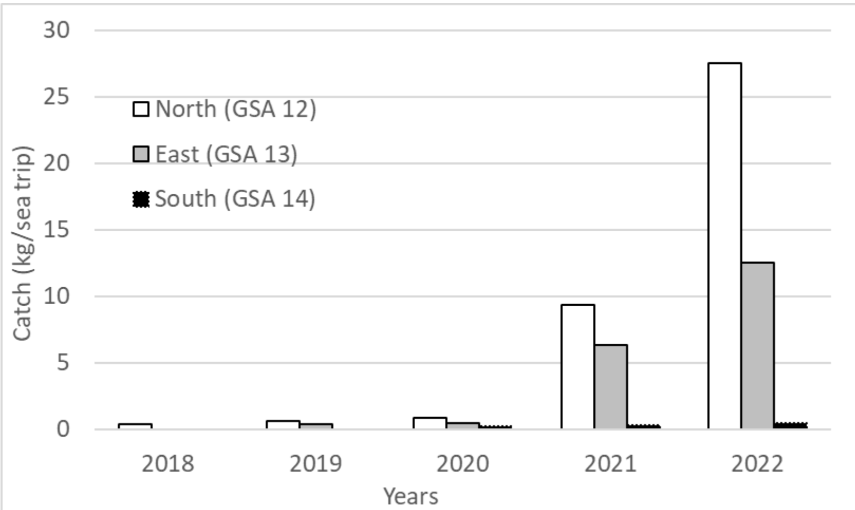

Fig.1. Evolution of the average catches of C. sapidus in Tunisian waters

In the Gulf of Gabès, catches of this Atlantic crab are still low and stable. However, according to surveys, the species seems to still be confined at the northern part of the Gulf of Gabès, where the frequency of occurrence is the most important and where the number of specimens captured is highest (figure 2). In the northern part of the gulf, *C. sapidus* seems to form, until the end of 2020, small concentrations particularly in the south and north-east of Kerkennah islands, but also at shallow area of Sfax and Skhira. In the southern part of the Gulf of Gabès, the occurrence of the species is rather rare to occasional.

In Tunisian waters, the occurrence of *C. sapidus* resulted to be higher in May and during July-September, when the temperature is highest.

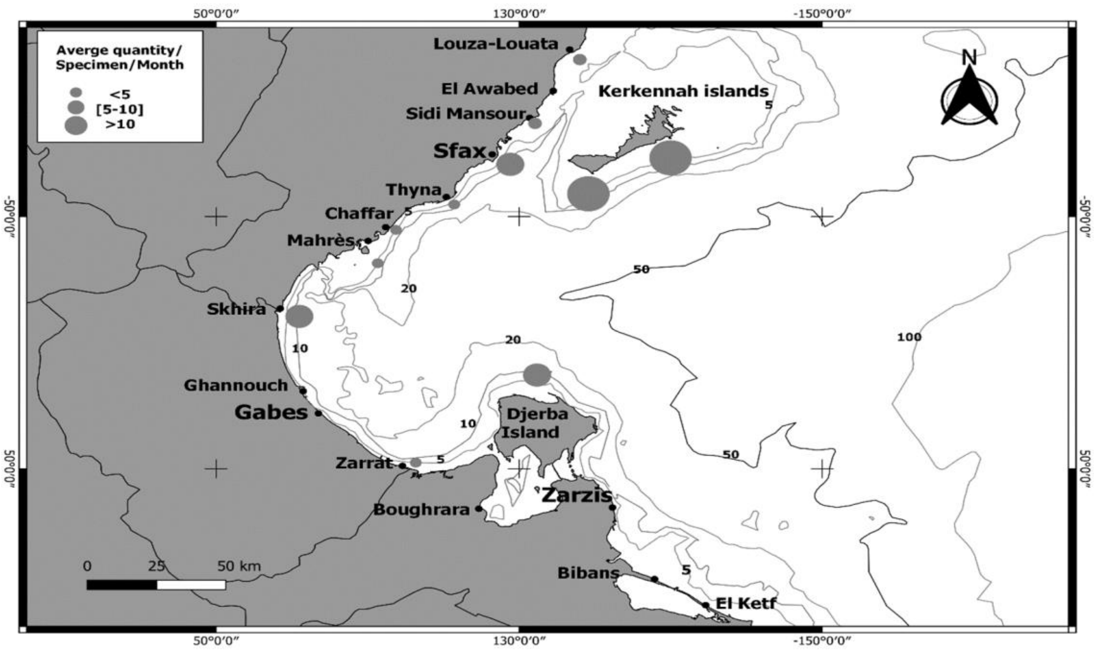

Fig.2. Density map of C. sapidus in the Gulf of Gabès (GSA 14)

Conclusion and discussions

C. *sapidus* seems to have established a permanent population along the northern coasts of Tunisia, where fishing activity targeting the species takes place. Intriguingly, in the United States the blue crab *C. sapidus* is considered a valuable seafood and supports an important fishery (Sharov *et al.*, 2003; Perry, 2015). In the last decade, several investigations have emphasised the high nutritional qualities of Mediterranean blue crab meat (Küçükgülmez and Çelik, 2008; Zotti *et al.*, 2016), and small *C. sapidus* fisheries are currently located in Turkey (Ayas and Ozogul, 2011) and northern Greece (Kevrekidis *et al.*, 2013). Moreover, the abundance of this crustacean is gradually increasing in other Tunisian locations. The species has negative interactions with fishing gears and damages the entangled fish. Economic impacts mostly resulted in the reduction of the profitability of fishing operations due to the deterioration in the quality of the catches and therefore the reduction in their value- Also the costs of replacing damaged parts of the fishing gear were mentioned by some interviewed fishermen.

Studies on the Atlantic blue crab are of great importance to assess the ecological and socio-economic impacts of this invasion. The strong swimming capacity (Spirito, 1972), its high fecundity and its aggressivity (Galil *et al.*, 2008) imply that the colonization of *C. sapidus* might take place in the near future in the investigated area, with significant effects on the existing local communities, as already experienced, following the introduction of *Portunus segnis* in the Gulf of Gabès.

Acknowledgment
We wish to express our sincere gratitude to all the fishermen for their support and for providing us with necessary information.

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LESSEPSIAN MIGRATION AND PARASITISM: ATYPICAL HISTORY OF INTRODUCTION

Abstract
Parasites can play various roles in the invasion of species, but these are still understudied in marine ecosystems. In this study, we investigated the role of Lessepsian fish parasites in the Mediterranean Sea. Prevalence, intensity and richness of parasite communities of exotic (Siganus rivulatus, S. luridus, Sphyraena chrysotaenia, Fistularia commersonii, Etrumeus golanii) and native fish (Sarpa salpa, Sparisoma cretense, Sphyraena sphyraena) were compared taking into account both, Mediterranean and Red Sea populations. Comparative approaches using GLM and multivariate analyses were used. The parasite examination of Sphyraenidae revealed the loss of two parasites, the co-introduction of three parasites and the acquisition of six parasites after the introduction. Parasite richness, prevalence and intensity were overall much lower in the invasive fish compared to the native ones. The parasite study of herbivorous fish, two exotic (S. rivulatus and S. luridus) and two native (S. cretense and S. salpa), revealed the absence of Digenea switching. The parasite investigation of S. rivulatus and S. luridus in the Mediterranean and Red Sea suggested the loss of 20 parasites in both fish, three parasites were co-introduced by S. rivulatus and five by S. luridus and four parasites were acquired by the two fish after their introduction in the Mediterranean Sea. A similarity of infecti.on levels in native and introduced ranges was found. Parasite diversity of F. commersonii in Mediterranean and Red Sea showed the loss of at least four parasites, the co-introduction of six parasites and the acquisition of nineteen native parasites. However, parasite richness, prevalence and intensity were overall much higher in the introduced range compared to the native one. Finally, for Etrumeus golanii the occurrence of three parasites was documented. Overall, these results illustrate the co-introduction history of invasive fish and their parasites into the Mediterranean is not typical but depends on the host invasion history.

Key-words: Lessepsian migration, fish, parasite spillover/spillback, Mediterranean Sea

Introduction
Parasites can play various roles in the invasion of non-native species, but these are still understudied in marine ecosystems. This also applies to Red Sea species that enter the Mediterranean Sea via the Suez Canal, the so-called Lessepsian immigrants. In this study, we investigated the parasites of a number of Lessepsian fishes. The objective of this work is to provide new information on the parasitofauna of these species and contribute to the understanding of host-parasite interactions and their ecological and evolutionary implications in marine ecosystems. More specifically, this research aimed to test the following three hypotheses: (i) loss of parasites during the process of introduction of exotic fish in the Mediterranean ("Enemy Release Hypothesis "); (ii) increased parasitism in native fish populations, through the introduction of exotic parasites ("spill-over" hypothesis) or the amplification of local parasites ("spill-back" hypothesis).

Materials and methods

Between February 2012 and May 2016, 594 exotic and native fish specimens were sampled from the Gulf of Gabès and the Gulf of Aqaba and examined. Parasites were identified to the lowest taxonomic level possible. We searched literature databases for published records of additional parasite species from the Mediterranean Sea and the Red Sea. Patterns of structure (prevalence, abundance, richness) of parasite communities of exotic (*Siganus rivulatus, S. lurius, Sphyraena chrysotaenia*, *Fistularia commersonii*, *Etrumeus golanii*) and native fish (*Sarpa salpa*, *Sparisoma cretense*, *Sphyraena sphyraena*). In the case of exotic fishes, comparative analyses were carried out through generalized linear models and multivariate analyses, comparing exotic (Mediterranean) and native (Red Sea) populations.

Tab. 1: Number of analised specimens, according to each species, sex and sampling localities during the study period (February 2012 and May 2016).

Gulf	***Sphyraena sphyraena* (native)**		***Sphyraena chrysotaenia* (invasive)**		***Etrumeus golanii* (invasive)**		***Fistularia commersonii* (invasive)**		***Siganus rivulatus* (invasive)**		***Siganus luridus* (invasive)**	
	F	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
Gulf of Gabès	50	29	68	57	7	-	28	15	53	51	48	44
Gulf of Hammamet	23	5	2	21	-	-	-	-	-	-	-	-
Gulf of Aqaba	-	-	-	-	17	14	23	7	18	12	2	-
Total	**73**	**34**	**70**	**78**	**24**	**14**	**51**	**22**	**71**	**63**	**50**	**44**

Results

The parasite examination of congeneric species (*Sphyraena chrysotaenia* and *S. sphyraena*) revealed the loss of at least two parasite species of the invasive fish. At the same time, the Lessepsian migrant has co-introduced three parasite species during the initial migration to the Mediterranean Sea, that are assumed to originate from the Red Sea of which only one parasite species has been reported during the spread to Tunisian waters. In addition, we found that the invasive fish has acquired six parasite species that are native in the Mediterranean Sea. However, parasite richness, prevalence and intensity were overall much lower in the invasive compared to the native fish host in the Mediterranean Sea.

The analysis of four herbivorous fish, two exotic (*Siganus rivulatus* and *S. luridus*) and two native (*Sparisoma cretense* and *Sarpa salpa*) did not support the expectations of a Digenea transfer among these species.

Comparative analyses between *S. rivulatus* and *S. luridus* populations in the Mediterranean and Red Sea, suggested that the invasion was associated with the loss of at least of 20 parasites in both fish. Three parasites were co-introduced by *S. rivulatus* and five by *S. luridus*. Moreover, four parasite species were acquired by the two fish in the Mediterranean. Infection levels in the two siganids showed similar values in both gulfs. All known Monogenea in the Red Sea were co-introduced with a high prevalence.

The composition of the parasite community of *Fistularia commersonii* in Mediterranean and Red Sea showed significant differences with the loss of at least four parasite species, the co-introduction of six parasite species and the acquisition of nineteen parasite species that are native from the Mediterranean Sea. Parasite richness, prevalence and intensity were overall much higher in the exotic range compared to the native one. The native population of *F. commersonii* maintained all its known Red Sea Digenea. Finally, the analysis of *E. golanii* allowed to identify three parasites at different developmental stages.

Tab.2 Parasite biodiversity of fish hosts studied in the Mediterranean Sea

Parasite taxa	Acanthocephala	Annelida	Cestoda	Copepoda	Digenea	Isopoda	Monogenea	Nematoda	Tot N of species
Sphyraena sphyraena	-	-	Tetraphyllidae	*Bomolochus unicirrus*	*Didymozoon sphyraenae*	*Gnathia* sp.	*Chauhanea mediterranea*	*Anisakis* sp.	6
Sphyraena chrysotaenia	-	Piscicolid sp.	-	*Caligus* sp.	*Lecithochirium* sp.	*Gnathia* sp.	*Pseudempleurosoma* sp.	-	5
Siganus rivulatus	-	*Piscicolid* sp.	-	-	*Thulinia microrchis*	*Gnathia* sp.	*Glyphidohaptor plectocirra* *Polylabris mamaevi*	-	5
Siganus luridus	-	*Piscicolid* sp.	-	*Hatschekia siganicola* *Nothobomolochus neomediterraneus*	*Thulinia microrchis*	*Gnathia* sp.	*Glyphidohaptor plectocirra* *Polylabris mamaevi*	-	7
Fistularia commersonii	*Sclerocollum* sp.	*Trachelobdella lubrica*	*Phyllobothriidae* *Nybelinia africana*	-	*Allolepidapedon petimba* *Neoallolepidapedon hawaiiense* *Ectenurus virgulus* *Lecithochirium* sp. *Stephanostomum* sp.	*Gnathia* sp.	-	*Hysterothylacium* sp.	11
Etrumeus golanii	-	-	-	-	*Lecithochirium jaffense*	*Gnathia* sp.	-	Anisakis larva	3

Tab.3 Parasite biodiversity of fish hosts studied in the Red Sea

Parasite taxa	Acanthocephala	Copepoda	Digenea	Monogenea	Nematoda	Tot N of species
Siganus rivulatus	*Sclerocollum rubrimaris*	-	*Hexangium sigani*	*Glyphidohaptor plectocirra* *Polylabris mamaevi*	-	4
Siganus luridus	-	-	*Gyliauchen volubilis*	*Glyphidohaptor plectocirra* *Polylabris mamaevi*	*Procamallanus elatensis*	4
Fistularia commersonii	*Sclerocollum* sp.	*Caligus fistulariae*	*Allolepidapedon petimba* *Neoallolepidapedon hawaiiense*	-	-	4
Etrumeus golanii	0	0	0	0	0	0

Discussion and conclusions

The parasite diversity in the exotic-parasite fish models revealed the non-uniformity of the infection parameters of host species examined. These results would suggest that Lessepsian migration affected native fish hosts by potentially altering the dynamics of native and invasive parasite-host interactions. In addition, the lower infection levels in the invasive fish may result in a competitive advantage over native fish hosts (enemy release hypothesis). Comparison of herbivores fish Digenea indicate that spill-back and spill-over of parasites are long processes.

The decrease in genetic diversity of the Mediterranean populations of both Siganidae and the absence of bottlenecks could explain the successful introduction of Monogenea and reflect a common history of high propagule pressure during initial colonization. However, the *F. commersonii* results strongly contradicted the enemy release hypothesis.

This study identified at least 18 co-introduced exotic parasites associated to invasive hosts in the Mediterranean Sea. Differences among the studied species suggested that the history of introduction of invasive parasites into the Mediterranean does not follow a general pattern and probably depends on the introduced host invasion history as well as other ecological, physiological and behavioral parameters that influence introduction success. Indeed, after its initial dispersal, naturally or by introduction, a parasite must colonize the host population in the new habitat by establishing new infestations. However, colonization is only possible if the requirements for biotic and abiotic factors are present. Abiotic factors may include, for example, salinity (Poulin et al., 2011a) or pH (Marcogliese & Cone, 1996), i.e., factors that can directly affect living stages and ectoparasites. Another important biotic factor, in addition to suitable host fish, is the presence of intermediate or final hosts required for a specific parasite to complete its life cycle. In more global context, the study of parasite communities of exotic fishes has highlighted the non-uniformity of infestation parameters observed during invasions according to the host and parasite communities considered. Indeed, if a significant reduction of the parasitic pressure was demonstrated during the invasion of exotic species, this process was however only visible in *S. chrysotaenia, S. rivulatus, S. luridus* on the other hand it was not verified in *F. commersonii.*

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A TRANSPLANT EXPERIMENT TO TEST THE BIOTIC RESISTANCE IN NATIVE FOULING COMMUNITIES TO BIOINVASION

Abstract

The introduction of non-indigenous species (NIS) poses a threat to marine ecosystems and is a major cause of biodiversity loss. Globally, shipping is considered one of the most important vectors of NIS introduction, and ports the relative hotspot areas. In ports, NIS can easily colonise artificial substrates and displace native species, becoming an important component of biofouling communities. The ecological interactions behind the success of a NIS introduction are still unclear, including the capability of communities characterized by high native species richness to limit the success of NIS (i.e., biotic resistance hypothesis).

To test this hypothesis, communities rich in native species from a "low-impact" site were transplanted in two marinas ("high-impact") in the Gulf of La Spezia (in 2019), expecting to assess a difference in NIS number and cover between transplanted and non-transplanted panels. A total of 25 PVC panels were deployed and after three months a subset of the panels that had grown in the low-impact sites was moved into marinas, for an additional period of two months. In general, fouling communities were significantly different among treatments (control, transplanted and not-transplanted panels) at the end of the experiment, suggesting the maintenance of peculiar assemblages in the transplanted panels. On the other hand, newly recruited species after the transplant were not significantly different between transplanted and no-transplanted panels, thus indicating a null effect of mitigation by native species to NIS settlement. In literature, contradictory results were obtained when testing the hypothesis of biotic resistance, but the use of native communities in eco-engineering infrastructures is an interesting future prospect and should deserve further investigation.

Keywords: Alien species, colonization success, biofouling, ports, Mediterranean Sea

Introduction

The marine urbanization sprawl is globally replacing natural habitats with hard artificial infrastructures, a physical aspect that can strongly modify the structure of marine communities (Airoldi *et al*., 2015). Ports are the main artificial habitats involved in marine urbanization and provide new available substrates for opportunistic and tolerant fouling species, which are mostly composed by NIS (Mineur *et al*., 2012). In fact, ports are considered hotspot areas for NIS, due to the influence of one of the most important vectors of introduction of these species, namely maritime shipping (including large commercial and small recreational vessels; Bailey *et al.*, 2015; Ulman *et al*., 2019). In particular, species can be transported by vessels into ballast waters or as fouling on their hulls; while the first vector is now regulated on large vessels in most countries, the second one is still largely underestimated and considered the most dangerous unregulated vector of NIS worldwide (Drake *et al*., 2021; Ulman *et al*., 2019). The recreational boating has been now largely acknowledged as an important vector of NIS spreading worldwide and the management of fouling should be implemented urgently,

as promoted by the GEF-UNDP-IMO GloFouling Partnership project (https://www.glofouling.imo.org, accessed July 2022). At the moment, the management of fouling on recreational boats is based on guidelines applied on voluntary basis by boat owners, and the management of fouling communities inhabiting ports has been poorly considered, while the design of artificial infrastructures able to limit NIS colonization is an aspect that needs further investigation (Hopkins *et al*., 2021). Elton's biotic resistance hypothesis (Elton, 1958) suggests that communities rich in native species should be more resistant to NIS settlement. Several experiments have been carried out to test this hypothesis, however reporting contradictory results (e.g., Stachowicz *et al*., 2002; Rius & McQuaid, 2009; Gestoso *et al*., 2014).
This study, inspired by the experiment carried out by Gestoso *et al*. (2017) in Macaronesia, aimed at testing the biotic resistance hypothesis on Mediterranean fouling communities through a transplant experiment.

Materials and methods

This study was carried out in three sites in the Gulf of La Spezia (Ligurian Sea, Italy) at different level of 'impact', in terms of NIS propagule pressure and occurrence: one low-impact site (Palmaria, a small island within the regional natural park of Porto Venere, with very low NIS richness) and two high-impact sites (the marinas of Le Grazie and Fezzano, with high NIS richness). During the transplant procedures from Palmaria to the two marinas, only 20 out of 25 panels were found.

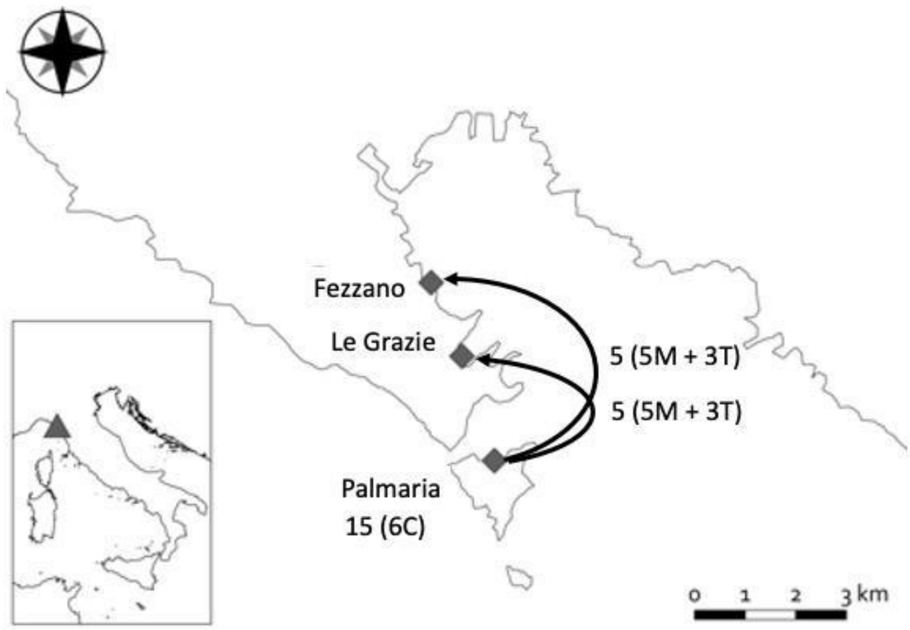

Fig.1: Map of the study area. Numbers indicate the panels initially deployed in each site, while in brackets the number of panels located in each site after the transplant experiment, specifying the treatment assigned: C (control, in Palmaria); T (transplanted panels from Palmaria to the marinas); M (no-transplanted panels in marinas).

In April 2019 a total of 25 PVC panels were deployed in the three sites, arranged as follow: 15 panels in Palmaria, 5 in Le Grazie and 5 in Fezzano). After three months of immersion (July), all panels were retrieved, photographed, and rapidly analysed in the

field to assess all the species colonizing each panel. Then, 10 panels from Palmaria were moved to the two marinas (5 in each marina; transplant treatment), while 5 remained in Palmaria (control treatment); the panels initially deployed in the two marinas were re-immersed there (marina treatment; Fig. 1) for two additional months of immersion. At the end of the experiment, all panels were retrieved, photographed, and preserved in 70% ethanol for the final species identification in the laboratory. All the panel images were then analysed to assess the percent cover of the fouling assemblage through an image analysis software (photoQuad), considering 50 random points.
The experimental units used for the sampling were constructed following the method proposed by the Smithsonian institute, with the PVC panels (14x14 cm) attached to a brick and facing downwards at 1 m of depth (Tamburini *et al*., 2021).
The statistical analyses were carried out with the software PRIMER 6 (with PERMANOVA+ add-on package) on the sessile component of the fouling assemblages and considering two different dataset: i) the percentage cover of each species at the end of the experiment; ii) only the new recruited species after the transplant.
The experimental design had to be slightly modified: six instead of ten panels were transplanted in the marinas, and four instead of five panels remained in Palmaria (Fig. 1).

Results

After three months of immersion and before the transplantation, the number of NIS was comparable between Palmaria and the two marinas (Fig. 2), but NIS had a lower abundance in Palmaria: 71±12% native species (including cryptogenic species) and 2±2% NIS were recorded in the samples from Palmaria; while 34±20% and 33±25% native species, 23±20% and 31±25% NIS were observed in the panels from Fezzano and Le Grazie, respectively (average percentage cover ± S.D.).
A two-way PERMANOVA test was performed considering the two sampling times (three and five months) and the three treatments (control in Palmaria; transplanted and no-transplanted panels in the marinas), and a significant difference was observed among treatments (Pseudo-F = 7.9747, P(perm) = 0.0001), sampling times (Pseudo-F = 4.1738, P[perm] = 0.0001) and their interaction (Pseudo-F = 1.8737, P [perm] = 0.0158). On the other hand, when considering only the newly recruited species after the transplant, a non-significant difference was observed between transplanted and no-transplanted panels (Pseudo-F = 3.5651, P(Monte Carlo) = 0.0641).

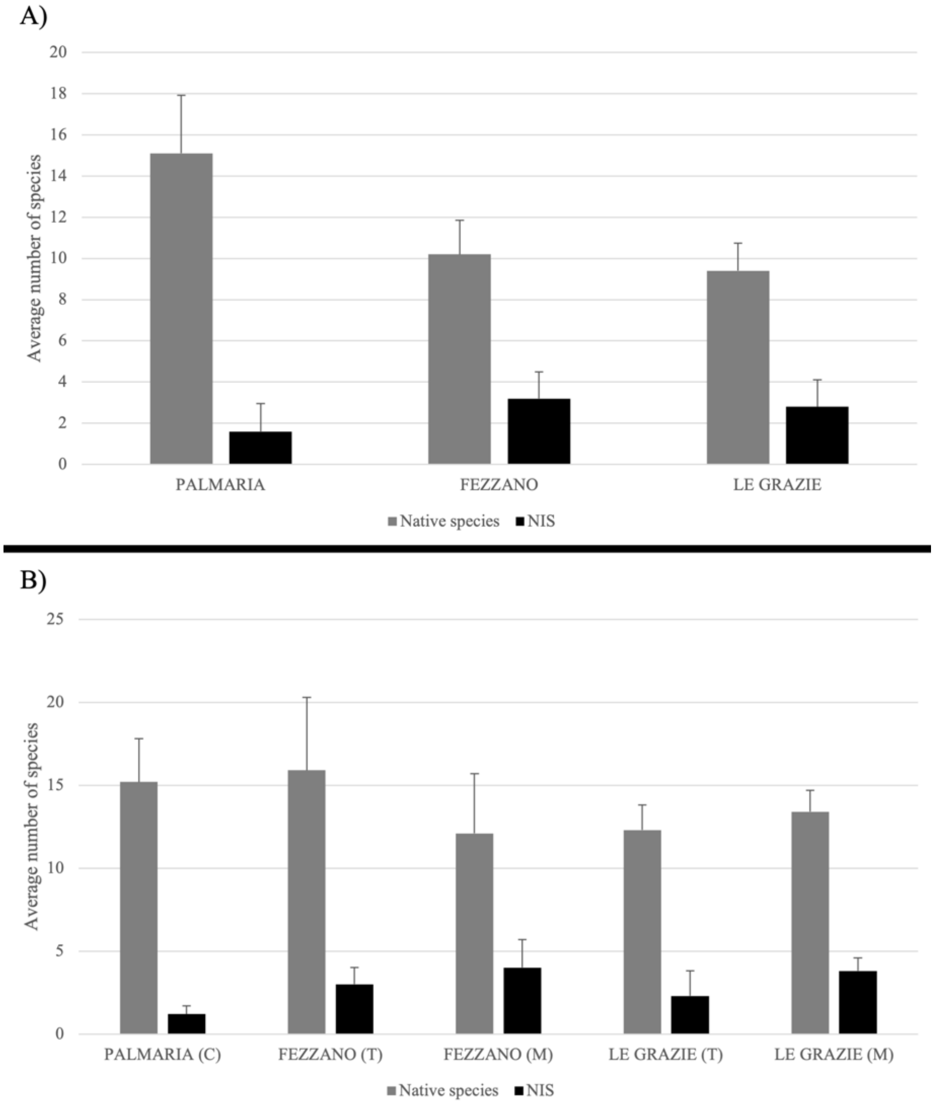

Fig. 2: Average number of native and non-indigenous species recorded after three (A) and five months (B) in the investigated sites. Letters in brackets refer to the different treatments of the experimental design: C (control, in Palmaria); T (transplanted panels from Palmaria to the marinas); M (no-transplanted panels in marinas).

Discussion and Conclusions

In this study, a transplant experiment was carried out to test the biotic resistant hypothesis in the context of Mediterranean port habitats: results show that native fouling communities do not exert significant effects in terms of establishment of new NIS. In general, fouling communities from Palmaria, the "natural" site, were highly covered by native species, with only a very limited abundance of NIS. After the transplant, the fouling assemblages from Palmaria demonstrated to maintain their own identity in the two following months of immersion inside the marinas. However, when considering the new recruitment after the transplant, the panels moved from Palmaria and the unmoved panels from the marinas were similarly colonized by new species.
Therefore, with this experiment, the biotic resistance hypothesis has not been observed as in other studies (e.g., Gestoso *et al.*, 2017); this result could be partially due to the

loss of panels occurred before the transplant procedures, which has reduced the number of replicates. Thus, additional experiments are recommended also in other localities and for a longer time of immersion, in order to collect more data and assess which are the factors that mostly promote the biotic resistance by NIS in fouling communities, as well as to shed light on the different results presented in the literature when testing this hypothesis (Kimbro *et al*., 2016; Marraffini & Geller, 2015).

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THE LEVANT - A HOTSPOT, A BEACHHEAD AND A DISPERSAL HUB OF MARINE ALIEN SPECIES

Abstract
The Israeli shelf serves as an important hotspot, beachhead and dispersal hub for Erythraean aliens. The spatial and temporal spread of Erythraean aliens in the Mediterranean Sea has advanced concurrently with successive enlargements of the Suez Canal, rise in mean seawater temperature, and prevalence, duration, and severity of MHWs increase. Rising temperatures have already had significant impacts on geographical and depth distribution of Erythraean aliens, while extirpating and reducing native sessile communities. The unceasing propagule pressure and rising establishment success of warm-water species, as well as the already present 'invasion debt', should be factored into strategic plans for managing Mediterranean nature, nature's contributions to people and 'good quality of life'.

Key-words: Erythraean aliens, Suez Canal, biodiversity change, invasion debt

Introduction
The Contracting Parties to the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) adopted in 1995 the Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean (United Nations Environment Programme, 2019). Article 13 of the Protocol states "The Parties shall take all appropriate measures to regulate the intentional or accidental introduction of non-indigenous… species to the wild and prohibit those that may have harmful impacts on the ecosystems, habitats or species in the area to which this Protocol applies… The Parties shall endeavour to implement all possible measures to eradicate species that have already been introduced when, after scientific assessment, it appears that such species cause or are likely to cause damage to ecosystems, habitats or species in the area to which this Protocol applies."
Meanwhile, many Erythraean species have become the most conspicuous denizens in across the Levant Basin, having displaced and replaced native species, thereby reversing marine conservation efforts and hampering stock recovery of key economically and ecologically important species. Yet, the ample scientific documentation of Erythraean bioinvasions in the Mediterranean Sea failed to elicit the implementation of effective management policies.
The study set out to present the current state of knowledge about the marine introduced species recorded along the Mediterranean coast of Israel, with particular consideration to introductions through the Suez Canal. We aim to elucidate their emergent patterns and consequences. Finally, we aim to draw attention to regional policy and management initiatives, now underway, and their possible impacts on transboundry amplification of the grave risk of invasions and associated impacts.

Materials and methods

Publications recording the presence of introduced species along the Mediterranean coast of Israel were re-examined, validated, expanded to capture both previously overlooked records and recently published ones (to June, 2022), while some records were deleted following reevaluation). With the exception of documented intentional introductions, the means and route of introduction are seldom known from direct evidence. These are inferred from the biology and ecology of the species, the habitats and locales it occupies in both native and introduced ranges, and its pattern of dispersal. The Suez Canal is recognized both as a pathway, through which biota may cross naturally, and a corridor for shipping-introduced biota. We document the spread of Erythraean aliens to regions within the Mediterranean Sea. It is recognized that data varies greatly, and even better studied regions experience geographic, temporal and taxonomical lacunae.

Results

A total of 440 multicellular species were recorded thus far off the Israeli coast, more than anywhere in the Mediterranean Sea (Galil et al., 2021), and when reckoned per coastline length the number is of calamitous proportions. The cumulative number of recorded introductions has been rising inexorably, the number tripled between 1970 (147 species) and 2022 (440 species). The most speciose taxa are molluscs, fish, crustaceans comprising 35%, 22%, 14%, respectively. The rate of reporting new records varied among taxa, reflecting the temporal irregularity of research efforts and dearth of expertise. The overwhelming majority of Israeli and Levanitine introductions are considered to have been entered through the Suez Canal. The geographic spread pattern of the Erythraean molluscs, fish and crustaceans is roughly similar: their number decline with distance. This pattern is amplified among these species which populations along the Israeli coastline are 'common' and 'abundant': larger populations ensure higher percentage of successful establishement. Examination of the major taxa of Erythraean aliens reveals a distinctive pattern: a high percentage of species recorded before 1960 have established large populations, whereas, for species recorded in the past three decades, rare species, or those known from single records, comprise an important portion. The geographic spread pattern of the major taxa of Erythraean aliens reveals a distinct temporal pattern as well. A high percentage of species recorded in the Southeast Levant before 1960 have spread westwards in the Mediterranean Sea (e.g., 84%, 32% in the Greater Levant and Central Mediterranean, respectively). For species recorded in the past three decades the percentages are distinctly lower (51%, 18%, respectively, despite the great enlargement of the Suez Canal and the rising sea water temperature in the Mediterranean Sea during this time period. It has long been assumed that populations of Erythraean aliens will be restricted to the upper shelf as the shallow depth of the Suez Canal would exclude deeper living organisms. Indeed, until the 1980s Erythraean biota along the Israeli shelf, with few exceptions, was confined to habitats shallower than 50 m. But post-millenium, Erythraean species, some newly arrived, some long-established, were collected on the Levantine lower shelf and upper slope: Erythraean fish and a brachyuran crustacean were collected beyond the shelf edge (Innocenti *et al*., 2017; Galil *et al*., 2019). The occurrence of aggressive, carnivorous and highly fecund lionfish *Pterois miles* and swimming crab *Charybdis longicollis* in mesophotic habitats likely interferes with the native biodiversity of these sensitive and geographically circumscribed communities.

Marine protected areas are viewed by the public as oases of natural biodiversity. Their true state is far different. Entire habitats overrun by Erythraean aliens were documented in the largest, oldest and most effectively managed marine reserve in Israel (Ramos-Esplá & Valle Pérez, 2004). These authors emphasized the abundance of Erythraean fish, which comprised over 37% of the total specimens recorded during their visual census surveys. In Lebanon surveys conducted in 2013 reported that a fifth of the identified species (56 of 268) were Erythraean aliens (RAC/SPA-UNEP/MAP, 2013). In 'bioblitz'-style surveys of fish and benthos conducted in 2015 it was found that the proportion of biomass of Erythraean aliens to native species was actually higher inside the reserve than in adjacent areas in the fall, as was the abundance of the Erythraean rabbitfish both spring and fall (Frid & Yahel, 2018). The lionfish has proliferated within the reserve (Stern & Rothman, 2018).

Discussion

All Mediterranean coastal states and the European Union are Contracting Parties to the Barcelona Convention (https://web.unep.org/unepmap/ viewed 28 March, 2020). The Convention forms the legal framework of the Mediterranean Action Plan developed under the United Nations Environment Programme Regional Seas Programme. A recent publication acknowledged "*The trend of new introductions of alien species in the Mediterranean has been increasing. About 1000 marine alien species have been reported in the Mediterranean .... Many of these species have become invasive with serious negative impacts on biodiversity, human health, and ecosystem services*" and pledges "*… to promote the development of coordinated efforts and management measures throughout the Mediterranean region in order to prevent as appropriate, minimize and limit, monitor, and control marine biological invasions and their impacts on biodiversity, human health, and ecosystem services*" (Action Plan concerning Species Introductions and Invasive Species in the Mediterranean Sea, 2017, p. 5).

The 2017 Mediterranean Quality Status Report emphasized "Corridors are the most important pathways of new introductions in the Mediterranean" (https://web.unep.org/unepmap/2017-mediterranean-quality-status-report, p. 280, viewed 19 July, 2022). A "Draft Guidelines for controlling the vectors of introduction into the Mediterranean of non-indigenous species and invasive marine species"[i] states that "the greatest influx of invaders resulted from the opening of the Suez Canal in 1869 that allowed entry of Indo-Pacific and Erythraean biota."

Recognizing that most factors that drive the introduction and dispersal Erythraean aliens are increasing, and a large-sized invasion debt already accrued, it is in the best interests of the EU and the Barcelona Convention signatories to proactively promote biosecurity in the Suez Canal, and work with Egypt and the international maritime industry, to address the threat to the Mediterranean biota, and by extension, to the economic and social wellbeing of Mediterranean populations.

Vector/pathway management is the most effective strategy for preventing translocation of species, thereby reducing introduction and spread of marine alien species. Egypt nationalized the Universal Company of the Suez Maritime Canal in 1956, undertaking all its assets, rights, and obligations. Egypt is a signatory to UNCLOS, signed and ratified CBD, and is a Contracting Party of the Barcelona Convention, but it has made no attempt to curb the influx of Erythraean biota into the Mediterranean. However, the present Egyptian government is in a position to reduce future introductions. Recently Egypt announced the development of 35 desalination plants, of which the first 16 plants will add 671,000 m3/d of new capacity, and a further 19 facilities and additional 682,000 m3/d (Myers, 2019). It is suggested that an environmental impact assessment evaluate the environmental consequences of utilizing the brine effluents from the large-scale desalination plants constructed in the vicinity of the Suez Canal to restore the salinity barrier once posed by the Bitter Lakes as against the continuation of the current situation.

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THE ROLE OF RECREATIONAL BOATING IN SHAPING THE STRUCTURE OF FOULING COMMUNITIES: COMPARISON AMONG LIGURIAN MARINAS WITH DIFFERENT BOATING TRAFFIC

Abstract
Ports are considered important sink and source areas of non-indigenous species (NIS) and the role of recreational boating in the spread of NIS is well known. The artificial structures of ports can host rich fouling communities, but the abundance of NIS can be influenced by several abiotic factors. With this study we aimed to assess if marinas with different levels of boat traffic host different fouling communities and NIS.
Fouling samples were collected with a hand-held rigid net at three closer marinas in San Remo area (Liguria, Italy): Porto Sole (a marina with international recreational boat traffic), Porto Vecchio (a marina for local fishing boats) and Ospedaletti (an incomplete and unused marina). A total of 85 species (including 2 NIS) were found in the Ospedaletti marina, while 45 species (8 NIS) and 47 (9 NIS) were observed in Porto Vecchio and Porto Sole, respectively. As expected, a higher number of native species (including cryptogenic species) was observed in Ospedaletti, while a higher number of NIS was observed in the two marinas attended by boaters. The differences in fouling structure among the sites were also highlighted by a one-way PERMANOVA test; and high dissimilarity values were observed between Ospedaletti - Porto Vecchio and Ospedaletti - Porto Sole.
A higher number of NIS was observed in the two busier marinas, while only two NIS were collected in Ospedaletti, underlining the key role of recreational boating in the spreading of NIS.

Key-words: alien species, maritime traffic, biofouling, ports, Mediterranean Sea.

Introduction
The introduction and spreading of non-indigenous species (NIS) via shipping is a well-known vector at global level and the monitoring of port habitats is considered an important measure to early detect new invaders (Olenin *et al.*, 2011; Seebens *et al.*, 2013). In the Mediterranean Sea, the primary role of recreational boats in introducing NIS, and not only as secondary spreading vector, has only recently been acknowledged, raising the urgency for the implementation of possible management strategies (Ferrario *et al.*, 2017; Ulman *et al.*, 2019a). The role of shipping in influencing NIS establishment in ports has been poorly studied in the Mediterranean Sea, mostly concerning the comparison between fouling communities inhabiting commercial ports and marinas (Ferrario *et al.*, 2017; Tempesti *et al.*, 2020, 2022), as well as the study of the biotic and abiotic factors favouring NIS introduction in ports (Ulman *et al.*, 2019b). The assessment of key factors facilitating NIS introduction would help in the identification of sites at highest risk of invasion, prioritizing their management. In this context, a preliminary investigation was carried out to assess if the establishment of fouling NIS

depends on the type of maritime traffic or not, considering three different marinas with different use destinations, including an incomplete marina that does not host boats yet.

Materials and methods

Fouling samples were collected in September 2020 in three marinas within the San Remo coastal stretch (Ligurian Sea, Italy): Porto Sole (a marina with international recreational boat traffic, including megayachts), Porto Vecchio (a marina for local fishing boats) and Ospedaletti (an incomplete and unused marina). Porto Sole and Porto Vecchio are closer and embedded in the same port area, while the locality of Ospedaletti is less than 10 km away. In each site, five samples of fouling communities were collected by scraping the vertical surface of the concrete docks with the use of a hand-held rigid net (Ferrario *et al*., 2017). The samples were preserved in ethanol 70% and then analyzed in the laboratory for the taxonomic identification of sessile and mobile macrobenthic fauna. A semi-quantitative approach was followed to assess the abundance of each species per sample (see methods in Ferrario *et al*., 2017) and statistical analyses were performed with the software PRIMER 6 (with PERMANOVA+ add-on package).

Results

A total of 85 species (including 2 NIS) were found in the Ospedaletti marina, while 45 species (8 NIS) and 47 (9 NIS) were observed in Porto Vecchio and Porto Sole, respectively. In all the investigated sites, Crustacea was the most abundant group in terms of number of species, followed by Mollusca in Ospedaletti and Polychaeta in the other two localities (Fig. 1). Interestingly, the port for local fishing boats, Porto Vecchio, displayed the highest number of bryozoans, but other taxonomic groups such as Porifera, Cnidaria, Sipuncula and Echinodermata were missing, (Fig. 1).
Considering the abundance of species, the one-way PERMANOVA test highlighted a significant difference among sites (Pseudo-F: 5.4174; P(perm): 0.0001).
In addition, the dissimilarity among the marinas were measured with a one-way SIMPER test: between Ospedaletti and Porto Vecchio was equal to 80.32%; between Ospedaletti and Porto Sole was 85.30%; while between Porto Vecchio and Porto Sole it was 67.46%.

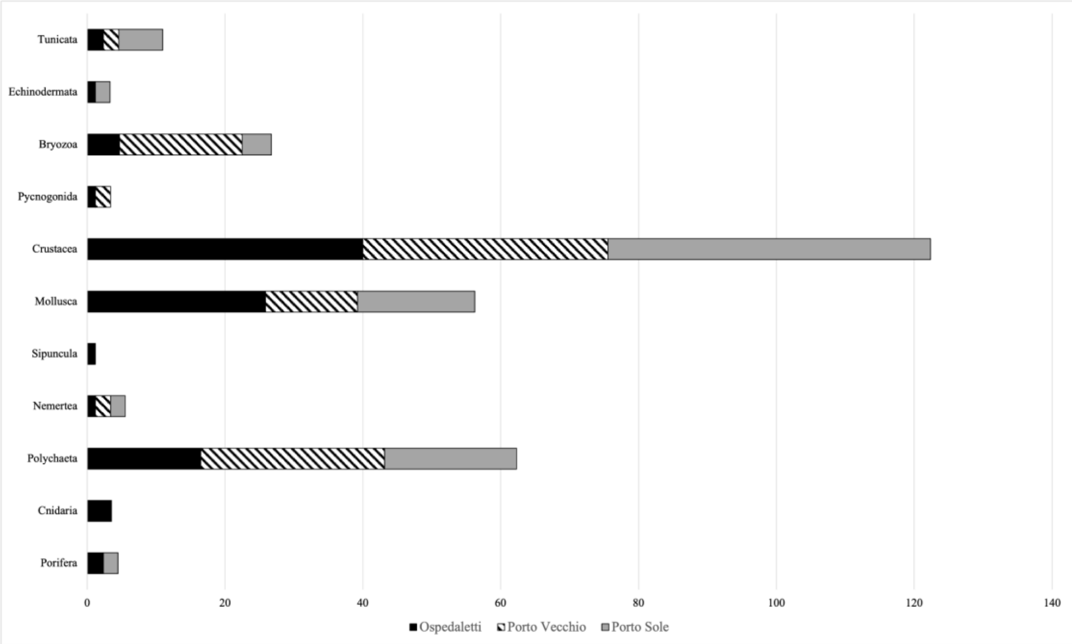

Fig.1: Percentage of species per taxonomic group in the three investigated sites.

Discussion and conclusions

This study allowed us to assess the fouling communities in three marinas within the coastal stretch of San Remo, a site not yet investigated for NIS presence. A total of 11 NIS was identified, but no new Mediterranean or country records were reported; all the NIS observed were already known from the Ligurian Sea (Ferrario *et al*., 2017; Tamburini *et al*., 2020). All the investigated sites were significantly different to each other, despite a low average dissimilarity was observed between the two active marinas (Porto Sole and Porto Vecchio). Apart from the higher number of species recorded in Ospedaletti in comparison to the other localities, the fouling community of the unused marina showed a higher abundance of species more common in natural habitats instead of ports, and with only two NIS. These results suggest that the presence of boats can strongly influence the structure of fouling communities, and that a higher propagule pressure can facilitate the establishment of NIS (Occhipinti-Ambrogi, 2007). It would be interesting to monitor the site of Ospedaletti once active, in order to verify if recreational boating will actually lead to an increase in the number of NIS.
Furthermore, the sites of Porto Vecchio and Porto Sole displayed similar fouling assemblages with comparable numbers of native and non-indigenous species. A different effect of the maritime traffic (fishing VS recreational boats) was not observed, but this could be also related to the proximity of the two sites, which inevitably influence each other. Further investigation should be carried out to test ports with different maritime traffic, including biotic and abiotic factors proper of each site, in order to identify the features of the ports most at risk of invasion.

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A LOCAL ECOLOGICAL KNOWLEDGE APPROACH FOR A COLLABORATIVE NIS MAPPING IN SARDINIA (ITALY)

Abstract
The Mediterranean basin is among those areas of the world most affected by the introduction of Non-Indigenous Species (NIS). NIS are a growing driver of change for terrestrial and aquatic ecosystems, but their management and presence evaluation within the decision-making process,is often constrained by the lack of spatially-explicit information, such as about their presence, abundance and spatial distribution (mapping). Therefore, considerable efforts are devoted in collecting this information, for example through biodiversity inventories, which are nevertheless demanding and complementary sources of information such as Local Ecological Knowledge (LEK) raise growing interest. The aim of this study is to investigate the spatio-temporal changes of the distribution of NIS in Oristano lagoons, Sardinia (Western Mediterranean Sea). This island, with more than 1.800 km of coastline and almost 500 km^{2}of wetlands (ca. 2% of the Sardinian territory) represents a biodiversity hotspot where field-based NIS inventories are demanding due to the extension of these ecosystems and the lack of in-depth research. NIS occurrence and temporal dynamics were quantified by eliciting the LEK of small-scale fishers, in order to evaluate the perceived distribution and historical changes. Local fishermen have been asked to draw the distribution of selected species on pre-printed maps, indicating areas characterized by different perceived abundances. The abundance of each species was rated on an ordinal scale, according to LEK standard protocol, from 0 (Absent) to 5 (Dominant, always in captures and abundant). Afterwards, maps were digitized on a GIS and abundances overlapped and synthesized in a unique layer per species. These findings will contribute to consolidate the use of participatory mapping approach in relation to the increasing ecological and socio-economic pressures posed by marine bioinvasions, providing new insights for spatially informed management of NIS in Sardinia coastal waters.

Key-words: *Callinectes sapidus*, Invasive species, LEK, citizen science, participatory mapping

Introduction
Coastal areas are systems where biota is subject to high anthropic pressure, both from the use and exploitation of habitats and resources (fishing, tourism, etc.) and from pollution due to the presence of human settlements and productive activities (industry, agriculture, etc.). Their high natural variability, the great productive potential, the role in the life cycle of many species of conservation and commercial interest, and the multiple uses that converge on coastal lagoons, represent a major challenge from the point of view of conservation, management and spatial planning (Garrabou *et al*., 2018). The Mediterranean basin is one of the most affected areas by the introduction of Non-Indigenous Species (NIS). They represent increasing changing factors for terrestrial and aquatic ecosystems, but their management and presence evaluation in decision-making processes are often limited by the lack of detailed information, such as their presence, abundance and spatial distribution (mapping) (Azzurro and Cerri, 2021). Indeed, a first

and valuable tool for managing NIS is an up-to-date knowledge on their location, which is crucial for conservation priorities (*e.g.* alien vs. protected species). For this reason, inventories and mapping of alien species are increasingly important, and a major international effort focused on collecting and summarizing data on the current status is crucial, so as to have a robust baseline for assessing historical changes occurring in ecosystems, Local Ecological Knowledge (LEK; Beaudreau *et al*., 2014) is the information that people have about local ecosystems where they spend most of time. This knowledge is acquired by individuals during daily activities such as fishery, therefore, considerable efforts should be taken into account *i.e.* through biodiversity inventories (which are, however, demanding in terms of sampling resolution) or complementary sources of information such as LEK, which have been attracting increasing interest in recent years (Azzurro *et al.,* 2019) .

In Sardinia Island (Italy), studies on the distribution of NIS and autochthonous species are rather rare or fragmentary, mostly attributable to temporally or spatially reduced surveys, as is the involvement of society in scientific research (Grech *et al*., 2020). This is particularly true considering that Sardinia is the main connection towards the western Mediterranean basin: an outpost of early detection of NIS on a large island poorly inhabited land with complex and fragmented coastline, where the participatory approach of the population is still in its infancy, but with a great increasing potential. Indeed, these actions, if properly supported, can also trigger important synergies, generating awareness in society and improving the exchange of information within the general public, something that is of paramount importance in the field of invasion biology (Alvito & Grech, 2021).

Some pioneering approaches about this topic have recently been presented by Azzurro & Cerri (2021) in the Lesina lagoon (Adriatic Sea), and similar social involvement initiatives are underway in projects sharing standardized protocols for Mediterranean-scale data collection in Marine Protected Areas (Garrabou *et al*., 2018; 2022).

The aim of this study is to investigate occurrence, abundance and spatio-temporal changes on the distribution of NIS in the study area of Oristano lagoons, Sardinia (Western Mediterranean Sea).

Materials and methods

The study area is located in the West coast of Sardinia, an island with more than 1,800 km of coastline and almost 500 km^2 of wetlands (ca. 2% of the territory) representing a biodiversity hotspot where field-based NIS inventories are demanding, due to the extension of these ecosystems and the lack of in-depth research.

NIS occurrence and temporal dynamics were quantified by eliciting the LEK of small-scale fishers in 6 coastal lagoons (Is Benas, Mistras, Cabras, Santa Giusta, S'EnaArrubia, Marceddì).

Local fishermen have been asked to draw the distribution of selected species on pre-printed maps, indicating areas characterized by different perceived abundances. Species were selected after targeted bibliographic research. The abundance of each species was rated on an ordinal scale, according to LEK standard protocol (Azzurro *et al*., 2019; Azzurro & Cerri, 2021) abundance values (0 = Absent, 1 = Rare, 2 = Occasional, 3 = Common, 4 = Abundant, 5 = Dominant) and if possible, to give a temporal score of abundance during years. Maps were then digitized applying a mesh on a GIS and abundances were overlapped and synthesized (mean) in a unique layer per species for an overall overview. Summarizing maps for the most abundant species were provided for the study area.

Results

This contribution allowed mapping the occurrence and abundance of NIS Oristano lagoons through the LEK of fishermen. In Sardinia at least 66 NIS taxa were reported in literature up to 2020, 71% of them were saltwater-brackish and 29% freshwater. Of these, 13 were found in this study (Fig. 1, Tab.1).

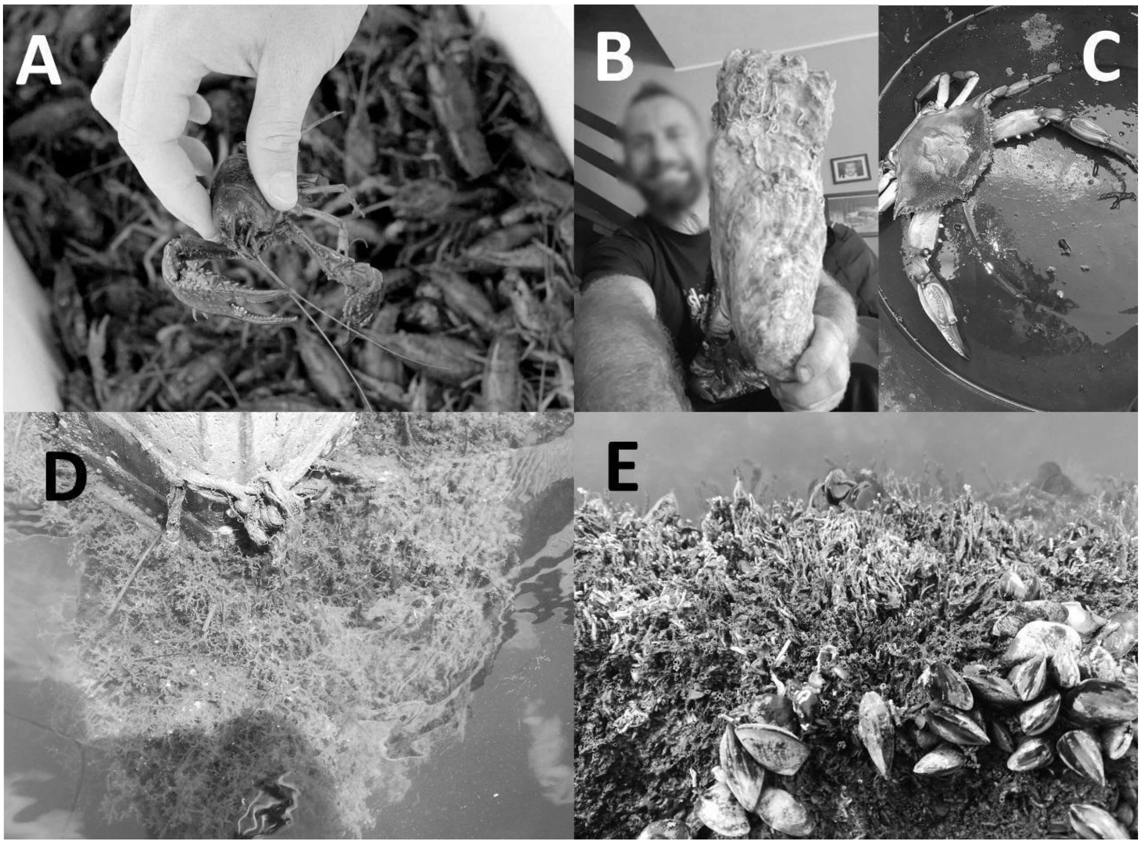

Fig.1: NIS in Oristano lagoons collected with LEK protocol. A: *Procambarusclarkii*, B: *Magallana gigas*, C: *Callinectes sapidus*, D: *Amathiaverticillata*, E:*Ficopomatusenigmaticus.*

For *Amathia verticillata* its detection represents the first record for the study area (Gulf of Oristano) and *Paraleucilla magna* was recently reported for the biogeographic area (Sardinia;Grech &Trainito, 2022).

Historical dynamics were reconstructed for *Callinectes sapidus* representing the species steeply increasing in distribution during last 10 years: from being totally absent for the Oristano lagoons since 2012, it appeared in S'EnaArrubia (2013), Cabras (2015), SantaGiusta, Is Benas, Marceddì (2018) and currently is very abundant in S'EnaArrubia. The most abundant reported species is *Ficopomatusenigmaticus,* present in Oristano lagoons since immemorial times. Anecdotal evidence is the fact that even older fishermen have known it for generations and in fact they erroneously consider it as a native species. *Mnemiopsisleidyi* has also steeply increased in abundance in Marceddìsince 2012. Other species that increased in abundance are *Ameiurus melas* (Cabras), *Magallana gigas* (S'EnaArrubia) and*Myocastor coypus* (all the lagoons, between 2000 and 2010).*Cyprinuscarpio*is perceived as stable for some interviewed and declining for others (Cabras, Santa Giusta and S'EnaArrubia). *Eichhornia crassipes* seems to have bloomed in Cabras between 2010 and 2011 and then disappeared, as for Santa Giusta between 2016 and 2018.

Tab.1: List of NIS in Oristano lagoons collected with LEK protocol.

Kingdom	*Phylum*	*Family*	*Species*	*Cabras*	*S'Ena Arrubia*	*Mistras*	*Santa Giusta*	*Marceddì*	*Is Benas*
Plantae	Tracheophyta	Pontederiaceae	*Eichhornia crassipes*	+			+		
Animalia	Porifera	Amphoriscidae	*Paraleucilla magna*				+		
	Ctenophora	Bolinopsidae	*Mnemiopsis leidyi*	+	+	+	+	+	
	Bryozoa	Vesiculariidae	*Amathia verticillata*	+	+	+	+	+	+
	Annelida	Serpulidae	*Ficopomatus enigmaticus*	+	+	+	+	+	+
	Mollusca	Mytilidae	*Arcuatula senhousia*				+	+	
		Ostreidae	*Magallana gigas*	+	+	+	+	+	
Animalia	Arthropoda	Cambaridae	*Procambarus clarkii*		+		+	+	
	Arthropoda	Portunidae	*Callinectes sapidus*	+	+	+	+	+	+
	Chordata	Cyprinidae	*Carassius auratus*	+					
		Cyprinidae	*Cyprinus carpio*	+	+		+	+	
		Ictaluridae	*Ameiurus melas*	+	+		+	+	
		Myocastoridae	*Myocastor coypus*	+	+	+	+		

C. sapidus is currently widespread in all the lagoons with more than 5 records per each basin (Fig. 2), except for Is Benas where only occasional specimens until 2021 have been reported. Moreover, a focus on 3 of the most abundant reported NIS is performed in Cabras(Fig. 3), where the highest number of interviews was collected (N=11).

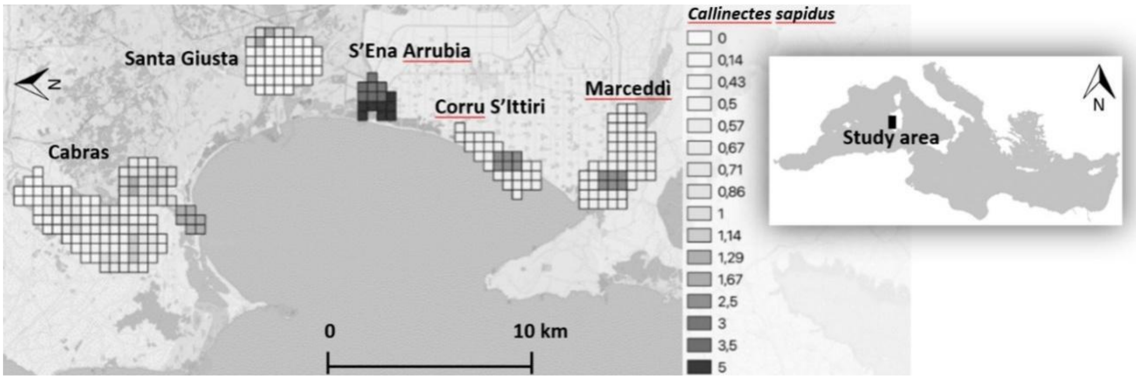

Fig. 2: Mean abundances of *C. sapidus* in Oristano lagoons

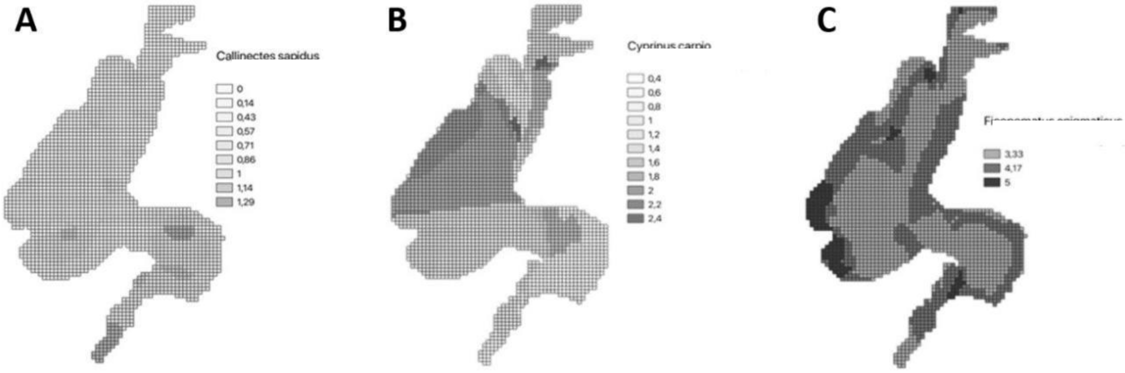

Fig. 3: Summary maps ofCabraslagoon (mesh size 100 m). Perceived mean abundance of *C. sapidus*(A, N=10);*C. carpio* (B, N=5);*F. enigmaticus* (C, N=6).

Discussion and/or conclusions

These findings depict the first comprehensive framework of NIS in Oristano lagoons through the LEK methodology. Despite the low number of interviews and/or the corresponding perception of each species, data can be considered reliable, it should be stressed that the interaction with fishermen was sometimes extremely complex, as was the verification of some of the information collected, due to the suspicious nature of Sardinian fishermen and the complex restrictions due to the Covid-19 pandemic period. Moreover, this approach allowed to gather both an overall information of NIS of the study area, but also for other common or widely distributed native taxa and rare ones (27 in total), contributing to the species inventory of this site with a relatively low sampling effort and disseminative feedbacks for the involved fishermen.

Aaccording to them, apart from a few striking cases (*e.g.M. leidyi*), NIS are not perceived as competitors of their target species , This is especially true for *C. sapidus*, which is usually not perceived as a threat to the exploited stocks.They only realize ecological issues after having been adequately informed, but pointing out mainly on the opportunity of economic profit on this species, nevertheless, the considerable negative economic effect of this species on natural stocks are widely known (Clavero *et al*., 2022 and references therein).

For these reasons, this work also pointed out the importance of an increase in public engagement and outreach on the issue of alien species, involving society on all sides. In this case, an important step has been taken towards fishermen, who were called to actively participate in scientific research through the share of their LEK heritage and active collaborative mapping.

This experience will contribute to consolidate the use of participatory mapping approaches in relation to the increasing ecological and socio-economic pressures posed by marine bioinvasions, providing new insights for spatially informed management of NIS in Sardinia and Mediterranean coastal waters.

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THE INTEGRATION OF ENVIROMENTAL DNA METABARCODING IN THE MONITORING PROGRAMMES FOR DESCRIPTOR 2 OF THE MSFD

Abstract
The environmental concerns associated with the presence of non-indigenous species (NIS) in marine waters led to the definition of Descriptor 2 in the Marine Strategy Framework Directive (MSFD). In Italy, the monitoring activities related to Descriptor 2 are carried out by the Sistema Nazionale Protezione Ambientale (SNPA) in most relevant hotspot areas such as ports and shellfish plants. Along with traditional monitoring, a new methodological approach (NISME: NIS identification in Marine Environment) based on environmental DNA (eDNA) metabarcoding has been developed by the Institute for Environmental Protection and Research (ISPRA) in collaboration with the Polytechnic University of Marche (UNIVPM) to improve NIS detection. The effectiveness of both molecular and morphological approaches for NIS surveillance could be compared, allowing the identification of challenges and perspectives of eDNA metabarcoding as a complementary tool to traditional monitoring.

Key-words: Marine Strategy Framework Directive, Non-indigenous species, environmental DNA, metabarcoding, Descriptor 2

Introduction

Following the Commission Decision (EU) 2017/848, the evaluation of Good Environmental Status for D2 within the MSFD is primarily focused on the reduction of new NIS (non indigenous species) introductions (criterion D2C1). This strategy implies on one hand the implementation of adequate measures by Member States and on the other hand, an efficient monitoring network to detect the new NIS introductions. In Italy, the monitoring activities related to Descriptor 2 of MFSD are carried out every year, since 2015, by the Sistema Nazionale Protezione Ambientale (SNPA) in hotspots areas such as ports with international traffic and, since 2021, in a few selected shellfish plants. Along with traditional monitoring, a new methodological approach (NISME: NIS identification in Marine Environment) based on environmental DNA (eDNA) metabarcoding has been developed by the Institute for Environmental Protection and Research (ISPRA) in collaboration with the Polytechnic University of Marche (UNIVPM) in order to improve NIS detection in compliance with the MSFD D2 Targets. In the present contribution we provide preliminary results of NIS identified through eDNA metabarcoding in samples collected in Trieste harbour (North-western Adriatic Sea) as pilot area.

Materials and methods

With the collaboration of SNPA agencies, water and sediments sampling was carried out at two stations in Trieste harbour. All samples were collected in the same stations where traditional sampling for benthos, phytoplankton and zooplankton is routinely carried out for MSFD-D2 implementation. Different trials were conducted to identify the optimal sample volume of seawater (1L, 2L, 3L) and filter's typologies of 0.45 µm pore size: mixed cellulose ester (MCE), Polyvinylidene difluorite (PVDF), Polyethersulfone (PES); besides this, multiple markers (e.g. *18S*, *COI*, *rbcl*) were tested for detecting NIS taxa belonging to different trophic groups.

Results

Overall, eDNA metabarcoding analyses carried out on all samples collected allowed the identification of 837 marine species validated according to WoRMS database (marinespecies.org). Preliminary results obtained by using different molecular markers on eDNA extracted from 2L of seawater filtered on MCE filters and from sediments, showed the genetic signature of >500 taxa. The taxa were matched against the Mediterranean NIS list (CIESM Atlas of Exotic Species in the Mediterranean https://www.ciesm.org and Zenetos *et al.*, 2022) and the Italian waters NIS list (provided by ISPRA). Among the 18 NIS identified only 8 are already reported from Italian waters, and only *Pseudodiaptomus marinus* Sato, 1913 and *Styela plicata* (Lesueur, 1823) have been found between 2015-2020 with traditional monitoring in the same area. According to our analysis, eDNA of 10 NIS that have never been identified in the Italian seas, was recovered.

Discussion and conclusions

The high-throughput sequencing of eDNA provides a valid complementary tool supporting traditional morpho-taxonomic analyses for investigating marine biodiversity (Pawlowski *et al.*, 2021). The advantage of eDNA metabarcoding for biodiversity analyses relies on its capacity to detect on a relatively short-time scale a broad range of species, including small size organisms that are difficult to identify by the classical taxonomic approach (Ruppert *et al.*, 2019). However, caution should be posed in using eDNA metabarcoding data for NIS surveillance given: 1) the inability of used gene regions to reliably detect some taxa at species level, 2) the incompleteness of reference databases (NCBI, BOLD) for marine organisms, 3) the transient nature of eDNA being produced and degraded with a different extent in different marine environments, and 4) errors that may occur in the reference data (Duarte *et al.*, 2021). Results presented in this study allowed the identification of 10 NIS that potentially can be recent new introductions or species undetected by traditional monitoring analyses. These findings encourage the use of eDNA to identify the presence of new NIS and/or harmful species, which can support the traditional monitoring and mitigation strategies for avoiding the spreading of alien species.

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AN EARLY INVASION INTERRUPTED BY A TSUNAMI: THE CASE OF *AMPHISTEGINA LOBIFERA* (FORAMINIFERA) IN MALTA, CENTRAL MEDITERRANEAN SEA

Abstract
Information on early invasion stages, whether successful or not, is often lacking because most invaders are discovered only once they have become abundant or have caused appreciable changes to the recipient environment. Moreover, when newcomers fail to establish self-sustaining populations, they are often not even documented.
Here we report an early invasion detected from two sediment cores radiometrically dated through ^{210}Pb chronology, collected in the Marsamxett harbour of Malta. The cores record at least the last 110 years and contain specimens of the Indo-Pacific benthic foraminifer Amphistegina lobifera Larsen 1976; showing that the species was already present in Malta at the beginning of the 20th century, but then it abruptly disappeared. We hypothesise that the disappearance was triggered by the 1908 Messina earthquake, with the following scenario: an anomalous tsunami wave reached the Marsamxett harbour and deposited over 20 cm of sediment. This depositional event suddenly buried the sea-bottom and its benthic community. Today, A. lobifera is abundant along the Maltese coast, likely as a result of a re-invasion.

Key-words: Foraminifera, invasion, sediment core

Introduction

Many factors have been suggested to drive the success or failure of biological invasions, among which propagule pressure and genetic structure of the introduced population, habitat match between source and recipient regions, biotic resistance of native communities (through competition, predation and parasitism interactions).
Unfortunately, the early stages of invasion, when the newly introduced population is facing all these challenges, are particularly difficult to detect and study: new species, in fact, are often noticed only after having developed well-established populations, especially in marine habitats and when small-sized taxa are concerned. The understanding of timing of first introductions and early stages invasion processes is therefore limited. A variety of approaches have been used to compensate for this gap and try to reconstruct the colonization history of alien species: reexamination of old museum collections (e.g. Ahnelt, 2016), analysis of published descriptions (e.g. Zullo, 1992), interviews to local fishermen (e.g. Bariche *et al*., 2014), molecular tools (e.g. Deldicq *et al*. 2019) and radiometric dating (e.g. Petersen *et al*., 1992; Albano *et al*., 2018). In particular, the analysis of historical records preserved in sediment cores allows to reconstruct the colonization history of alien species and to identify the first introduction events, as well as possible early failed invasions.
In this study, we document a peculiar case of early introduction, followed by an abrupt failed invasion, occurred over one century ago in the Central Mediterranean Sea after a

catastrophic geological event: the 1908 Messina earthquake. The alien species with this unique invasion history is the benthic foraminifer *Amphistegina lobifera* Larsen, a unicellular organism with a small-sized carbonatic test, which had entered the Mediterranean Sea through the Suez Canal (Prazeres *et al.*, 2020).

Materials and Methods

The research was carried out in Marsamxett Harbour (35°54'16.7"N; 14°30'27.5"E), a natural bay on the western coast of Malta island where a well-established population of the Indo-Pacific foraminifer *Amphistegina lobifera* was already known since 2006 (Yokeş *et al*., 2007; Guastella *et al*., 2019). This site was selected after multiple and careful inspections, because it displays the required condition of sediment grain size, shelter, depth and absence of human activities that could have altered sedimentation on the sea-floor.

Two sediment cores were collected in May 2018 and September 2019 at 16 and 17 m depth, respectively, using a hand-corer operated by scuba divers (Fig. 1A). As core samples reflect the sedimentation pattern on the sea floor that has occurred in the past decades, our cores were expected to record the colonization history of *A. lobifera* in the area. After collection, both cores were vertically sectioned in two halves (Fig. 1B), photographed, lithologically described and finally crosscut at each centimeter, thus obtaining 41 sediment samples from the 2018 core (Fig. 1C) and 50 sediment samples from the 2019 core (Fig. 1D).

Grain size analysis was performed using five overlapped sieves of 1 mm, 500, 250, 125, and 63 µm mesh size. Sediment porosity was calculated from the loss of water between wet and dry sediments, by weighing samples before and after drying sessions at 55 °C and applying Berner (1971) equations.

The analysis of foraminiferal content was carried out on samples from one half of each core, to assess occurrence and quantify absolute abundance of *A. lobifera* at each centimeter of sediment. The other core half was utilized for radiometric analysis, in order to identify an age model corresponding to the different depth levels of the cores.

For foraminiferal analyses, sediment samples were prepared as washed residues following the standard procedures suggested by the FOBIMO protocol (Schönfeld *et al*., 2012), which involves the following steps: oven-drying at 40 °C for one day, washing over a sieve of 63 µm mesh size and then oven-drying at 40 °C for another day. Discrete sample aliquotes obtained with a micro-splitter were then analysed at the dissecting microscope. Specimens of *A. lobifera* were identified following Hottinger *et al*. (1993) and counted as number of individuals per gram of dry sediment.

The most common technique to date recent sediments (up to 100-150 years) applies the radiometric decay of ^{210}Pb isotope, a natural radio-nuclide (Incarbona et al., 2016) and ^{137}Cs, an artificial radio-nuclide introduced in atmosphere after the nuclear tests around the world starting from the 1950s and is used as an independent tracer for validation of the ^{210}Pb chronology (Smith, 2001).

Results

Foraminiferal analyses revealed a similar pattern of *A. lobifera* occurrence and abundance along both cores. The upper portions of the cores, corresponding to recent times, exhibit high abundances of the alien foraminifer. In the central part of both records, the species is absent, but reappears in the lower portions, that correspond to older times. This is more evident in the 2018 core where the species is present from cm

34.5 below sea floor (bsf) down to cm 39.5 bfs, while in the 2019 core, only a few individuals are observed at cm 28.5 bsf (Tab. 1). This pattern reveals that the species had two separate introduction events: an earlier one, that lasted for a limited amount of time and resulted in a failed invasion, and a later one, still ongoing.

The radiometric dating of the two cores also reflects very similar patterns of ^{210}Pb activity, showing the typical activity-depth profile, with higher values at the upper levels of the core that rapidly decrease down core. The obtained age models were also similar: in the 2018 core, ^{210}Pb isotope halved within the first 20 cm bsf; this interval was used to calculate a constant sediment accumulation rate of about 0.2 cm yr$^{-1,}$ corresponding to an estimated time interval of about 5 years for each centimetre of sediment. In the 2019 core ^{210}Pb activity halved within the first 35 cm bsf, providing a more accurate estimation of sediment accumulation rate (0.22 cm yr^{-1}) and consequently a second more constrained age model, where each centimetre of sediment core corresponds to about 4.5 years.

Following these age models, we should conclude that the two cores represent sediments accumulated in Marsamxett Harbour in about 200 years, and that *A. lobifera* first occurred in Malta around the year 1840. However, this scenario must be discarded, since *A. lobifera* is a Red Sea species that entered the Mediterranean Sea through the Suez Canal, which opened only in 1869 (Prazeres *et al*., 2020).

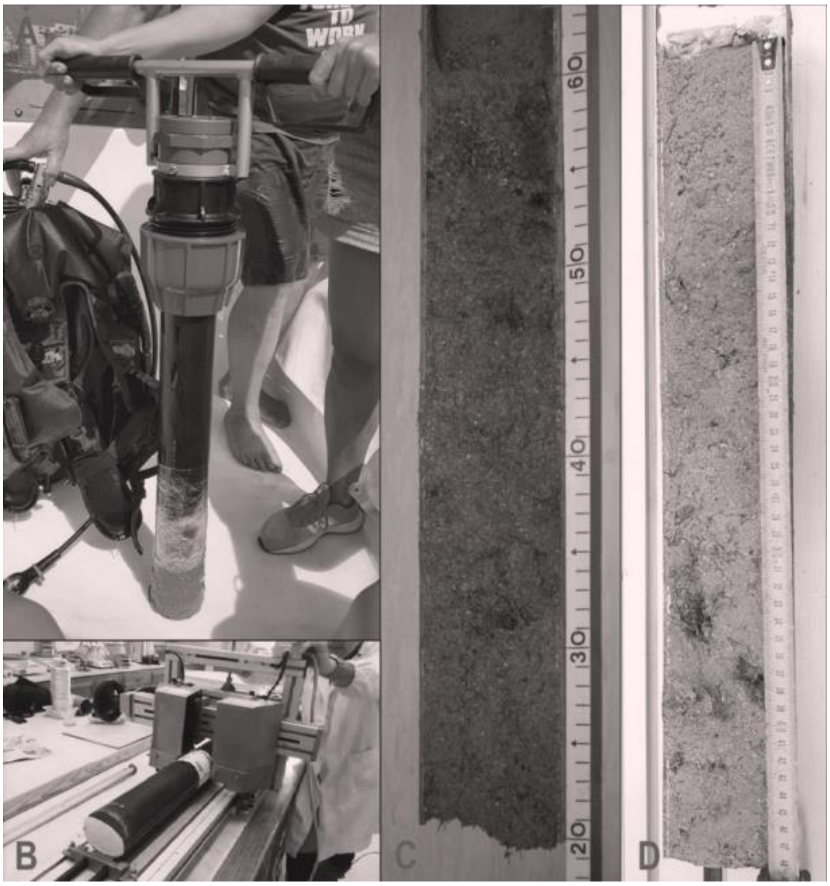

Fig. 1: A) Hand-corer used to collect the sediment cores; B) Instrument used for core sectioning; C) sectioned core (from 2018 sampling); D) sectioned core (from 2019 sampling).

Tab. 1: Absolute abundances of *Amphistegina lobifera* recorded along decreasing depth levels of the two cores portions (upper and lower), collected in 2018 and 2019.

Upper core			*A. lobifera* abundance ($N g^{-1}$)			Lower core			*A. lobifera* abundance ($N g^{-1}$)		
Depth level (cm bsf)	from cm	to cm	2018 core	2019 core		Depth level (cm bsf)	from cm	to cm	2018 core	2019 core	
0.5	0	1	3.70	3.83		25.5	25	26	0	0	
1.5	1	2	2.70	5.99		26.5	26	27	0	0	
2.5	2	3	3.96	7.06		27.5	27	28	0	0	
3.5	3	4	3.68	4.81		28.5	28	29	0	0.13	
4.5	4	5	3.40	2.02	Current invasion	29.5	29	30	0	0	
5.5	5	6	2.82	2.97		30.5	30	31	0	0	Early failed invasion
6.5	6	7	1.12	2.31		31.5	31	32	0	0	
7.5	7	8	1.40	1.18		32.5	32	33	0	0	
8.5	8	9	0.85	1.02		33.5	33	34	0	0	
9.5	9	10	0.28	0.38		34.5	34	35	0.28	0	
10.5	10	11	0.28	0.26		35.5	35	36	0.57	0	
11.5	11	12	0.57	0.65		36.5	36	37	0.28	0	
12.5	12	13	0.57	0.39		37.5	37	38	0.14	0	
13.5	13	14	0.28	0.38		38.5	38	39	0.57	0	
14.5	14	15	0.28	0.39		39.5	39	40	0.28	0	
15.5	15	16	0	0		40.5	40	41	0	0	
16.5	16	17	0	0.13		41.5	41	42		0	
17.5	17	18	0	0		42.5	42	43		0	
18.5	18	19	0	0		43.5	43	44		0	
19.5	19	20	0	0		44.5	44	45		0	
20.5	20	21	0	0		45.5	45	46		0	
21.5	21	22	0	0		46.5	46	47		0	
22.5	22	23	0	0		47.5	47	48		0	
23.5	23	24	0	0		48.5	48	49		0	
24.5	24	25	0	0		49.5	49	49,5		0	

An alternative age model was developed after observing results of grain-size and porosity analyses. In both cores, in fact, sediment grain-size and porosity were remarkably different in the upper and lower parts of cores: the upper portion exhibited sediments mainly composed of very coarse sands and, in equal proportion, of coarse and medium sand, while fine and very fine sands and mud were subordinated. Particularly, the mud fraction had percentages between 5% and 18%. Porosity ranged between 0.42 and 0.59. Mollusc shells occurring in this portion were typically depigmented, eroded and bio-perforated.

Conversely, sediments from the lower portion of both cores mainly consisted of fine and very fine sands and mud (60-70%) and were characterised by a dark grey colour, probably due to hypoxic conditions of the sediments (Fig. 1A-B). This was suggested by both the smell of hydrogen sulphide present during the core sectioning and the very high abundance of undecomposed *Posidonia oceanica* remains, mainly rhizomes well visible to the naked eye (Fig. 1D), which were missing in the upper core. Porosity was higher, ranging between 0.52 and 0.74. Sediments from this core portion also contained abundant mollusc shells with an unusually well-preserved pigmentation and with unsmoothed, sharp edge fractures.

These differences are consistent with the hypothesis of a sudden depositional event occurred around cm 25-24 bsf, i.e., around the years 1905-1910.

Discussion and Conclusion

Stochastic events, such as forest fires, ice storms, hurricanes and typhoons, that can abruptly change local environmental conditions, may be involved in invasion failure, although this has been rarely documented in the literature (e.g., Carlton & Eldredge, 2009). Our data suggest that an earlier population of the Red Sea foraminifer *Amphistegina lobifera*, occurring in Malta's Marsamxett Harbour at the beginning of the

20th century, was wiped out by an abrupt event which occurred at that time in the Central Mediterranean Sea.
This region actually experienced a catastrophic earthquake in 1908, with an epicentre off the coast of Messina (northeastern Sicily). This event is known to have generated tsunami waves that reached the coast of Malta (Mottershead *et al*., 2017).
The consequent shift in sediments grain size and the sudden burial of *Posidonia oceanica*, as observed in our cores, likely created an unsuitable habitat for *A. lobifera* that is known to prefer vegetated bottoms with coarser substrata (Guastella *et al*., 2019).
The reappearance of the species in Marsamxett samples can be dated around the 1940s, hence after a considerable lag-time after the first failed invasion.
In conclusion, the analysis of historical records preserved in sediment cores radiometrically dated allows to reconstruct the invasion dynamics of alien species and to detect possible early failed invasions, leading to a better understanding of biological invasion mechanisms.

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ASSESSMENT OF SESSILE BENTHOS IN MARINAS OF GREECE WITH A FOCUS ON NON-INDIGENOUS SPECIES

Abstract
Marinas receive a multitude of anthropogenic impacts such as marine pollution, high turbulence and biological invasions via transport-stowaway and can function as stepping stones for the dispersal of non-indigenous species (NIS). Although Greece has a high number of marinas, little is known about their benthic communities. The current study aims to quantitatively and qualitatively describe the benthic community patterns on artificial hard substrates and to record NIS and cryptogenic species (CRY) found in four marinas across the Greek Seas. Thus, during the last two years, a rapid assessment survey was applied in three sampling events using non-destructive sampling (photoquadrats) while complementary qualitative samples were also collected. In total, 45 photoquadrats were analyzed resulting in significant differences in the community structure of the marinas, despite the highly similar macroalgal coverage. Additionally, visual surveys provided new data regarding the presence of twelve NIS and CRY, including four invasives. Additional sampling on a regular basis and further investigation of the collected samples will provide a better understanding of this highly affected ecosystem and can also be an effective tool for preventing secondary dispersion of NIS.

Key-words: Benthic communities, photoquadrats, hard substrate, marinas, alien species

Introduction
Greece constitutes a sea hub that unites five marine ecoregions (Adriatic Sea, Aegean Sea, Levantine Sea, Ionian Sea and Black Sea, *sensu* Spalding *et al*., 2007). Commercial, recreational and fishing vessels are active all year round while their hulls and ballast waters act as a means of transport for many non-indigenous species (NIS) (Zenetos *et al*., 2020). Sessile species can attach to the hulls of ships and reach various marinas while ballast waters can carry various species' larvae or grown individuals. Several ports and marinas in Greece have been characterized as eutrophic environments with a high number of anthropogenic pressures (Chatzinikolaou *et al*., 2018), generating a strong impact on the local assemblages. A particular pressure occurring in marinas is biological pollution, or, in other words, the presence of NIS, since they can overcome environmental constraints and be frequent visitors (Nunes *et al*., 2014). Artificial structures, such as docks and floating pontoons found in marinas, provide suitable habitats to host opportunistic fouling species (Ferrario *et al*., 2017) and can function as stepping stones for NIS' dispersal in nearby natural habitats. In addition, the seawater warming in the Mediterranean Sea (as a result of global climate change) seems to enhance such NIS dispersal (Bianchi *et al*., 2019).
The present study was carried out in the framework of AlienPorts project (https://alienports.hcmr.gr/) and focuses on four high maritime traffic marinas of

southern Greece. The goal was to investigate the biodiversity of sessile benthos in these marinas with visual census methods for the first time, with special emphasis on NIS.

Materials and Methods

Four marinas of southern Greece were sampled in September 2020, June 2021 and October 2021, namely Heraklion (HER), Patras (PAT), Zea (ZEA) and Rhodes (RHO) (Fig. 1). In the first sampling event, randomly selected quadrats (25 x 25 cm) were photographed from the vertical walls of all studied stations as well as below floating artificial platforms when present (in HER and RHO). In total, 45 quadrats were photographed with a Sony RX100V camera (15 from HER, 5 from PAT, 10 from ZEA and 15 from RHO) to assess the benthic community structure. Qualitative samples and *in situ* supplemental close-up photos of selected taxa were also collected in all sampling periods for their taxonomic identification.

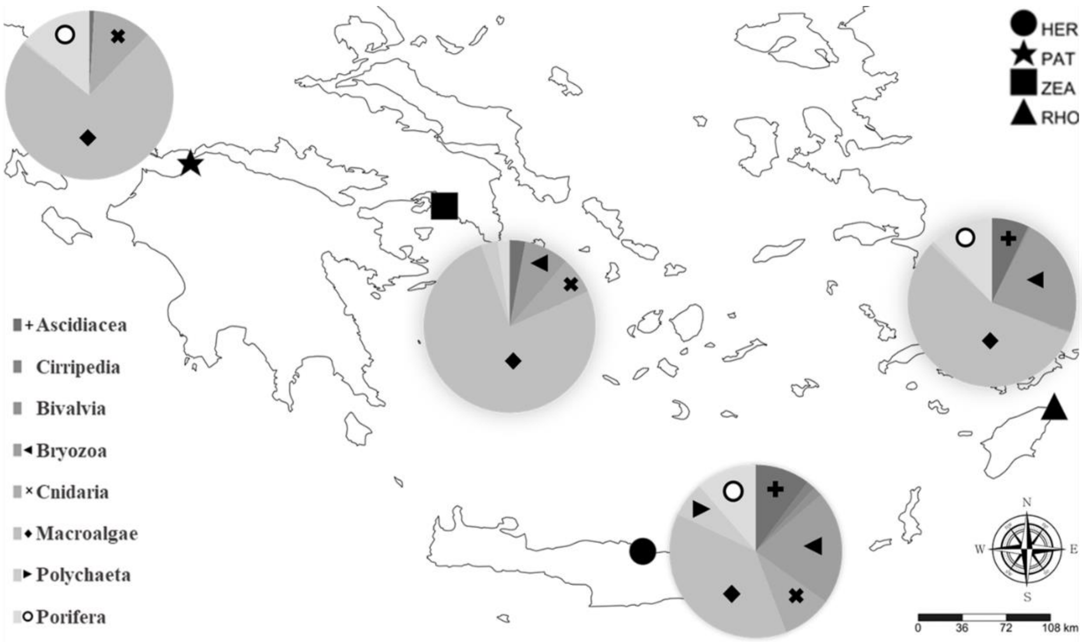

Fig. 1: Map of southern Greece with the four studied areas and the percent coverage of the different groups of identified taxa (indicated by different symbols when > 8%).

Quadrat analysis was applied through PhotoQuad software to calculate the taxa coverage percentages in each area (Trygonis & Sini, 2012). After creating a species / taxa library, 100 points were randomly spawned in each photo frame and assigned to a specific taxon or morpho-functional group (e.g., Turf-forming algae, encrusting Rhodophyta). The points that could not be safely assigned to a particular taxonomic group (<15%) were assigned as Unidentified Biogenous Substrate (UBS) and removed from the statistical analysis (Gerovasileiou *et al*., 2017; Dimarchopoulou *et al*., 2018; Rallis *et al*., 2022).

The statistical analysis was performed on taxa coverage data with the PRIMER- 6 software package and the PERMANOVA+ add-on. Data were transformed under the fourth root formula and standardized in order to mitigate the contribution of the most abundant species. Then, a triangular matrix was created based on the Bray-Curtis similarity index. The resemblance matrix was compared using permutational multivariate analysis of variance (PERMANOVA), with the fixed predictive factor of sampling locations, while similarity percentages analysis (SIMPER) was used to reveal which taxa contributed the most to the sessile community pattern of each marina. Finally, a non-metric multidimensional scaling analysis (nMDS) was used to depict spatial patterns in community structures for the studied marinas.

Results

In total, 60 benthic taxa were recorded in the studied marinas, 45 of which were identified to the species level (or species complex), 12 to genus level and 13 to higher taxonomic and/or morpho-functional categories. The taxa were classified into Chlorophyta (4), Rhodophyta (6), Phaeophyta (3), Porifera (12), Cnidaria (4), Polychaeta (2) Mollusca (7), Crustacea (1), Bryozoa (7) and Ascidiacea (14). The highest species richness was recorded in HER and RHO marinas (48 and 37 taxa, respectively) and the lowest in PAT and ZEA (25 and 26 taxa, respectively). Ascidiacea was the most diverse group with 13 taxa in HER, but only 3 to 5 in the other studied areas. In terms of coverage, macroalgae (in descending order: Turf-forming algae, Rhodophyta, Chlorophyta and Ochrophyta) prevailed in all marinas (mean coverage: 60.84%), mainly on vertical substrates, while sciaphilic benthic taxa dominated below horizontal floating platforms in HER and RHO marinas. Statistical analysis revealed differences among the examined areas and different dominant taxa. PERMANOVA showed that there were significant differences (p-value < 0.05) among the four sampling locations (also in pairwise comparisons). SIMPER revealed the similarity of samples within the marinas with 39.94% in RHO, 41.88% in HER, 45.53% in ZEA and 46.69% in PAT. Different taxa combinations contributed to the similarity in every site with Turf-forming algae contributing more than 25% in each area. The nMDS analysis revealed a scattered pattern with no distinct grouping for the quadrats of different studied areas, despite the significant differences among them (Fig. 2).

In total, four (7%) sessile CRY and eight (13%) sessile NIS were recorded in the studied marinas, four of which are characterized as invasive (INV) (nine in HER, five in PAT, three in ZEA and six in RHO). These were the macroalga *Stypopodium schimperi* (INV), the sponge *Paraleucilla magna* (INV), the anthozoan *Oculina patagonica* (CRY), the bivalve molluscs *Brachidontes pharaonis* (INV), *Dendostrea folium* (INV) and *Pinctada radiata* (INV), the erect bryozoans *Amathia verticillata* (CRY) and *Bugula neritina* (CRY) and the ascidians *Ecteinascidia turbinata* (CRY), *Herdmania momus*, *Phallusia nigra* and *Styela plicata*. Four species were constantly present in the study areas (*D. folium*, *H. momus*, *B. neritina* and *A. verticillata*) while another four species were present in only one marina (*P. magna*, *B. pharaonis*, *P. radiata* and *E. turbinata*). Additionally, the crypto-expanding crab *Percnon gibbesi* and the two non-indigenous echinoderms *Diadema setosum* and *Synaptula reciprocans* (INV) were recorded in the marinas during the SCUBA surveys.

Discussion and conclusions

Visual census methods can provide useful information for mega- and macro-benthic taxa and can also be a cost-effective and time-efficient tool for the assessment of biodiversity in marinas (Bianchi *et al*., 2004; Chebaane *et al.*, 2022). The analysis of photoquadrats consists a non-destructive approach and is widely used for the studying and monitoring sessile communities in both natural and artificial substrates (Gerovasileiou & Voultsiadou, 2016; Sedano *et al*., 2019; Rallis *et al*., 2022).

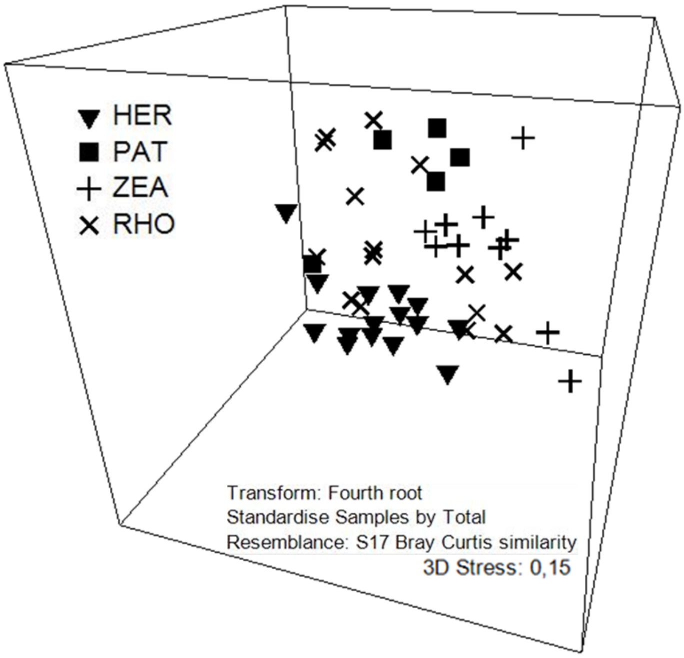

Fig. 2: 3-D nMDS plot based on the biodiversity data from the first sampling event (September 2020) for the four studied marinas

According to our results, the four studied marinas presented differences in their benthic community structure with many taxa records in each area. In any case, due to the unequal sample size, the biodiversity of the marinas with fewer samples may have been underestimated. The PERMANOVA resulted in significant differences among the sites even if the nMDS (Fig. 2) does not reveal a clear clustering among samples. This pattern may be attributed to the high coverage rates of the macroalgae taxa that prevailed in most photoquadrats. Among the study sites, the marina of HER was the largest in terms of surface (0.87 km^2), entrance opening (280 m) and has more extensive shipping activity, since it also functions as a commercial port (Chatzinikolaou & Arvanitidis, 2016). Additionally, marinas of HER and RHO provided a sciaphilic environment below artificial floating platforms. These features may explain to some extent the higher recorded biodiversity in these two study areas. The CRY and NIS records were also higher in HER and RHO, probably due to their geographic location closer to the Suez Canal than the other two study areas (Tsirintanis *et al*., 2022).

Twelve out of the 45 identified sessile species were CRY and NIS with four of them being also invasive. Yet, the status of NIS is constantly under review (Thessalou-Legaki *et al*., 2012). The high recorded variety of CRY and NIS was expected since the marinas are prone to host such opportunistic species (Tempesti *et al*., 2020) and especially ascidians, which are abundant in port environments (López-Legentil *et al*., 2015). Specifically, in the marina of Heraklion, three ascidians were reported on the hard artificial substrates (*H. momus*) and the hanging submerged ropes (*S. plicata* and *P. nigra*) (Fig. 3). Furthermore, the ascidian *S. plicata* and the bryozoan *A. verticillata* are typical of ports and marinas (Tempesti *et al.*, 2020). However, these two species were absent from the photographic samples of RHO despite their previous records in the area (Ulman *et al*., 2017). On the other hand, the sponge *P. magna* that has only recently been recorded in Greece (Gerovasileiou *et* al., 2017) was reported for the first time in the marina of Heraklion.

The findings of the present study confirm the strong presence of CRY and NIS in port environments. Monitoring biodiversity in ports and marinas and recording NIS during their early succession stages, could prevent a secondary dispersion and limit the degradation of the nearby natural habitats (Ulman *et al*., 2019). Visual census methods, when performed on a regular basis, can be a useful tool for this purpose.

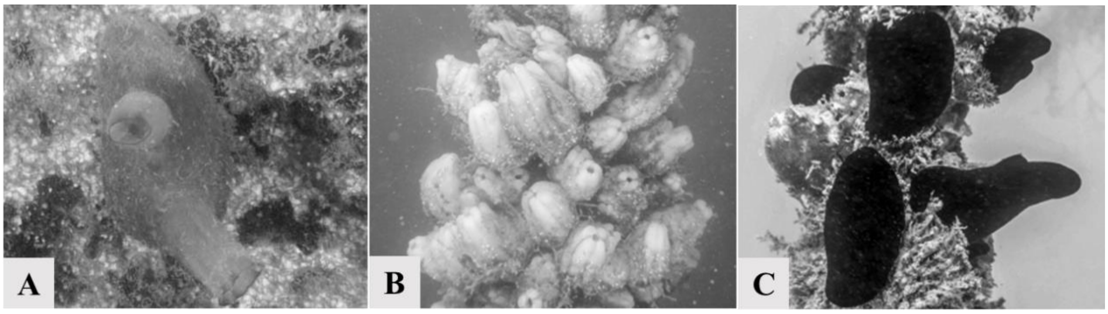

Fig. 3: Three non-indigenous ascidians recorded in the marina of HER. A: *Herdmania momus* on the artificial vertical wall and piles, B: *Styela plicata* and C: *Phallusia nigra* attached on hanging submerged ropes.

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EAT ALIEN INVASIVE BLUE CRABS: YES, BUT WITHOUT RUNNING HEALTH RISKS!

Abstract
The non-indigenous blue crabs (Portunus segnis and Callinectes sapidus) represent an emerging fishery in Tunisia, which can effectively contribute to food security in the context of local resources scarcity. In 2019, catches reached 14 000 T in this country, and were mainly exported worldwide. These crabs have a high nutritional value and are considered as a new source of proteins for Tunisian consumers. Nevertheless, some trace elements may constitute a threat to human health at high concentrations and heavy metals are toxic even at very low contents (bioaccumulation via the food chain). This work aims to evaluate and compare the concentration of nine trace metals (Fe, Cu, Zn, Mn, Ni, Co, Cr, Cd and Pb) in various tissues (muscles, hepatopancreas and exoskeleton) of C. sapidus using Inductively Coupled Plasma Mass Spectrometer. Samples (n=33) were collected in Tunis southern lagoon by fyke nets during eel fishing campaign (from October 2020 to July 2021). Results highlighted that metal concentrations differed significantly according to tissues (p<0.05). The exoskeleton of the crab accumulated the highest levels for all the elements followed by hepatopancreas and pereiopods' muscles. The Metal Pollution Index was 3.73± 0.19, 0.26± 0.04 and between 0.11 and 0.16 respectively for exoskeleton, hepatopancreas and muscles. Zn was the most abundant in the different tissues with a highest concentration in the exoskeleton (11.19± 2.49 mg/100g, 14.69± 1.10 mg/100g, 13.74± 0.98 mg/100g and 168.95± 19.1mg/100g respectively in claws, locomotion pereiopods, hepatopancreas and exoskeleton). Regarding highly toxic metals (Pb and Cd), concentrations were under the admissible limits of the European Union except for hepatopancreas and exoskeleton with a significant difference between them (p<0.05). Indeed, the most consumed tissues (muscles) are the least affected and targeted by Pb and Cd. More controls are strongly needed to guarantee human health safety. Biomonitoring must cover all fishing sites, especially polluted areas, and must take into account seasonal variations, sex and crab physiology.

Key-words: Bioinvasion, Heavy metals, *Callinectes sapidus*, Food safety, Invasivorism

Introduction
Biological invasions have been considered worldwide as a major driver of change in Mediterranean marine biodiversity (Azzurro *et al.,* 2019). Managing invasive non-indigenous species (NIS) represent a challenge for biodiversity conservation. Different approaches are used for control such as mechanical and physical removal (Nunez et al., 2012). Furthermore, eating edible NIS or “invasivorism” to reduce invasive populations is gaining popularity (Franke, 2007). In addition, turning invasive species into gourmet meals may contribute to food security and is an opportunity to boost local economy (Nunez *et al.,* 2012).This approach was presented as a win-win for consumers and environmental stakeholders (Franke, 2007). Several promoting campaigns for NIS consumption, namely “Eat Those Invasives”, “Eat Lionfish” and “Fighting invasive species, one bite at a time” were carried out (Nunez *et al.*, 2012). In addition, many cookbooks focused on recipes based on NIS (Franke, 2007; Leone, 2020). Alien portunid crabs are regularly caught and landed by small-scale fisheries in Tunisia since 2015 for

Portunus segnis (Forskål, 1775) and later for the Atlantic crab *Callinectes sapidus* (*C. sapidus*) Rathbun, 1896 (Ben Souissi *et al*., 2017; Katsanevakis *et al*., 2020). Actually, more than 28 processing units and export companies have been implemented in Tunisia and crab exports increased 100 times between 2015 and 2019 to more than 20 destinations all over the world (GIPP, 2019). The exported quantities reached 3500 tons for more than 12 million euros in 2019 (export price is between 2.5 and 4 €/kg depending on products) (GIPP, 2019). These crabs have a high nutritional value and considered as a new source of minerals for Tunisian consumers. Some trace elements are essential for the metabolism of the organism but may constitute a threat to human health at high concentrations. Other metals have no known biological functions and are toxic even at very low contents because of their toxicity, and biomagnification in the food chain (Barat Kumar *et al.*, 2019). *C. sapidus* is omnivorous predator, feeding on bivalves, fish, gasteropods, and other crustaceans, as well as algae (Kampouris *et al.* 2019). Since accumulation of trace metals in aquatic organisms has been correlated to their feeding habits (Barat Kumar *et al*., 2019), it seems necessary to evaluate trace element contents, especially heavy metals, in crab tissues to estimate his potential toxic effect. Therefore, this work is the first one dealing with trace elements uptake on the invasive decapod *C. sapidus* in Tunisia.

Materials and methods

Crab sampling and preparation: Specimens of *C. sapidus* (n=33) were collected from the Southern lagoon of Tunis (north eastern of Tunisia) by fyke nets of 18 mm mesh size during eel fishing campaign (from October 2020 to July 2021). Samples were then transferred alive to the laboratory to be measured and classified into males and females. After morphometric caracterisation, adult crabs were euthanized by thermal shock (-20°C for 10 min) and divided randomly into three homogeneous groups. Then, exoskeleton, hepatopancreas and muscles from thoracic sterna, claws (chelipeds) and locomotion pereiopods of each group were hand-picked and blended to homogenize (Khamassi *et al*., 2022).

Trace element analysis: The metal analysis was made according to Canli and Atli (2003). The carapace, hepatopancreas and muscle samples (0.1 g dry weight each) were dried at 150 °C until reaching constant weight. Then concentrated nitric acid (4 mL, Merck, Darmstadt, Germany) and perchloric acid (2 mL, Merck) were added to the samples, which were put on a hot plate set to 150 °C until all tissues were dissolved. Inductively coupled plasma mass spectrometer ICP-MS, Agilent, 7500ce Model) was used to determine elements (Fe, Cu, Zn, Mn, Ni, Co, Cr, Cd and Pb) concentrations. detected as µg metal g^{-1} dry weight. The mean value of the measured concentrations in the crab tissues are used in this work. High purity multi standard (Charleston, SC, USA) was used for determination of the metals.

Statistical analysis: A one-way ANOVA was performed after normal distribution data analysis. Duncan's multiple range test was assessed to separate differences among means. All statistical analysis was tested at the 0.05 level of probability with the software Statistical Analysis System (SAS 9.1.3, 2002-2003, Institute Inc., Cary, NC, USA).

Results

First occurrences of *C. sapidus* in the Mediterranean Sea are summarized in Figure 1. Actually, this species is present in 19 out of the 23 countries surrounding the Mediterranean Sea. Since its first record in 1949, the crab has colonized almost the entire Mediterranean basin (Figure 1).

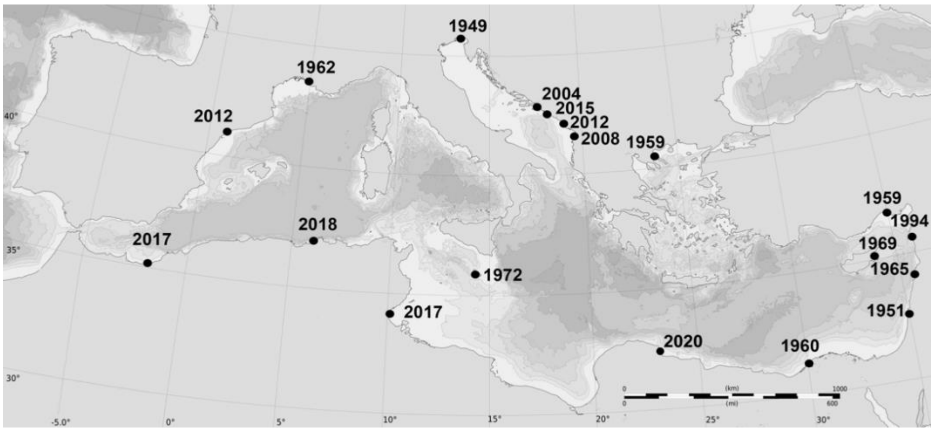

Fig. 1. Chronological occurrences of *Callinectes sapidus* in the Mediterranean Sea (Ben Souissi *et al.,* 2017; Falsone *et al.,* 2020; Corsini-Foka *et al.,* 2021 ; Mancinelli *et al.*, 2021)

Adult males and females were measured and weighed (Fig. 2). The mean carapace width CW, carapace length CL, and total weight TW of adult specimens were, respectively, 135.38 ± 10.34 mm, 59.75 ± 5.48 mm, and 167.41 ± 34.37 g for females and 134.51 ±17.09 mm, 62.34 ± 7.17 mm, and 185.19 ± 65.15 g, respectively, for males. Total muscles, hepatopancreas and exoskeleton yields represented respectively 39.45 ± 4.69%, 3 ± 1.08% and 30.2 ± 2.8%.

Fig. 2. *Callinectes sapidus* (scale bar=1cm)

Trace element concentrations (mg/100g WW) in *C. sapidus* collected from the southern lagoon of Tunis are presented in table 1**.** Results highlighted that metal concentrations differed significantly according to tissues ($p<0.05$). The exoskeleton of the crab accumulated the highest levels for all the elements followed by hepatopancreas and muscles (Tab. 1). The Metal Pollution Index was 3.73± 0.19, 0.26± 0.04 and between 0.11 and 0.16 respectively for exoskeleton, hepatopancreas and muscles. Zn was the most abundant in the different tissues with a highest concentration in the exoskeleton. It represented 11.19± 2.49 mg/100g, 14.69± 1.10 mg/100g, 13.74±0.98 mg/100g and 168.95± 19.1mg/100g respectively in claws, locomotion pereiopods, hepatopancreas and exoskeleton (Tab. 1). Furthermore, heavy metals contents (Pb and Cd) were higher in the exoskeleton than in the hepatopancreas and muscles ($p<0.05$). The lead concentration in the carapace reached 0.43 ± 0.05 mg/100 g.

Tab.1. Trace element concentrations (mg/100 g WW) in *Callinectes sapidus* collected from the southern lagoon of Tunis

	Mn	Fe	Co	Ni	Cu	Zn	Cr	Cd	Pb	MPI
Ch	0.04± 0.002[c]	1.21± 0.45[b]	0.03± 0.01[b]	0.12± 0.04[b]	0.041± 0.02[d]	11.19± 2.49[b]	0.03± 0.002[c]	0.04± 0.01[b]	0.03± 0.01[c]	0.11± 0.01[c]
LP	0.1± 0.04[b]	1.01± 0.25[b]	0.025± 0.01[b]	0.05± 0.02[b]	1.56± 0.66[b]	14.69± 1.10[b]	0.03± 0.01[c]	0.03± 0.04[b]	0.02± 0.01[c]	0.16± 0.04[c]
He	0.07± 0.01[b]	0.62± 0.16[b]	0.06± 0.01[b]	0.07± 0.02[b]	0.42± 0.2[c]	13.74± 0.98[b]	0.48± 0.04[a]	0.32± 0.14[a]	0.07± 0.004[b]	0.26± 0.04[b]
Ex	12.54± 1.9[a]	131.1± 0.07[a]	0.41± 0.08[a]	3± 0.26[a]	13.92± 0.67[a]	168.95 ±19.1[a]	0.28± 0.02[a]	0.26± 0.01[a]	0.43± 0.05[a]	3.73± 0.19[a]

Data are mean ± SD; Significant differences between analyzed tissues are detected at 5%; MPI: Metal Pollution Index; Ch: Chelipeds; LP: Locomotion pereiopods; He: Hepatopancreas; Ex: Exoskeleton

Discussion and conclusions

C. sapidus is eurythermal and euryhaline. It has a wide ecological tolerance and can inhabit estuaries, lagoons, rivers and other coastal habitats (Taybi & Mabrouki, 2020). In addition, it has high fecundity and strong swimming capacities. It is considered as an opportunistic and aggressive predator (Kampouris *et al.*, 2019). Such characteristics have contributed to its establishment and expansion of its distribution range in the Mediterranean Sea since its first record in 1949 (Falsone *et al.*, 2020).

Since its first record in 2017 in the gulf of Gabès (Ben Souissi *et al.*, 2017), *C. sapidus* has expanded its distribution northwards (Shaeik *et al.*, 2021). Currently, it is recorded almost ubiquitously along northern coasts of Tunisia with an increasing exponential rate. This may lead to ecological and socio-economic impacts on the local fisheries by damaging nets and catches. In this context, fishermen confirmed a decline in their eel catches, since blue crabs captured in fyke nets in Tunis lagoon, damaged the fishing gear. Therefore, eels were released. To turn this biological explosion from a threat into an opportunity, many scientists have proposed and supported the approach of controlling NIS through gastronomy (Franke, 2007; Nunez *et al.*, 2012; Rjiba-Bahri *et al.*, 2019; Khamassi *et al.*, *in press*). In addition, analysis of the nutritive value of *C. sapidus* sampled in the Tunis southern lagoon revealed that muscle yield of this species is higher than other commercial crabs (between 36 and 42% of the total weight) with high protein content (>23%) and low fats (<5%) (Khamassi *et al.*, 2022). Crabs can absorb minerals directly from the aquatic environment through gills and body surfaces. Therefore, thay can accumulate high levels of metals leading to biomagnification through the food chain (Barath Kumar *et al*., 2019). Heavy metals may pass to humans through food chain, causing serious health problems (Zhang *et al*., 2011). Although blue crab exports increased since its first record in Tunisia, there is no studies dealing with trace metal uptake in this species. Only few papers focused on heavy metals in *P. segnis* collected in Tunisian coasts as bioindicator of ecosystem pollution and not a health risk for consumers (Annabi *et al.*, 2018; Bejaoui *et al.*, 2021). In this study, the exoskeleton of the crab accumulated the highest contents of all trace metals followed by hepatopancreas and muscles. Many studies have shown that metal contents in crabs are variable according to body tissues, with the highest contents in the exoskeleton, gills and hepatopancreas (Béjaoui *et al*., 2021). Since Cd have an ionic radius similar to that of the calcium, it is progressively accumulated in the exoskeleton via calcium uptake routes with other trace elements such as Mn, Pb, and Zn (Annabi *et al*., 2018).

The hepatopancreas contained the highest levels of Cr and Cd. It is the key site of metals storage and detoxification in crustacean and particularly crabs (Bejaoui *et al.*, 2021). Although the muscles receive the major portion of hemolymph, metal accumulation was found to be relatively low. The zinc was the most abundant metal in the different tissues followed by iron and copper with the highest concentrations in the exoskeleton. Same results were observed with *Portunus pelagicus* from the Persian Gulf but with highest levels in the hepatopancreas (Haidarieh *et al.*, 2013). However, Annabi *et al.* (2018) found that Cu contents in *P. segnis* from the gulf of Gabès were higher than Zn which was accumulated especially in muscles.
Some of trace metals such as Zn, Fe, Cu, Co, Ni and Mn play a vital role in the metabolic activity of living organisms (a component of hormones and enzymes) and can be toxic only at relatively high concentrations. In fact, Zn and Mn are important components of several enzymes and play an essential role in a number of biological processes involved in growth and development. Iron is one of the very important essential trace elements since it serves as a carrier of oxygen to the tissues from blood. Besides, copper serves as an essential co-factor for several oxidative stress-related enzymes and Cr is required for carbohydrates metabolism (Barath Kumar *et al.*, 2019).
Results have shown that all trace metals concentrations were within the permissible limits of FAO/WHO (1984), except for exoskeleton which exceeds those limits in the case of Mn, Cu, Cd and Pb. In addition, the hepatopancreas also resulted in high contents of Cd, which exceeded the permitted threshold, highlighting the need of monitoring these metals . This is in line with the European Food Safety Authority (EFSA) which advises to limit crab hepatopancreas consumption because it accumulates high amounts of Cd. This bioaccumulation may be due to the site of collection, in fact, Tunis lagoon borders a strongly urbanized area in continuous expansion. It has long served as a receiving environment for domestic and industrial discharges from the city of Tunis and neighboring agglomerations. Therefore, sediments of the southern lagoon may contain high concentrations of trace metals (Pb, Cd and Cu) (Derouiche, 2016) .
Indeed, the most consumed tissues (muscles) are the least affected and targeted by toxic metals (Pb, Cd). More controls are strongly needed to guarantee human health safety. Biomonitoring must cover all fishing sites, especially polluted areas, and must take into account seasonal variations, sex and crab physiology.

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INVASION ECOLOGY THROUGH RISK ASSESSMENT QUESTIONS: A MULTIVARIATE COMPARISON AMONG NON-INDIGENOUS SPECIES TRAITS IN THE MARINAS OF TUNISIA

Abstract
Marinas are highly modified habitats, hotspots of non-indigenous species (NIS) and hubs for NIS introduction, acclimation and spread. Although most of the NIS that thrive inside marinas do not have an invasive behavior in nearby natural coasts, they have the potential to become invasive in these areas and generate ecological and socio-economic impacts. Therefore, it is imperative to assess the risk of invasion for these species to inform management choices. The NIS and cryptogenic species assessed in this study were identified during a rapid assessment survey conducted in 6 marinas of Tunisia in August 2021. A multivariate dataset was generated from the Aquatic Species Invasiveness Screening Kit (AS-ISK) questions answered to assess the risk of invasion of each of these species. In this study, the answers to 37 AS-ISK questions were used as variables to assess the Euclidean distances among NIS and cryptogenic species and identify the traits related to their invasiveness potential. The main contributors to the risk of invasion were the questions on the taxon capacity for (1) generate undesirable effects in the risk assessment area, (2) exploit and sequestrate ecosystem resources and (3) reproduction and dispersal. Active predators (decapods) scored highest in these traits, followed by sessile filter feeders and ecosystem engineers. Overall, a subset of the questions provided a fairly accurate assessment of the invasion risk and findings will be useful to understand the traits shared by potentially invasive species and for the management of coastal NIS hotspots.

Key-words: Marinas, AS-ISK, Non-indigenous Species, Cryptogenic Species, Invasiveness

Introduction
The Mediterranean Sea is considered one of the main global hotspots of marine biodiversity, as it represents less than 1% of world aquatic surfaces, but it hosts 18% of its macroscopic marine species, from which about 30% are endemic (Bianchi & Morri, 2000). However, it is also considered one of the most impacted marine regions of the world (Lejeusne *et al*., 2010). Among the main threats that jeopardize its native biodiversity and ecosystems functioning and services are the effect of human activities (e.g., habitats destruction, fragmentation, and degradation), the effects of climate change and the impacts derived from marine invasions (Bianchi & Morri, 2000; Lejeusne *et al*., 2010).
Marinas are hubs for non-indigenous species (NIS) introduction, acclimation and spread (Molnar *et al*., 2008). These structures constitute the nodes of a network of highly modified water bodies (HMWB) connected by recreational maritime traffic (Pineda *et al*., 2011; Ros *et al*., 2020). Therefore, it is imperative to monitor NIS in these habitats and assess the risk of invasion that these species pose to nearby areas (Chebaane *et al*., 2019; Rondeau *et al*., 2022). Ultimately, a better understanding of NIS ecological risk will improve the management strategies against biological invasions in coastal habitats

(SPA/RAC - ONU Environment/PAM, 2017).
Although our ability to predict whether a NIS will become invasive is still limited, several tools, such as the Aquatic Species Invasiveness Screening Kit (AS-ISK), can produce estimations, which can assist the decision-making processes on NIS management (Hewitt & Hayes, 2002; Copp *et al*., 2016). The AS-ISK tool assess the invasiveness potential of NIS within a certain area. It follows the EU Regulation No 1143/2014 on invasive alien species of EU concern (EC, 2014), and is particularly designed to work with marine autotrophs, invertebrates and fish.
A continuous update of NIS spatial distribution, temporal occurrence and abundance, especially in high-risk areas, is recommended by the Mediterranean Action Plan concerning Species Introduction and Invasive Species (UNEP/MAP-RAC/SPA, 2008) and the National Monitoring Program for Biodiversity and Non-indigenous Species of Tunisia (SPA/RAC - ONU Environnement/PAM, 2017), which is in line with the requirements of the Integrated Monitoring and Assessment Program of the Mediterranean Sea and Coast and Related Assessment Criteria (UNEP/MAP, 2016a, 2016b; PNUE-PAM-CAR/ASP, 2017). In this context, this study aims to identify the topics and questions, among those provided by AS-ISK, that are most relevant for the risk assessment of the invasiveness potential of NIS in the Mediterranean Sea, with special focus on Tunisian coasts.

Materials and methods

A risk assessment was conducted using AS-ISK (Copp *et al*., 2016) on 20 taxa identified on rapid assessment surveys conducted in six marinas of Tunisia in summer 2021 (see Chebaane *et al*., 2022 for more details). A total of 55 questions were answered for each taxon during the AS-ISK screening protocol. These include 49 questions for a Basic Risk Assessment, which outputs the baseline and most accurate risk rank (Vilizzi *et al*., 2022) and verses on the biological, biogeographical, ecological and socioeconomic aspects of the scanned taxon. Also, 6 questions for a Climate Change Assessment, which evaluates how future predicted climate conditions are expected to affect the risks of the taxon introduction, establishment, dispersal, and impacts. The AS-ISK questions were answered based on bibliographic information extracted from peer-reviewed literature whenever possible, and a confidence level, ranging from ‘very high’ to ‘low’, was given to each answer, based on the amount and quality of the information available to answer (Copp *et al*., 2016).
After conducting the AS-ISK for all species (Chebaane *et al*., 2022), a subset of 37 suitable AS-ISK questions was selected and their answers were transformed into ordinal variables, ranging from 0: negative response with the highest confidence to 7: positive response with the highest confidence. These variables were then used to calculate Euclidean Distances resemblances among the 20 assessed taxa. We used SIMPROF to search for statistically homogeneous groups among these taxa and BEST to relate taxa similarity with the summed scores of each group of questions by topic, as defined for AS-ISK by Copp *et al*. (2016). Results were visually displayed using a Principal Coordinates Ordination (PCO) and those topics with a Spearman correlation higher than 5.0 with any PCO axis were overlayed as vectors on the PCO plot. Analyses were conducted using Primer-e v6 +PERMANOVA.

Results

The topics grouping questions on the taxon capacity for: (1) generate undesirable effects in the risk assessment area (e.g., disrupting the food web structure), (2) exploit and sequestrate ecosystem resources and (3) reproduction and dispersal, were the most important in structuring the assessed taxa (Rho = 0.67; P(BEST) = 0.001). The assessed taxa were segregated into 9 groups by SIMPROF, with mobile decapods and autotrophs grouped separately, and sessile invertebrates further divided into 7 groups (Fig. 1).

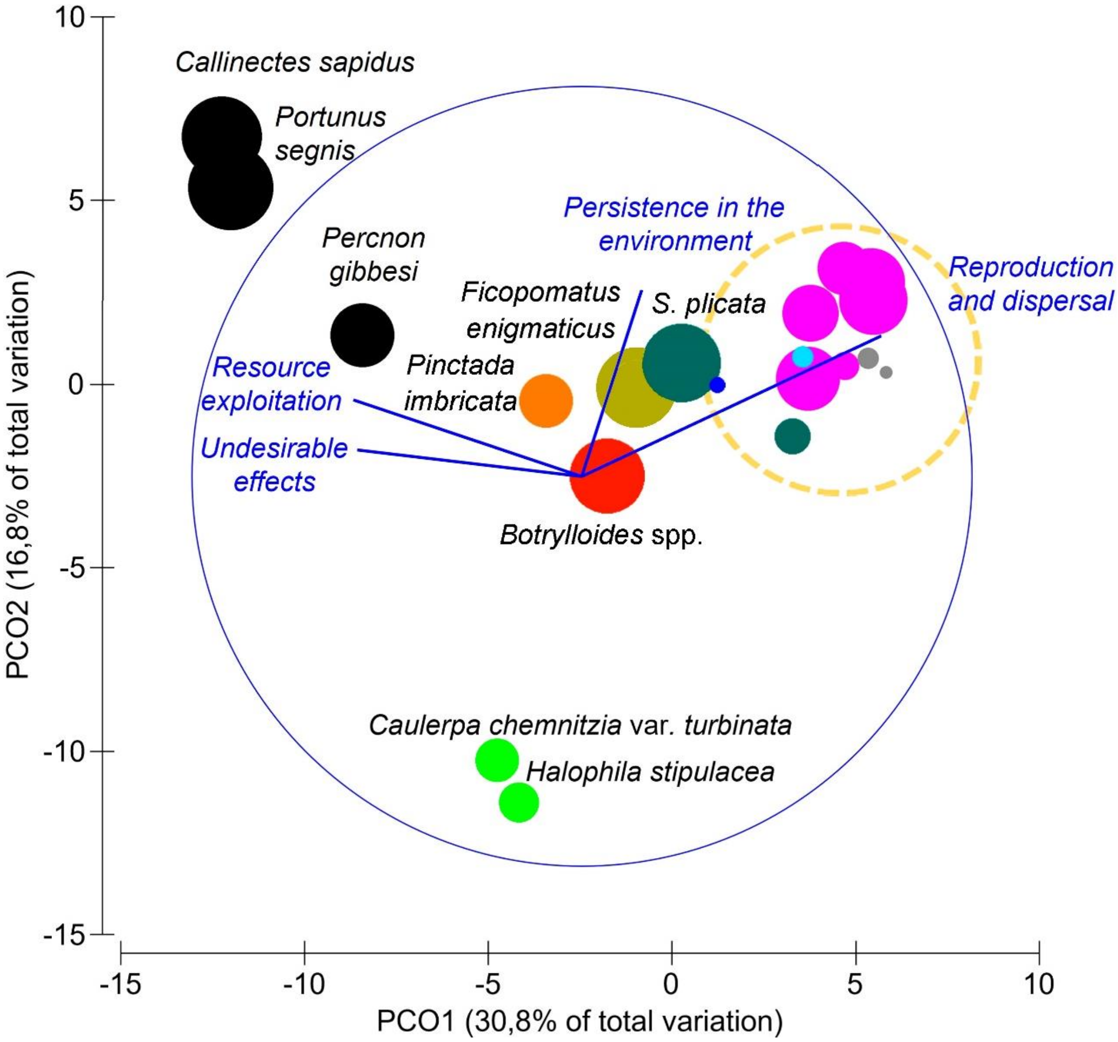

Fig. 1: Principal Coordinates Ordination displaying the 20 taxa assessed using AS-ISK (dots). Dot size represents the AS-ISK Basic Risk Assessment Score output and color indicate statistically homogeneous groups of taxa according to SIMPROF (threshold = 0.05). Taxa within the yellow dashed line, which names are not displayed, include fouling invertebrate species: barnacles, bryozoans and ascidians.

Overall, the AS-ISK identified active predators as high impact-risk, indicating a high potential for exploit ecosystem resources and generate undesirable effects (Fig. 1). Among sessile invertebrates, some filter feeders with high-risk scores could also generate undesirable effects through resource-exploitation and sequestration. Nevertheless, most of the fouling invertebrates identified in the marinas of Tunisia had high reproduction and dispersal, as well as tolerance and persistence traits, regardless of the Basic Risk Assessment scores obtained by AS-ISK.

Discussion and conclusions

In the Mediterranean Sea, previous studies have ranked NIS by their risk and impact, which has provided important information for resource allocation on NIS management (Streftaris & Zenetos, 2006; Ojaveer *et al*., 2015). To this matter, AS-ISK and other screening tools could allow a complementary information on NIS invasiveness (Copp *et al*., 2016; Vilizzi *et al*., 2022). This is occurring for some non-indigenous fishes (Filiz *et al*., 2017a, b; Bilge *et al*., 2019; Glamuzina *et al*., 2021; Yapici, 2021) and pelagic invertebrates, such as jellyfish (Killi *et al*., 2020) and tunicates (Glamuzina *et al*., 2021). Among benthic organisms, it has been carried out for some vagile molluscs and arthropods (Glamuzina *et al*., 2021; Tarkan *et al*., 2021; Stasolla *et al*., 2021; Tomanić *et al*., 2022). However, the risk posed by marine fouling NIS, which constitutes a high percentage of the Mediteranean NIS diversity, has not been yet assessed, but for only a few molluscs and barnacle species, such as *Crassostrea gigas* and *Amphibalanus improvisus* (see Glamuzina *et al*., 2021; Stasolla *et al*., 2021). All these assessments have been carried out in the European coasts of the Mediterranean. Therefore, Chebaane *et al*. (2022) constitutes, to our best knowledge, the first assessment for the African coasts, and the first focused on the communities in marinas, which are among the most invaded marine habitats of the Mediterranean Sea.

Results indicate that screening protocols, such as AS-ISK, can be a valuable tool to assess the risk by NIS in marinas, including species that are not yet present in the Mediterranean Sea but have risk of introduction (Copp *et al*., 2016; Vilizzi *et al*., 2022). These include species prone to be introduced by known vectors, such as the aquaculture of mussels, which has been introducing macroalgae and other NIS from the Pacific Ocean into the Mediterranean Sea during the past decades (Katsanevakis *et al*., 2013; Verlaque & Breton, 2019; Ruitton *et al*., 2021). Nevertheless, exhaustive and documented screening of a high number of species requires a considerable effort. To this topic, this study suggests that a subset of the AS-ISK questions can provide an accurate risk assessment for the assessed species. As risk screening protocols are starting to be implemented in the Mediterranean Sea, the identification of the most relevant questions could expedite the assessment when working with a high number of NIS or when performing a quick scan on recently introduced species. Therefore, these results might set the ground for a screening protocol focused on the species on marine artificial coastal habitats in the Mediterranean region towards an improvement of NIS management.

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POSTERS

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INDIGENOUS *VS* EXOTIC: STUDY OF THE FAUNA AND FLORA ASSOCIATED WITH THE VERMETID REEFS ALONG THE LEBANESE COAST

Abstract
Non-Indigenous Species (NIS) are significantly increasing along the Lebanese coast. This study aims to evaluate the impact of NIS on the fauna and flora associated with the vermetid reefs. Accordingly, benthic communities of vermetid reefs were collected by rock scraping using a hammer along four sites of the Lebanese coast. The results show a significant dominance in the abundance of NIS in all the monitored sites. In addition, this study confirms the invasion status of newly introduced species along the Lebanese coast (e.g. the Macroalgae/ Ochrophyta Dictyota acutiloba, and the Mollusca rayed dwarf-turban Turbo radiatus). Based on this finding, it is essential to establish a sustainable monitoring program of NIS along the Lebanese coast to evaluate their impact on the local communities and the whole habitat.

Key-words: Non-Indigenous Species, Benthic communities, *Dictyota acutiloba*, Turbo radiatus, Lebanese coast

Introduction

The Lebanese coast, which at its southern end is about 400 km distant from the Suez Canal, is fully exposed to the arrival of marine “lessepsian” species. Indeed, the number of alien species is still increasing along the Lebanese waters (Badreddine, 2018 and references therein). Invaders represent a threat to local flora and fauna, as in the case of the Indigenous Species (IS), the Mollusca *Mytilus galloprovincialis*, which was abundant in the past, according to Gruvel (1931), and is now totally replaced by the invasive NIS *Brachidontes pharaonis* and *Cerithium scabridum*. This study aims to evaluate the impact of NIS on the fauna and flora associated with the vermetid reefs along the Lebanese coast.

Material and Methods

The study was performed in May 2021 along four sites (Tab. 1) characterized by large vermetid reefs along the Lebanese coast. Subsequently, the assessment of benthic communities was performed by random 10 x 10 cm quadrats. At each sampling site, five 10 x 10 cm plots were randomly placed on the inner and outer edges of the vermetid reefs, and the benthic assemblage was collected from each plot by the destructive methods of rock scraping using a hammer. Each sample was stored in a plastic zipper bag with a mixture of seawater and 10% formalin solution until their identification.

Tab. 1: Sites considered for the assessment of vermetid reefs along Lebanese coast, their coordinates, and status*

Sites	Latitude, Longitude	Status
Nakoura	33° 8′25.15″N, 35° 9′14.82″E	Not-Impacted
Tyre	33°16′33.58″N, 35°11′34.64″E	Moderately-Impacted
Beirut	33°54′9.15″N, 35°29′2.02″E	Impacted
Palm Island Nature Reserve	34°29'45.20"N, 35°46'26.24"E	Moderately-Impacted

* The status was evaluated based on several pressures (i.e. urbanization, agricultural and industrial activities, sewage outfalls, commercial harbors, freshwater input).

Results and Discussion

A total of 80 taxa, belonging to 8 Phyla, were observed and counted to 24312 individuals. The main group was Mollusca with 30 taxa and 12,236 individuals, Arthropoda with 16 and 10,430 individuals, Annelida with 10 taxa and 1136 individuals, Cnidaria (with 10 individuals) and Nemertea (with 500 individuals) with 2 taxa each. Regarding the macroalgae, the main represented group was Rhodophyta with ten taxa, Ochrophyta with seven taxa, and Chlorophyta (3 taxa). Concerning Non-Indigenous Species (NIS), 35 taxa were recorded: 16 Mollusca, 13 Annelida, 2 Chlorophyta, 1 Arthropoda; 1 Rhodophyta, and 3 Ochrophyta. Subsequently, and in terms of abundance, the number of individuals NIS (with 13,300 individuals, mainly Mollusca) was more than the IS counted (with 11,012 individuals, mainly Arthropoda). In addition, it was remarkable the abundance of the Mollusca *Brachidontes pharaonis*, *Conomurex persicus*, *Ceritium scabridum*, *Trochus radiatus*, Annelida *Pseudonereis annulata*, *Timarate punctata*, Artropoda *Charybdis helleri*, especially in the moderately-impacted and impacted sites. The Lebanese waters, like the entire Levantine Sea, are increasingly exposed to the continuous entrance of NIS species, primarily through the Suez Canal. In this context, updating the checklist of non-indigenous species along the Lebanese coast is recommended through an efficient monitoring program targeting the most invasive NIS already established along the Lebanese coast.

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THE MARCHICA LAGOON: A COASTAL ECOSYSTEM AT RISK OF BIOINVASIONS ON THE MEDITERRANEAN COAST OF MOROCCO

Abstract
The Marchica lagoon, the unique lagoon on the Mediterranean coast of Morocco, is under pressure due to a complex mixture of stressors, among which Invasive Alien Species are becoming one of the leading and most rapidly growing threats in the lagoon. This paper documented and discussed the occurrences of Non Indigenous Species in the Marchica lagoon considered as a hotspot of bioinvasion.

Key-words: Non-indigenous, Invasive, Coastal lagoon, Impacts, Morocco, Mediterranean.

Introduction

The Marchica lagoon is under pressure of a complex mixture of natural and anthropogenic stressors, among which Invasive Alien Species (IAS) are becoming one of the leading and most rapidly growing threats in the lagoon. Here we present the current knowledge of non-indigenous species (NIS) recorded in the lagoon.

Materials and methods

Scientific literature, websites and unpublished data were analysed to establish an updated list of the alien marine species reported from the Marchica lagoon. For each species, the date of first record, pathway and establishment success were presented.

Results

Up-to-now, four NIS have been recently recorded in the Marchica lagoon and are well established. These are (1) the Mollusc *Bursatella leachii* Blainville, 1817 (a circumtropical mollusc widely distributed both in the Atlantic and the Indo-Pacific, including the Red Sea) (2) the American blue crab *Callinectes sapidus* Rathbun, 1896 (native to western Atlantic estuaries) (3) the Amphipod *Caprella scaura* Templeton, 1836 (native to the Pacific coast of North and Central America) and (4) the isopod *Paracerceis sculpta* Holmes, 1904 (native to the Pacific coast of North and Central America). The Mollusc *Bursatella leachii* was first recorded in the lagoon in 2016 and can be even considered as becoming a nuisance because it clogged fishing net (Selfati *et al.*, 2017). The American blue crab *Callinectes sapidus* was first recorded in 2017 (Oussellam and Bazairi *in* Chartosia *et al.*, 2018) and its impacts in the lagoon have shown a variety of impacts scoring the "Minor Impacts" following the new SEICAT methodology (Oussellam *et al.*, 2021). The Amphipod *Caprella scaura* and the isopod *Paracerceis sculpta* were recorded first in 2005 and are leading the benthic communities in the Marchica lagoon in terms of abundance and biomass (El Kamcha *et al.*, 2020; 2021).

Shipping activity was the most likely vector for the introduction of these species into the Marchica lagoon.

Fig. 1: The American blue crab *Callinectes sapidus* from the Marchica lagoon on the Mediterranean coast of Morocco

Discussion and conclusions

The recent bioinvasions in the Marchica lagoon reveal that this coastal ecosystem is at risk of biological invasions. Accordingly, the lagoon has to be monitored regularly to better understand the effects of these bioinvasions on native biodiversity.

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ADDITIONAL OCCURRENCE OF COMMON LIONFISH, *PTEROIS MILES* IN THE NORTHERN TUNISIAN COAST

Abstract

The record of common lionfish Pterois miles (Bennet, 1828) (Osteichthyes: Scorpaenidae) from the Tunisian coast confirms the occurrence of the species in Northern Tunisia (Western Mediterranean). This is the second reported capture of P. miles in northern Tunisia and the northernmost observation in this country. These records are not sufficient to state that the species is substantially established in the area, but investigations are rapidly needed to avoid unfavorable consequences for the local environment and economy.

Key-words: invasion, *Pterois miles*, geographical expansion, Tunisia, occurrence record.

Introduction

The invasion of the Indo-Pacific lionfishes *Pterois volitans* (Linnaeus, 1758) and *P. miles* (Bennett, 1828) outside of their native range has been reasonably well documented throughout the past decade (Bariche *et al*. 2013, Azzurro *et al* .2017). In the last two decades, they rapidly spread through the Western Atlantic and Caribbean Sea (Schofield, 2009), becoming a major problem for coastal environments and representing one of the world's top conservation issues (Azzurro *et al* .2017). In the Mediterranean Sea, a lionfish was first detected in July 1991 by Golani & Sonin (1992), who identified it as *Pterois miles*. Despite the conspicuous appearance of this fish, no further observations were made until 2012, when two new *Pterois miles* specimens, confirmed by molecular analyses, were recorded (Bariche *et al*. 2013). Recent findings in the Gulf of Tunis in 2015, indicate that two specimens of *P. miles* were recorded for the first time in Northern Tunisia (Ounifi Ben Amor & Ghanem 2016). The first specimen was captured in June 2015, in the waters surrounding Zembra Island (Gulf of Tunis) by gill net (28mm mesh size), on sandy bottom at a depth of 5m. The second fish was photographed by a diver in Haouaria (Cape Bon), in September 2015, in a similar habitat and very shallow waters.

Materials and Methods

In the wake of collaboration with Tunisian fishers and special regard to investigations concerning local ichthyofauna, we were informed that a specimen of *P. miles* was captured on 21 October 2020 by trammel-nets, off Ras Jebel (Gulf of Tunis) (37,2701° N, 10,1029° E) at a depth of about 18-22 m, on rocky bottoms partially covered by seagrass and algae. The specimen was preserved in 10% buffered formalin and deposited in the Ichthyological Collection of the National Institute of Marine Sciences and Technologies, under the catalog numbers: INSTM-miles-02.

Results and discussion

The Tunisian *Pterois miles* measured 225mm total length and weighed 125g. It was identified by the following combination of characters: body slightly compressed; head angular with spiny protrusions, tentacles and cirri around eyes and mouth; large mouth with villiform teeth; dorsal fin feathery with spines longer than body, and membranes incised almost to the base; anal and caudal fins rounded; pectoral fins wing-like with separate broad, smooth rays; small cycloid scales. Color of head and body with red and black stripes alternating vertically; dorsal spines, pectoral and ventral fins alternately banded with black, red and pink; dorsal soft rays, anal and caudal with series of dark spots. Golani *et al.* (2021) assigned *P. miles* to Lessepsian migration. Therefore, the most suitable hypothesis is that this species had entered the Mediterranean from the Red Sea, through the Suez Canal. Multiple evidence (e.g. Albins & Nixon, 2008) showed that the occurrence of lionfish displayed negative effects on reefs by a decreased recruitment of species in these areas, mainly those having an economical interest. Lionfish impacts on tourist recreational activities have been observed and some locations have posted warning signs advising potential envenomation by lionfish (Morris *et al.*, 2009). Further research is needed to verify if this invasive species has established a permanent population along the northern Tunisian coasts. They should be regularly monitored in order to avoid negative impacts on the local environment and economy.

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FIRST DATA ON THE BIOLOGY AND DYNAMICS OF THE AMERICAN BLUE CRAB *CALLINECTES SAPIDUS* IN MELLAH LAGOON, ALGERIA

Abstract
This study is a contribution to the knowledge of the biology and population structure of an invasive American blue crab *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: Portunidae). A total of 525 individuals, represented by 312 females (38.9 < CLf < 80.3 mm, 77 < WTf < 173 g) and 213 males (43.7 < CLm < 90.5, 80 < WTm < 175 g) was sampled between February 2021 and February 2022. The following parameters were determined: size structure, sex ratio, reproductive period, size at first sexual maturity, relationship between size and weight. The overall sex ratio was in favour of females (SR = 0.68, χ^2 = 18.66). This dominance was maintained during all seasons, with the exception of Summer when the two sexes were numerically balanced. The first ovigerous females appeared in May and became the majority in July (63.8%). The size frequency distribution (LC) highlighted 5 different cohorts, which would correspond to ages 1 to 5, with respectively 4.4 cm, 5.5 cm, 6.8 cm, 7.8 cm, 8.3 cm of carapace length (CL). The CL / total body weight (TP) relationship resulted for both sexes: W = 4.82 CL -163.7 (r2 = 0.493). Females reach their first sexual maturity at 7.05 cm.

Key-words: *Callinectes sapidus*, population structure, reproduction, Mellah lagoon, Algeria.

Introduction

The American blue crab *Callinectes sapidus* is native to the North Atlantic and distributed from Nova Scotia, Maine and Massachusetts to Argentina, Bermuda and the West Indies (Tavares, 2002). It was reported along the European Atlantic coast in the year 1900 in France (Bouvier, 1901). In the Mediterranean, *C. sapidus* is an exotic species reported for the first time in 1949 (Mizza, 1993). High fecundity, great swimming ability and pronounced aggressiveness have favoured its establishment (Millikin and Williams, 1984). In Algeria, *C. sapidus* was reported for the first time in the mouth of Oued Zhor (Skikda) in 2019 (Benabdi *et al.*, 2019). Hamida and Kara (2021) and confirmed and documented its presence the same year in Mafragh Estuary (El Tarf). The species developed a highly invasive population in Mellah lagoon since 2021 (Kara et Chaoui, 2021). This work provides the first information on the sexual cycle and dynamics of *C. sapidus* in Mellah lagoon.

Materials and methods

This study was carried out in Mellah lagoon, which is characterized by a surface area of 865 ha, with an average depth of 3.5 m. Temperature and salinity in this lagoon vary annually between 10–30.2°C and 25– 34.8‰, respectively (Draredja and Kara, 2004). Crabs are fished with fyke nets (L = 8 m) placed at a depth of about 2.5 m where the temperature. Here we examined 525 individuals, with a width between 77 mm and 178 mm, sampled between February 2021 and February 2022. The measurements made it possible to study the overall allometric relationships linking the different metric and mass characters (total carapace length CL, total wet weight W).

Results

Of the 525 individuals examined, 312 were females and 213 were males. The specimens analysed have an average CL of 70.01 mm and an average W of 173.80 g. Carapace length (CL) distribution frequencies indicated 5 different cohorts with the following mean lengths: 4.4 cm, 5.5 cm, 6.8 cm, 7.8 cm, 8.3 cm of CL). The mean annual *sex ratio* is in favour of females (SR = 0.68, chi-square = 18.66). The latter numerically dominated during all seasons, except in Summer when the population is numerically balanced (SR_{winter} = 0.21, $\chi^2 w$ = 38.22; SR_{spring} = 0.44, $\chi^2 s$ = 9.61; SR_{autumn} = 0.43, $\chi^2 a$ = 15.36; SR_{summer} = 1.18, $\chi^2 s$ = 1.93). The first ovigerous females appeared in May and became the majority in July (63.8%). The relative growth of length in relation to weight shows that the value of the allometry coefficient b (7.22) differed significantly from 3 at the 5% threshold. The relation established is allometric majorant.

Conclusion

This study provided the first information on the biology of *C. sapidus* in North Africa, providing original data on the status of the population studied. Future studies can contribute investigating impact on the environment and its interactions with resident species.

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DECORATING BEHAVIOR OF THE INVASIVE CRAB *LIBINIA DUBIA* H. MILNE EDWARDS, 1834 IN TUNISIAN WATERS

Abstract
Invasive species display the ability to thrive and establish populations while adapting to a host of novel pressures in new environments. In this context, spider crabs often decorate themselves to hide and protect against predators. This behavior is considered a kind of camouflage and operates either by avoiding detection (pre-detection defense) or by reducing recognition or consumption when the animal is already detected (post-detection defense). In this study, we evaluated the decoration behavior of 4469 specimens Libinia dubia *collected from the Gulf of Gabès from January 2015 to December 2016. The sample comprises 2189 juveniles (1658 males and 531 females), and 2280 adults (616 males and 1664 females). In both sexes, juvenile crabs engaged more in decoration, and attached various materials to hooked setae on their carapaces such as algae, Magnoliophyta, sponges, Bryozoa and other organisms. They showed a marked self-decorating behavior with higher level of carapace coverage compared to adults. Moreover, we recorded a variation in the body covering pattern between the two sexes. Decorative materials were more commonly distributed on male carapaces. In general,* L. dubia *juvenile use fragments of algae and Magnoliophyta to decorate their carapaces rather than other organisms. Decorating behavior was proportional to the carapace width increase, rendering crabs less vulnerable to predators, thus reducing their need for decoration. Behavioral changes exhibited by* L. dubia *resulted as "sexually dimorphic ontogenetic shifts", that is, decorative behavior shifts depending on the crab's age and gender.*

Key-words: Brachyura, Tunisian marine waters, NIS, Camouflage, Geographic comparison.

Introduction
The Western Atlantic native spider crab *L. dubia* H. Milne Edwards, 1834 has only been observed in southern Tunisian coasts since its occurrence in the Mediterranean Sea in 1997 (Enzenross & Enzenross, 2000). Here, we report the decorating behavior of *L. dubia* in order to investigate possible difference modalities and geographic variation in masking behavior between the native and invasive populations.

Material and methods
Sampling was conducted monthly over two years (2015-2016) where 4469 crabs were caught from the Gulf of Gabès (Tunisia), sexed and classified into juveniles or adults. For each specimen, the Carapace Width (CW, 0.01 mm) was measured and the percentage of coverage was assessed.

Results
Overall, *L. dubia* presented a clear preference for algae and seagrass as decorative materials (Fig. 1). Juveniles engaged more in decoration compared to adults. Moreover, camouflage materials were more abundant in male carapaces with higher mean %

carapace cover (Fig. 2). Concealment was proportional to carapace width increase. T test revealed highly significant results for all groups ($t_{cal} > t_{th}$, $p < 0.05$) except for combined males and combined females.

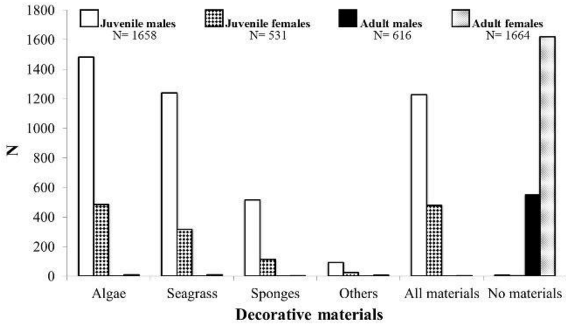

Fig.1: Distribution of decorative materials according to age/sex

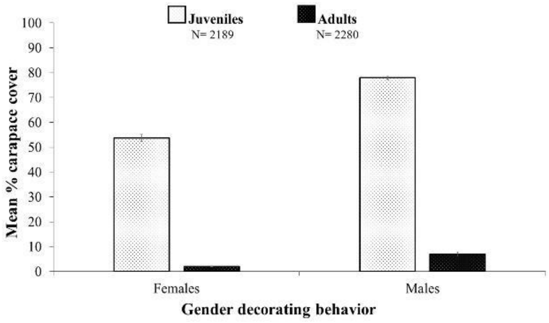

Fig.2: Mean % carapace cover ± SE *versus* gender

Discussion and conclusions

In spider crabs, size constrains the evolution of camouflage, that is, decoration behavior decreases with ontogeny (Hultgren & Stachowicz, 2009). Past studies on *L. dubia* decorating behavior from the Atlantic coasts, reported that smaller crabs usually decorate more than larger ones and regardless of gender. They exhibited strong camouflage preferences for plant materials in particular algae with chemical defense against omnivorous consumers and other predators (Stachowicz & Hay, 2000; Wortham, 2012). Likewise, *L. dubia* from the Gulf of Gabès used all available materials to conceal carapace with predilection for algae and seagrass. In both sexes, juveniles showed higher camouflage intensity. Nevertheless, males of the invasive population increased their percent carapace cover, which probably could be an ecological strategy of adaptation in the new recipient environment.

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TU-NIS-RISK: ASSESSING THE RISK OF INVASION FOR NON-INDIGENOUS AND CRYPTOGENIC SPECIES IN THE MARINAS OF TUNISIA

Abstract
The introduction and spread of non-indigenous marine species (NIS) have significant threats to global biodiversity, natural resources, and human health and constitute a priority for the protection and management of coastal areas. Harbours and marinas have been identified as NIS hotspots within the coastal infrastructure. NIS can acclimatize and increase the chances of spreading to natural areas and generate ecological and socio-economic impacts in these areas. Therefore, the introduced NIS inside the marina that managed to establish should be prioritized when categorizing "who comes first" when allocating resources for management. In this regard, decision-support tools for identifying potentially invasive NIS can be helpful. The Aquatic Species Invasiveness Screening Kit (AS-ISK) is a tool developed for this purpose and can assess the invasiveness potential of NIS within a specific area. This tool follows the EU Regulation No 1143/2014 on invasive alien species of EU concern and is mainly designed to work with marine autotrophs, invertebrates and fish. In this study, the AS-ISK is carried out for those NIS and cryptogenic species identified during a rapid assessment survey carried out in 6 marinas of Tunisia in August 2021. The assessment results highlight the taxa with a higher risk of invasiveness to the Tunisian natural coasts and protected areas. Therefore, these results will help inform managerial decisions and resource allocation for the early eradication of those NIS and cryptogenic species with a higher risk of causing environmental and economic problems.

Key-words: Marinas, AS-ISK, Non-indigenous Species, Cryptogenic Species, Invasiveness

Introduction
The introduction and spread of non-indigenous marine species (NIS) have significant threats to global biodiversity, natural resources, and human health and constitute a priority for the protection and management of coastal areas (Molnar *et al.*, 2008). Therefore, it is imperative to monitor these NIS and assess their risk of invasion to nearby areas to improve management and conservation strategies in coastal habitats (Copp *et al*., 2016).

Materials and methods
A rapid assessment survey (RAS) of 2 hours was conducted in six marinas of Tunisia (Fig. 1). For the taxa identified during RAS, a risk assessment was conducted using AS-ISK, answering up to 55 questions and obtaining scores for Basic Risk Assessment (BRA) and BRA plus Climate Change Assessment (BRA+CCA - Copp *et al*., 2016).

Results
Twenty taxa were identified and assessed, with arthropods, bryozoans and tunicates being

the most diverse groups. A total of 258 bibliographical sources were used to answer the AS-ISK questions. The taxa with higher risk of invasion included mobile predators (decapods) and filter-feeder invertebrates, including ecosystem engineers (Tab. 1).

Discussion and conclusions

The present study constitutes the first AS-ISK study for coastal non-indigenous marine species (NIS) in North Africa. These results will help inform managerial decisions and resource allocation for the early eradication of those NIS and cryptogenic species with a higher risk of causing environmental and economic problems (SPA/RAC - ONU Environment/PAM, 2017).

Fig. 1: Sampled marinas in Tunisia.

Tab. 1: Top-ten high-risk species according to AS-ISK for the Basic Risk Assessment (BRA) and BRA plus climate change assessment (BRA+CCA)

Taxa	BRA	BRA+CCA	Taxa	BRA	BRA+CCA
Portunus segnis	42	50	*Bugula neritina*	38	48
Callinectes sapidus	40.5	48.5	*Balanus trigonus*	37	45
Ficopomatus enigmaticus	40	48	*Tricellaria inopinata*	36	46
Percnon gibbesi	39.5	45	*Amathia verticillata*	34	44
Botrylloides spp.	39	47	*Pinctada imbricata*	33	41

Aknowledgements

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THE USE OF SOCIAL MEDIA AS A CITIZEN SCIENCE TOOL FOR NON-INDIGENOUS MARINE SPECIES MONITORING IN TUNISIA

Abstract
Citizen science approaches are emerging as an efficient way to improve data collection and help monitor the invasion stages of non-indigenous species (NIS). In the Mediterranean Sea, UNEP/MAP strategies and all the regional documents and frameworks for conserving nature recommend Citizen Science to boost the capacity to monitor NIS distribution under the goals of better management and large-scale biodiversity conservation. During the COVID pandemic in 2020, a Facebook group "TunSea" was created to bridge the gap between Tunisia's scientific community and civil society. This group became popular in a short timeframe, congregating policymakers, scientists and stakeholders, such as fishers, aquaculture workers and sea users. Currently, the group has more than 35,000 members, who generated nearly 7 million interactions in 2021/2022 (posts, comments, likes, and shares). The group was used to share knowledge about Tunisian marine species, stimulate debates about marine conservation, share interests and contribute stories. Non-indigenous species were a common topic in the group and NIS pictures were frequently shared by the TunSea members asking for taxonomical identification or complaining about their direct impact. To share knowledge about NIS and encourage the community engagement, a total of 20 pictures of NIS were posted in TunSea during July 2021, asking the members to investigate these species and provide its name in the Tunisian language. The results were positive, as the discussion on NIS species reached more than 50,000 people and generated 601 comments, providing useful information on these NIS local names, distribution and impacts in Tunisia, and the valorization possibilities for some NIS. Overall, his data contributes to the knowledge on NIS distribution and impacts in Tunisia.

Key-words: Outreach, Community Engagement, Invasive species, Tunisia.

Introduction

In the past two decades, citizen science has become a global interest tool to collect data over vast areas and long periods of time. It has contributed to raise knowledge on ecological conservation and monitoring, such as non-indigenous marine species (NIS) (Encarnação *et al.,* 2021). Therefore, the use of citizen science as a tool to engage citizens in gathering data on NIS distribution and their impact can represent an effective early warning system for possible biological invasions.

Materials and methods

In 2020, a group called "TunSea" was created in the social media platform Facebook to build a network of scientists, policymakers, fishermen, business managers, and any citizens with a direct relationship with the sea. These parties can share and exchange useful data for management, such as information on marine pollution, and the distribution of marine species in Tunisia (e.g., cetaceans, turtles, and NIS). During July 2021, a methodology based on open questions was used to collect information on 20 pre-selected NIS from the group members. These included Tunisian common names for

NIS species and information on their impacts and distribution. Two posts were published daily for each species (40 post in total): the morning post was a photograph of the NIS and a question: “How do you call this species?”, the afternoon post answered the question with the NIS scientific and common names, and information of interest on NIS.

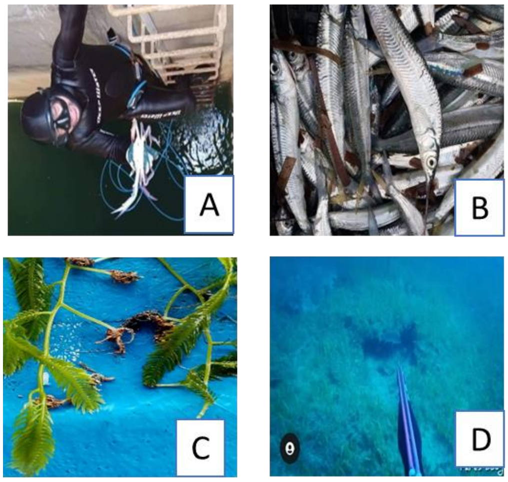

Fig. 1: Pictures of non-indigenous species in Tunisia uploaded in the comments of TunSea posts, A: *Portunus segnis*; B: *Hemiramphus far*; C and D: *Caulerpa taxifolia*

Results

A total of 50,010 people were reached by the 40 posts published in TunSea and 601 comments were posted by the members of the group. Some comments included pictures of NIS from different areas of Tunisia (Fig. 1). The discussion generated information on the habitat, distribution, and impacts of the species. It also revealed the common Tunisian name of 13 species.

Discussion and conclusions

People interact with NIS very frequently, as these are present in almost all earth ecosystems (Vitousek *et al.*, 1997). In fact, Tunisians have set a local name for a considerable number of NIS, which reflects their need to refer to them as a sizeable part of their lives. Apart for their outreach and sensibilization capacity, social media platforms are a valuable tool to collect data on NIS invasive status and calculate NIS impact on the social economic sector (Encarnação *et al.*, 2021).

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CONTINUED EXPANSION OF THE LESSEPSIAN INVADER MYTILID *BRACHIDONTES PHARAONIS* ALONG THE TUNISIAN COAST

Abstract
The Red Sea mussel, Brachidontes pharaonis is an invasive species in the Mediterranean. This species was observed for the first time in Tunisia (Zarzis, southern Tunisia) in August 2007. A monitoring covering the entire shallow coastline of Tunisia was carried out to collect quantitative and qualitative data on the mytilid between 2019 and 2022 by the authors and reinforced by recorded through citizen science Since its first record, B. pharaonis has rapidly extended its geographical range to the lagoon of Bougherara, then to almost Tunisian coast reaching more than 700 km from its initial area of first detection. B. pharaonis is present in the Bizerte lagoon (northern Tunisia), most likely facilitated by a combination of commercial and recreational boat traffic. Brachidontes pharaonis was found and colonized both natural habitats and artificial hard structures such as, rocky shores, aquaculture cages, harbours docks, with a high level of frequency and abundance, especially in lagoons (e.g., the lagoon of Boughrara) where extensive beds of B. pharaonic were found with individual abundances exceeding 10 000 specimens per square meter. The continuing spreading of B. pharaonis indicates the species is one of the most aggressive invaders and should be added to the list of invasive non-indigenous species to be monitored by the National monitoring programme for biodiversity in Tunisia.

Key-words: *B. pharaonis, invader, citizen science, Tunisia.*

Introduction
Brachidontes pharaonis from Indian Ocean origin is widely spread throughout the Red Sea. This bivalve was among the first non-indigenous species (NIS) introduced and noticed in the Mediterranean Sea. The occurrence of *B. pharaonis* in Tunisia was first signed in August 2007 (Ounifi *et al*., 2016). This work aimed to study the new distribution of *B. pharaonis* along the Tunisian coast and to assess its invasion rate by conducting targeted surveys and supporting records through citizen science to collect quantitative and qualitative data on this NIS.

Material and methods
The authors conducted the targeted surveys in the following localities: Tunis Northern lagoon la Goulette, Gammarth (Tunis), Fratelli Islands, Bizerte lagoon, Cani, Plane, Pilou Islands, Rafraf, Sidi Ali Makki (Bizerte), Bir Jday, Haouaria, Hammam Ghzez, Sidi Mahrsi (Nabeul), Chott Mariem, Marina Cap Monastir, Monastir touristic area, Kuriat Island, Hmem Island, Teboulba (Monastir), Sidi Frej –Kerkennah (Sfax), Djerba Island (all the coast of the Djerba Island), Boughrara port (Mednine). Along these locations, The intertidal zone and the rocky beaches were targeted and surveyed by snorkeling or by visual assessing the coast. Densities were classified as follow (low, medium, high, or very high. Additionally, a campaign was launched via the social media Facebook using the citizen science group TunSea and some naturalists were contacted to track the presence of *B. pharaonis* in other locations. Moreover, Malloula (Tabarka), Sidi Mechreg, Ghar El Melh lagoon, the shellfish farming in the Bizerte lagoon (Bizerte), aquaculture facilities in Bni Khiar, Kelibia, Kerkouene (Nabeul), aquaculture facilities in Teboulba (Monastir), Sidi Mansour (Sfax) and Ghannouche (Gabes)

were monitored by citizens and sea users. Reports of the presence of mytilid were subsequently verified using photos and videos sent to the authors.

Results

Our studies show that *B. pharaonis* is currently present in at least 7 locations in Tunisia as follows: Gammarth beach (in 2020 with low density), Bizerte lagoon (in 2021 with Low density), Tunis Northern lagoon: Berges du lac and Kheireddine canal (in 2022 with high density), Boughrara port (in 2022 with very high density), Djerba island: from Ajim in the southwest to the Roman road in the southeast (in 2022 with very high density). In addition, via the citizen science group TunSea two reports on the presence of *B. pharaonis* on the lines of structures of the aquaculture facility in Bni Khiar (in 2021 in low density) and Ghannouche beach (in 2022 in low density) (Fig 1).

Fig.1: *B. pharaonis* on the lines of structures of the aquaculture facility

Discussion and conclusions

This study based essentially on citizen science highlights that *B. pharaonis* continues its expansion (up to the Bizerte lagoon, the northernmost point at the moment). Regarding the various locations of occurrences, of ecosystems such as lagoons, ports and the distance between surveyed localities, this suggests that the introduction could probably occurs through these vectors: aquaculture (Bni Khiar), ballast water and shipping transport (Bizerte lagoon), fouling (boats, beings live), floating debris (Ghannouche beach stranding). The highest density was observed in Djerba (Ajim) covering all rocks of the intertidal zone and forming large beds. Density exceeding 10,000 individuals per square meter, twice as high compared to observations of Hamza *et al.* in 2019. In Tunis Southern Lagoon, *B. pharaonis* density was increasingly high going towards the lake, suggesting that this mollusc prefers sheltered areas. These observations agree with those of Safriel et al. (1980). *B. pharaonis* may aggressively overrun additional Tunisian areas in the future, threatening indigenous bivalve species such as the native Mytilaster minimus and leading to economic losses if it reaches the shellfish facilities of Bizerte lagoon that would be unable to compete with *B. pharaonis* in terms of density. This species should be upgraded to invasive non-indigenous species, be a priority for monitoring, and be included in Tunisia's national biodiversity monitoring program.

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COMPARISON AMONG METHODS FOR THE ASSESSMENT OF NON-INDIGENOUS SPECIES IN FOULING COMMUNITIES

Abstract
Ports are considered hotspot areas for the introduction of non-indigenous species (NIS) and their continuous monitoring is essential to promptly detect new arrivals. Unfortunately, there is still a lack of coordination among countries for the application of a common protocol to assess NIS presence. In 2018, a standardized method developed by the Smithsonian Institute (SERC, USA) was applied for the first time in the Mediterranean Sea (Gulf of La Spezia, Italy) and some efficiency in detecting NIS was observed. Currently, no specific studies have been conducted to select the "best" method for monitoring NIS in fouling communities. In this context, three different methods were tested in summer 2021, in a marina of the Ligurian region. Fouling communities were collected: i) on horizontal PVC panels (SERC protocol); ii) vertical panels; iii) by scraping a quadrat on vertical substrata in snorkelling. A total of 58 sessile taxa was identified, including 9 NIS. A one-way PERMANOVA test showed that samples collected by the different methods were significantly different, confirming some variability in species detection. In particular, 40 species were identified using the SERC protocol (including 9 NIS), 34 on vertical panels (7 NIS) and 29 in snorkelling (5 NIS).
Similar to previous studies carried out in the USA, the SERC protocol seems to be the most effective method to detect NIS, as well as the entire fouling community, but it has to be highlighted that snorkelling allowed to collect additional taxa not observed on the panels, and to obtain a more detailed description of the community. Additional tests in other localities would be recommended to confirm these preliminary results and promote the use of the SERC method in port monitoring.

Key-words: Alien species, sampling methods, Mediterranean Sea, fouling communities, ports

Introduction

The Mediterranean Sea is considered the most invaded sea in the world, with high establishment rates of non-indigenous species (NIS; Galil *et al.*, 2018) and high propagule pressure, partially due to intense commercial shipping and recreational boating activities (Seebens *et al.*, 2013; Ulman *et al.*, 2019). Thus, the monitoring of high-risk areas for NIS introduction is highly recommended for the detection of these species, but a common approach among European countries has not been agreed yet. This study aims to compare different methods suggested for baseline port monitoring, in order to identify their advantages and limitations and suggest the most effective one for NIS detection. In particular, we focussed on sessile macrofauna.

Materials and methods

This study was carried out in summer 2021 in the "Marina del Fezzano", a marina located in the Gulf of La Spezia (Italy, Western Mediterranean Sea) and hosting 250 berths for boats up to 25 m. Three different methods were compared: i) horizontal PVC fouling panels (as in the protocol developed by SERC – Smithsonian Environmental

Research Center; Marraffini *et al*., 2017); ii) vertical fouling panels of the same structure; iii) underwater scrapes of quadrat frames from vertical substrata. All methods involved a standard surface of 14x14 cm. Both horizontal and vertical panels were deployed at the end of April and retrieved in July, concurrently with the scrapes. The biogeographical status of the species identified in the lab was carefully analysed to distinguish NIS from native species (including cryptogenic species). Multivariate statistics were based on presence/absence data.

Results

A total of 58 sessile taxa were identified, including 9 NIS. In particular, 40 species were identified from the horizontal panels (including all 9 NIS), 34 from vertical panels (7 NIS) and 29 from scrape samples (5 NIS). A significant difference among sampling methods was observed through a one-way PERMANOVA test (Pseudo-F= 5.0259; P(perm)= 0.0001; Unique perms= 9919), and the pairwise test confirmed a significant difference between pairs of methods (P(perm)<0.05).

Discussion and Conclusions

The comparison of three selected sampling methods to collect fouling communities revealed a better performance of the SERC method in detecting NIS, as well as the native sessile species. This result reflects the observations of a previous study carried out in USA, even if in our experiment it was not possible to collect samples under the floating pontoons (i.e., scraping horizontal surfaces), for the impossibility of obtaining authorizations (Marraffini *et al*., 2017). The downward horizontal position of the PVC panels strongly limits the growth of algae, favouring the settlement of fouling macrofauna. Yet, scrape samples collected in snorkelling had additional taxa not observed on the panels. Other similar tests would be recommended in other localities to confirm the preliminary results of this study.

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HERE TODAY, GONE TOMORROW – THE LEVANTINE POPULATION OF THE BROWN MUSSEL *PERNA PERNA* DECIMATED BY AN UNPRECEDENTED HEATWAVE

Abstract
The newly established population of Perna perna (Bivalvia, Mytilidae) surveyed at four sites along the Israeli Mediterranean coast averaged between 2155 and 8022 specimens/m² in June 2021. The population suffered catastrophic mortality following a succession of heatwaves culminating in a prolonged heatwave in August (ambient temperature 36 to 38 °C, sea surface 32 °C). Yet, as an opportunistic species, ecosystem engineer, and fouling threat with rapid growth rate, the profuse presence of P. perna raised the possibility of ramifications for intertidal community structure.

Key-words: Invasion, population size-frequency, marine heat wave, mortality

Introduction

The brown mussel *Perna perna* (Linnaeus, 1758), a widely invasive mytilid mussel, was first reported from the Mediterranean coast of Israel in 1965. No living specimens of *P. perna* were documented in Israel in the next 55 years. The first documented evidence of the reappearance of *P. perna* along the Israeli coast consisted of photographs of small densely packed mussel patches north of Haifa port taken in May 2020. Subsequent surveys conducted along the Israeli shore in summer 2020 revealed patchy but dense aggregations at several locations. An opportunistic species, ecosystem engineer, and fouling threat with rapid growth rate, the profuse presence of *P. perna* on the Mediterranean Israeli coast raised the possibility of ramifications for intertidal community structure.

The study set out to document and compare the status of *P. perna* population at four sites, comprising basic data on population density, size structure, growth rate, and epibiota within one year of their initial detection. The study plan was upended by an unprecedented heatwave that annihilated the entire population at the sampled sites and throughout the Israeli coastline**.**

Materials and methods

Samples for population size structure analyses were collected at four sites, spanning approximately 140 km of coastline. Samples from Haifa A (32°49′59.4″N; 35°03′04.2″E) and Ashdod B (31°44′49.5″N; 34°36′03.6″E) were collected from semi-isolated small rocky outcrops separated by shallow troughs from public bathing beaches; from Ashdod A, a stretch of beach rock situated north of Ashdod Port (31°51′16.5″N; 34°39′39.3″E) and from Haifa B, concrete barrier blocks protecting the seaward fence of the Petroleum and Energy Infrastructures (PEI) facility, adjacent to Haifa port (32°49′35.1″N; 35°02′42.8″E). Mussels were scraped from within three randomly placed replicate 30 × 30 cm quadrates in June 2021.

An extreme heatwave in August 2021 eradicated the *Perna* population at the sampling sites - empty shells washed ashore at all sites, with the exception of the Haifa B site, where dead shells still attached by their byssus to the concrete surface were collected *in-situ*. Specimens are deposited at Steinhardt Museum of Natural History, Tel Aviv University (SMNH – MO 100507–100511).

Results

A total of 5691 live *P. perna* were collected. The number of individuals and population size structure reveal considerable differences among the four sites, abundance ranging from 2155 to 8022 per m². Average shell size was greater for individuals collected in Haifa sites than in the southern sites. Alone among the sample sites, Haifa B samples were clearly bi-modal, the largest size cohorts measuring 22.6–25.0, 57.1–60.0 mm and 27.6–30.0, 67.6–70.0 mm, respectively. Mussels of > 50 mm shell length were extremely rare in Ashdod A, B and Haifa A sites (7 of 5303 specimens), but were well represented at Haifa B samples, where a third of the mussels attained this size. A total of 147 dead shells were collected off the concrete slabs at Haifa B following the August 2021 heatwave. The sample size–frequency distribution was unimodal with the largest size cohort (≥ 10%) 77.6–80.0 mm, and largest shell length 101.8 mm. Shell sizes differed greatly between June and August: in June 66% of the shells were < 50 mm (average size 38 mm, ±19.7 SD, n = 194), while in August only 3% were < 50 (average size 75 mm, ± 12.5 SD, n = 174).

Discussion

The decimation of the Israeli population within 2.5 months of its first and only live-sampling left us with fragmentary but tantalizing data. Yet, the remaining *in-situ* shells were significantly larger than those collected in June – the largest specimens being 101.8 in shell length compared to 82.7 mm shell length in the earlier sample. The postulated growth rate is high but aligns with figures from other warm water locales. Marine heatwaves (MHWs) in the Mediterranean Sea were linked to extirpations of hard substrate macroinvertebrates. Mortalities of marine intertidal mussels have led to studies on thermal Exposure, though few documented heatwave-induced mortalities in introduced mussels. The sole documented case of heat-induced mortality in introduced *P. perna* concerns the population in Texas, USA, which collapsed when mean summer seawater temperature rose in 1997, but reappeared in 2000 when the average summer surface-water temperature was 28.5 °C. Over the past two decades, concurrent with long-term persistent warming, the mean MHW frequency and duration in the Eastern Mediterranean increased by 40% and 15% (Garrabou *et al*., 2022), respectively, and is projected to increase further, favoring thermally-tolerant species and those adapted to warmer waters. It is likely that native stenothermal biota unable to shift their range to deeper and/or colder water may endure increasing stress and demographic attrition, and replacement by warm water invasives, likely introduced through the Suez Canal.

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INADEQUATE: ISRAELI MONITORING SURVEYS' FAILURE TO ASSESS ALIEN BIOTA

Abstract
The shelf ecosystem along the Mediterranean coast of Israel is impacted by mainly Erythraean introductions through the Suez Canal, in addition to regional and local stressors, - causing significant changes in the biodiversity locally and across the Mediterranean Sea. The present study evaluates the current National Monitoring Program of Israel's Mediterranean waters.

Key-words: Alien species, Survey, Management

Introduction

"About 1000 marine alien species have been reported in the Mediterranean Sea" (SPA/RAC 2017), more than 400 of them reported from the Israeli coast (Galil et al. 2021). The flood of the alien species is an ongoing process that heavily affects biodiversity throughout the Mediterranean as well as the economy and public health. An analysis of a comparative study of two habitats along the Israeli coast revealed the inadequacy of the current National Monitoring Program, highlighting an urgent need for a comprehensive sampling program that would faithfully assess actual status of alien populations.

Materials and methods

Diurnal and nocturnal surveys, using an otter trawl at about 30m depth, were conducted in June and November-December, 2012, at sites (Haifa, Nitzanim) about 160 km apart, 18 tows at each site in total. Fish were identified to species (with few exceptions), counted and weighed. Analysis of Data carried out using Primer 7 statistical package.

Results

A total of 142,805 fish were identified to 105 species, of which 93,708 fish (66%) were identified to 36 non-indigenous species (NIS) (34%). At the northern site (Haifa), NIS fish abundance comprised 23% of the haul in June and 77% in November-December, whereas in the southern site (Nitzanim), NIS comprised 37% in June and 92% in November-December.
A similarity analysis of the sites and seasons revealed that the similarity between autumn and spring samples was ca. 50%, similarities between autumn and spring samples in the sites were ca. 65% in autumn and ca. 55% in spring.
A comparison of the number, proportion and abundance of NIS revealed considerable differences in spatial, seasonal and diurnal samples (Fig. 1, Nitzanim ver. Haifa; spring ver. autumn; day ver. night).
The differences were also expressed at the specific level. For example, 8,378 individuals of the native Axillary seabream *Pagellus acarne* were collected in daytime

in Haifa, none at night, whereas in Nitzanim only 76 individuals were collected in daytime, solely one at night. Not a single individual of the invasive striped eel catfish *Plotosus lineatus* was collected in Haifa in daytime, though 2,360 individuals were collected at night, whereas in Nitzanim 7 individuals were collected in daytime and 24,943 at night.

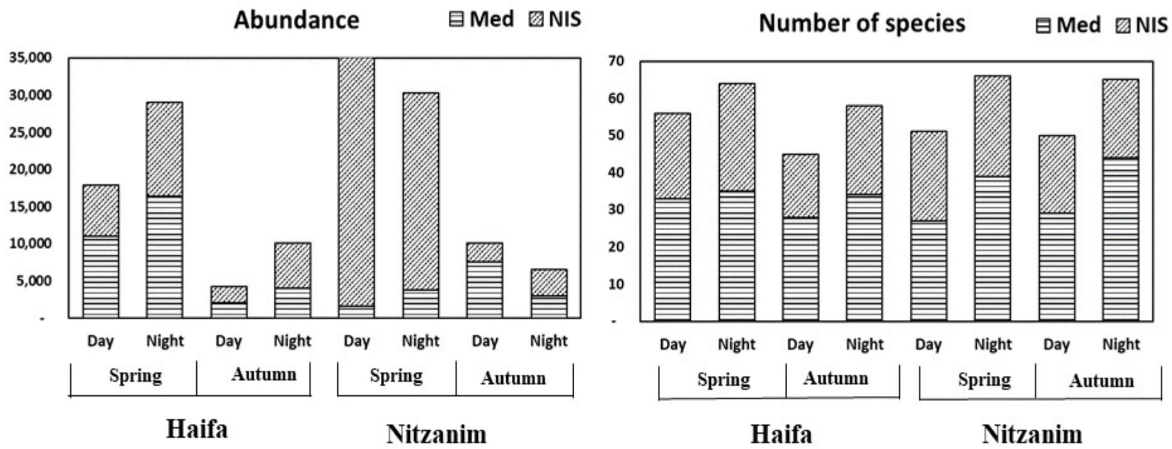

Fig. 1: Spatial, temporal and diurnal comparison of the abundance and number of species

Discussion

Surveys and monitoring are essential precursors for governance, policy, and management actions. The current monitoring methodology provides only partial information that fails to adequately represent the critical spatial and temporal variability of upper shelf NIS populations. The resulting data is insufficient, likely misleading, and useless as base for sustainable management actions. The southeastern Levantine Basin is recognized as an important way station and breeding ground for Erythraean NIS, and NIS establishing large populations are likely to spread and set up successfully in the Central and even Western Mediterranean Sea (Galil *et al*. 2021). A comprehensive NIS monitoring action plan within the National Monitoring Program of Israel’s Mediterranean waters would provide timely and valuable forewarning.

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NON-INDIGENOUS SPECIES (NIS) SURVEYS AND COMMUNITY ASSESSMENT IN RAVENNA HARBOR (ADRIATIC SEA) BASED ON SHORT-TERM DEPLOYMENTS OF AUTONOMOUS REEF MONITORING STRUCTURES (ARMS)

Abstract
To evaluate and monitor the presence of non-indigenous species (NIS) in harbors and marinas, Autonomous Reef Monitoring Structures (ARMS) were deployed in two sites, a commercial harbor and a touristic marina in the port of Ravenna (Italy, northern Adriatic Sea), in two seasons and left about 2 m underwater for three months. Significant differences in sessile and vagile community structures were found between sites and times. Out of 44 taxa identified, two sessile and five vagile NIS were found. Among them, the polychaete Schistomeringos cf. japonica (Annenkova, 1937) could represent the first Mediterranean record if confirmed by further analyses.

Key-words: ARMS, Mediterranean Sea, marine invertebrates, invasive species, monitoring

Introduction
Non-indigenous species (NIS) are organisms that have been introduced outside of their natural past or present distribution range through direct or indirect anthropogenic vectors, such as commercial and touristic vessels. Because of the low habitat heterogeneity and complexity, harbors and marinas can facilitate NIS colonization. As a result, methods for monitoring benthic communities in these environments are required to early detect new NIS. Autonomous Reef Monitoring Structures (ARMS) are standardized passive collectors that are used to assess marine cryptobenthic communities (Leray & Knowlton, 2015). Here we use an integrative approach based on 3-month ARMS deployment to characterize the benthic communities (with a special focus on NIS identification) of two sites along the Ravenna harbor (Adriatic Sea) in two different seasons.

Materials and methods
Ravenna (North Adriatic Sea) is one of the largest commercial seaports in Italy, with a canal port stretching 11 kilometers from the city center to the touristic marina. ARMS were deployed in two sites, the Ravenna harbour (H) and the touristic marina (M), at two times (spring and summer 2021) and left underwater for three months (Fig. 1). The colonizing sessile communities were assessed using photoQuad image analyses and vagile fauna (> 2 mm) was identified morphologically using a stereomicroscope and light microscope, with a particular focus on NIS detection.

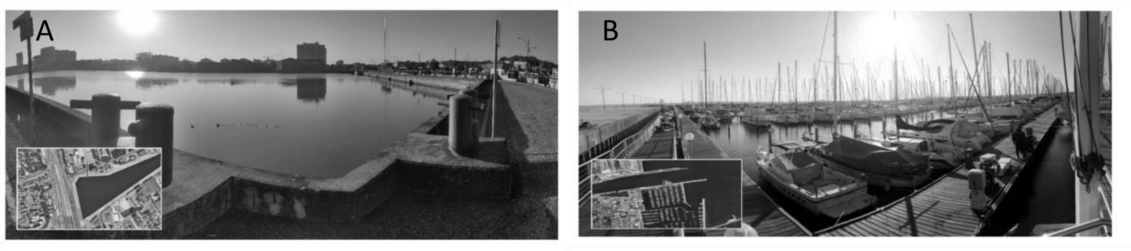

Fig.1: A) Ravenna Harbour (H) and B) touristic Marina (M) with associated ARMS deployment point (Google Maps)

Results

Overall, NIS accounted for 20.5 percent of all taxa. The Annelida Polychaeta *Ficopomatus enigmaticus* (Fauvel, 1923) had the highest percentage coverage, reaching an average of 25.2% (±0.7) and 22.8 (±0.3) in both sites in summer, and 19.4% (±0.8) and 7.4% (±1.1) in spring. Chordata Ascidiacea *Ciona robusta* Hoshino & Tokioka, 1967 was found only in M and covered an average of 11.7% (±0.8) in spring and 1% (±0.5) in summer. Among the 29 vagile taxa detected, five were NIS: isopods *Paranthura japonica* (Richardson, 1909) and *Paracerceis sculpta* (Holmes, 1904), amphipod *Caprella scaura* (Templeton, 1836), polychaetes *Podarkeopsis capensis* (Day, 1963) and *Schistomeringos cf. japonica* (Annenkova, 1937). The highest average number of NIS was recorded in M where, for both seasons, it was 2.6 (±0.6), followed by 2 (±0) in H, in spring. No NIS in H were observed in summer.

Discussion and conclusions

Differences in the NIS presence between sites and seasons are probably due to the different abiotic conditions, habitat complexity and types of human impacts. The Harbor site is in a confined area, with low water recirculation, high turbidity, and high pollutant accumulation. The Marina site is located at the very entrance of Ravenna canal port, separated from the sea only by breakwaters. All the NIS detected are already observed in different Mediterranean harbors and marinas except for *S. japonica*: it is native to Yellow Sea (Western Pacific Ocean) and it was recently reported for the first time outside its native range, in Pakistan (Khatoon *et al.*, 2020). In Ravenna harbor, 22 specimens of this species were found in spring. Because of its similarity to *S. rudolphi*, a species distributed in the Atlantic Ocean and the Mediterranean Sea, including the Adriatic, and frequently found in harbor environments, more morphological and molecular analyses are required to confirm the taxonomy of *S. japonica*. This work lays the basis for a long term spatio-temporal monitoring of community diversity and NIS presence in Ravenna's harbor using ARMS.

Acknowledgments

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ASSESSMENT OF THE IMPACTS OF THE SILVER-CHEEKED TOADFISH *LAGOCEPHALUS SCELERATUS* (GMELIN, 1789) ON NORTHERN TUNISIAN FISHERIES

Abstract
The silver-cheeked toadfish Lagocephalus sceleratus *is one of the recent Lessepsian invaders in the Mediterranean Sea, where it was recorded in various locations and has rapidly colonized the eastern basin. This toxic fish presents a serious risk for public health. In Tunisia,* L. sceleratus *was recorded for the first time in 2010 in the Southern coasts (Gulf of Gabes), then in 2012 in the Northern coasts. However, the impacts of* L. sceleratus *in Tunisian waters have not been assessed. Thus, surveys based on LEK were carried out at the main ports and landing points in the northern coasts of Tunisia. Results showed that, despite its low abundance, the silver-cheeked toad-fish negatively interacts with fishing gears (setnets and lines), causing damages on catches. We concluded that this Lessepsian immigrant has already started generating a reduction in the profitability of the fishing activity of small scale fisheries.*

Key-words: *Lagocephalus sceleratus,* impacts, Northern Tunisia, Central Mediterranean.

Introduction
Invasive Lessepsian species, can have serious impacts on marine life, fisheries, human well-being and human health in the Mediterranean Sea. Of these species, the silver-cheeked toad *Lagocephalus sceleratus* (Gmelin, 1789) is of particular concern. In Tunisian waters, *L. sceleratus* was recorded for the first time in the Gulf of Gabes (Central Mediterranean) in December 2010 (Jribi and Bradai, 2012). Then, additional occurrences of the species were recorded in the central and northern coasts (Ben Souissi et al 2014). Actions of awareness were taken in Tunisia, to alert consumers and fishers on the potential danger of this toxic species. However, the impacts of *L. sceleratus* in Tunisia have not been assessed. Thus, by this preliminary work, we tried to fill this gap and investigate the consequences of this invasion on the fishing activity of northern Tunisian coasts.

Material and methods
In order to collect information on the spread and impacts of the silver-cheeked toad-fish *Lagocephalus sceleratus* in Nothern Tunisia, field surveys based on the Local Ecological Knowledge (LEK) were conducted in the main ports and landing points of fishery products in the area (figure 1).

Results
Almost all the interviewed fishers were able to correctly identify *L. sceleratus*. They were also informed on the associated threats to human health, when consumed. A large percentage of them stated that this non-indigenous fish had negative interactions with fishing gears, representing a major problem for coastal biodiversity and for the profitability of their fishing activities. Indeed, according to interviews conducted with 45% of fishermen in northern Tunisia, *L. sceleratus* could cause nuisance because it

damages fishing gears by attacking fish, mainly cuttlefish *Sepia officialis* caught in nets, and lines, cutting lines and nets using its strong teeth.
The resulting economic impacts were related to a reduction in the profitability of fishing operations due to the deterioration in the quality of the catches and therefore the reduction in their commercial value. Another issue mentioned by the fishers was the costs of replacing damaged parts of the fishing gears.

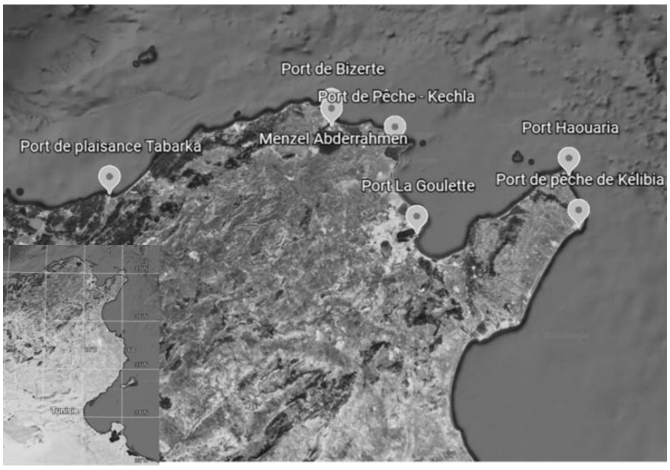

Fig.1: Locations of LEK surveys in Norther Tunisia

Conclusion and discussions

The silver cheeked toadfish occurred in Tunisian waters for about a decade. The species, despite being caught accidentally and in low abundance, seems to generate negative interactions with the fishing gear, mainly on nets and lines used by small scale fishers, generating reasonable concern and complaints. Similar results have been reported in eastern Mediterranean, where the occurrence of *L. sceleartus* has negatively affected fishing operations and incomes of small-scale fishers in Turkey (Ünal et al., 2015), but also in Egypt, Lebanon, Cyprus and Greece (Nader *et al.*, 2012).

Acknowledgement

We wish to express our sincere gratitude to all the fishermen for their support and for providing us with necessary information.

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FIRST RECORD OF THE NON-INDIGENOUS BROWN ALGA *STYPOPODIUM SCHIMPERI* IN THE ADRIATIC SEA

Abstract
The Indo-Pacific brown alga Stypopodium schimperi (Kützing) Verlaque & Boudouresque 1991, was introduced into the Mediterranean Sea through the Suez Canal. Initially mistaken for other species, it is uncertain which is the first record for the Mediterranean Sea, probably in 1973 in Israel as Spatoglossum asperum. Today, S. schimperi is widely distributed in the eastern part of the Mediterranean Sea, where it is considered an invasive species. In this study, we report the first record of Stypopodium schimperi in the Adriatic Sea, and the northernmost record for the Mediterranean Sea to date. Morphological analysis and DNA barcoding based on chloroplast regions rbcL, psbA, and psaA confirmed species identification. Our finding indicates exceptional spreading dynamics and high invasive character of S. schimperi in the environment of the central Adriatic Sea. With high annual increase in coverage, high biomass, total substrate overgrowth and large dimensions of the thalli, this species has the potential to become one of the most invasive benthic non-indigenous species in the Adriatic Sea but also in the western part of the Mediterranean Sea, once it arrives in this region.

Key-words: Adriatic Sea, *Stypopodium schimperi*, invasive species, marine macrophytes

Introduction

Stypopodium schimperi (Kützing) Verlaque & Boudouresque, 1991 is a brown alga of Indo-Pacific origin. It is a Lessepsian migrant species and was most likely detected initially in Israel in 1973 as *Spatoglossum asperum* (Verlaque *et al.*, 2015). To date, *S. schimperi* has been commonly recorded in the eastern and central Mediterranean, including the Aegean and Ionian Seas of Greece (Verlaque & Boudouresque, 1991; Verlaque *et al.*, 2015). The species has become abundant along the Levantine coasts and occurs from the surface to a depth of 45 m. Moreover, based on expert opinions and visual observations, it is considered an invasive species (Boudouresque & Verlaque, 2002; Bitar *et al.*, 2017). Here, we present the first record of *S. schimperi* in the Adriatic Sea (September 2020, Vis Island) and provide evidence of its high invasiveness.

Materials and methods

Ten transects were conducted in Komiža Bay (Vis Island, Adriatic Sea) via SCUBA diving to define the abundance and spatial and bathymetric distribution of *S. schimperi* from the time of its initial observation until May 2022. Specimens were collected for morphological and molecular analysis. To analyze the algal coverage, a total of 33 photoquadrat images of the rocky bottom (area of 50 x 50 cm) between 8 m and 12 m were collected (three photos taken every 10 m) along a 100 m long and 10 m deep transect. The average percent cover (25%, 50%, 75% and 100%) per species was estimated for each of the six sub-quadrats (digitally divided for each photograph) (Bianchi *et al*., 2004). Three plastid genes, psbA, psaA and rbcL, were used for DNA barcoding. The obtained sequences were compared with the *S. schimperi* sequences recovered from GenBank.

Results and discussion

The main morphological and anatomic characteristics of the specimens collected in this study were comparable to the existing data on *S. schimperi* (Verlaque & Boudouresque, 1991; Verlaque *et al.*, 2015). Additionally, the specimens obtained were large (several specimens up to 40 cm) and approximately twice the size of those recorded previously. The sequences of the Adriatic specimens showed high similarity (96.65% rbcL, 99.78% psbA and 99.7% psaA) to the *S. schimperi* sequences obtained from GenBank. *Stypopodium schimperi* was observed on a rocky bottom, primarily between 5 m and 15 m, whereas non-attached thalli were detected at 35 m. The number of thalli increased from a total of 20 in five transects in 2020 to several thousand in 2021. Regarding the reproductive organs, only sporophytes with tetrasporangia were recorded. Further sampling and examination are required to verify the development of a gametophyte. In more than 50% of the photoquadrats, *S. schimperi* covered >75% of the photoquadrat surface, with an average coverage of approximately 70% in the 100 m transect. Coverage of 100% results in the entire displacement of native erect algal species. Other algal species with significant coverage in the area were *Dictyota* spp., *Taonia* spp., *Padina pavonica* and *Halopteris scoparia*, whereas those with negligible coverage were *Caulerpa cylindracea*, *Acetabularia acetabulum*, *Laurencia* spp. and *Amphiroa rigida*. This study demonstrated a distinct shift from native infralittoral erect algae to the dominance of *S. schimperi*. Based on the recorded spreading dynamics, we believe further expansion of this species will occur. The population of *S. schimperi* examined herein represents the northernmost record for the Mediterranean Sea, and the results suggest a high potential for further dispersion within the Adriatic Sea. Additionally, due to the sea temperature similarity and its presence in the Central Mediterranean (Shakman *et al*. 2019), *S. schimperi* may also spread to the northwestern Mediterranean.

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THE STORY OF A SUCCESSFUL INVASION: *CAULERPA CYLINDRACEA* IN THE MARINE PROTECTED AREA OF PORTOFINO (LIGURIAN SEA)

Abstract
The green alga Caulerpa cylindracea, one of the worst invasive species in the Mediterranean Sea, was first recorded in the Ligurian Sea (NW Mediterranean) in 1996 and in the Portofino Marine Protected Area in 2008. Monitoring at Portofino between 2012 and 2017 showed that it was widespread between 1 m and 45 m depth. A new period of monitoring, 2018 to 2021, roughly confirmed previous information, but also evidenced that in the infralittoral zone C. cylindracea cover exhibited little intra- and interannual variability, while in the circalittoral zone it was more variable within and among years. A reduction in cover was observed at all depths in 2019. On the whole, our results indicate that C. cylindracea is now fully established on the rocky reefs of Portofino MPA.

Key-words: Alien alga; Monitoring; Algal reefs; Coralligenous reefs; NW Mediterranean Sea.

Introduction
The green alga *Caulerpa cylindracea* Sonder, 1845, of SW Australian origin, is one of the worst invasive species in the Mediterranean Sea: first recorded in 1990 off Libya, it reached the Ligurian Sea in 1996 (Montefalcone et al., 2015). Its presence remained mostly restricted to anthropized areas until 2008, when the species was observed also in the Marine Protected Area (MPA) of Portofino, especially on biodetritic bottoms (Morri et al., 2019). A yearly monitoring activity between 2012 and 2017 showed that it was widespread between 1 m and 45 m depth, with the highest substratum cover (up to 25%) at about 20 m (Morri et al., 2019). Here we report on the results of a second monitoring period, 2018 to 2021, in order to see whether *C. cylindracea* has somewhat receded after a first bloom or has remained similarly abundant as in the previous period.

Materials and methods
Cover data of *C. cylindracea* were estimated visually by scuba diving between 1 m and 45 m depth. Data were analyzed separately according to two different depth ranges: the infralittoral zone, 1 m to 20 m, and the circalittoral zone, 21 m to 45 m.

Results
The information obtained during the monitoring activity between 2018 and 2021 confirmed that *C. cylindracea* has remained roughly as abundant as in the previous period. However, different trends between infralittoral algal communities and circalittoral coralligenous communities were apparent. In the infralittoral zone (Fig.1a), *C.*

cylindracea exhibited little intra- and interannual variability: from 2008, the year of its first finding, its cover has increased from 4% to about 12% in 2012-17 and remained around this figure in the second period of monitoring, 2018 to 2021. In the circalittoral zone (Fig. 1b), its cover resulted more variable within and among years and tended to decrease over time: in 2008 its cover was 21%, to decrease to around 15% in 2012-17. Notwithstanding wide interannual variability, cover returned to 15% in 2021. A reduction in *C. cylindracea* cover was observed at all depths in 2019, after the severe storm that occurred in the autumn of 2018, which caused the removal of algal thalli.

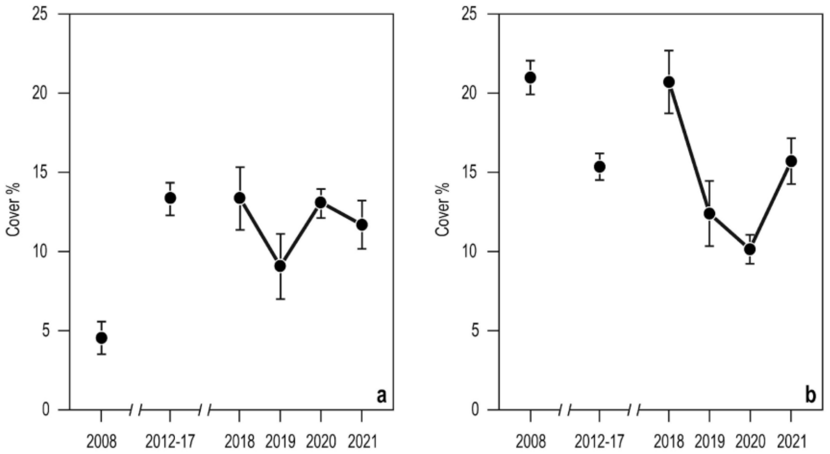

Fig. 1: Mean (± se) cover of *Caulerpa cylindracea* on the rocky reefs of the Portofino MPA, in three time periods: 2008, when it was first recorded in the area; 2012-17, first period of yearly monitoring (see Morri *et al.*, 2019, for detail); and 2018 to 2021, recent monitoring results. (a) Infralittoral zone (1 m to 20 m); (b) Circalittoral zone (21 m to 45 m)

Conclusions

Although primarily designed to manage fisheries, MPAs have been proven to be capable of improving the health status of benthic habitats (Bianchi et al., 2022). Nonetheless, our results show that the protection regime of Portofino MPA has been ineffective in keeping reef communities in such a state of health as to enable them to prevent biological invasions. In only 13 years, *C. cylindracea* has colonized the entire area and is now fully established on the rocky reefs of Portofino MPA. Long-term monitoring remains the only way to illustrate the naturalization trend of *C. cylindracea* and its consequences on the native communities of Portofino MPA.

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EXPLORING DISLODGEMENT RISK OF MOBILE EPIFAUNA DURING VESSEL TRANSPORT

Abstract
Vessel hull-fouling is responsible for most biological invasion events in the marine environment. We evaluated the performance of mobile epifauna during vessel transport via laboratory simulations, using the well-known invasive Japanese skeleton shrimp (Caprella mutica), and its native congener C. laeviuscula as case study. The invader did not possess any advantage in comparison to the native species in terms of inherent resistance to drag. Instead, its performance was conditioned by the complexity of secondary substrate. Dislodgement risk was significantly reduced as we progressively added sessile fouling basibionts; which provided refugia and boosted the probability of Caprella mutica remaining attached from 7% to 65% in laboratory flow exposure trials. The invader exhibited significantly higher exploratory tendency than its congener at zero-flow conditions. Implications in terms of en-route survivorship, invasion success and macrofouling management are discussed.

Key-words: basibionts, vector, hydrodynamics, non-indigenous species, boldness

Introduction
The accumulation of organisms attached to or associated with man-made underwater or wetted surfaces is responsible for approximately half of the currently established non-indigenous species (NIS) in coastal waters worldwide. Since experts agree that biosecurity needs to operate with a precautionary approach, the study of the early phases of the invasion process becomes of critical importance. The goal of this study was to evaluate the response of mobile invasive epifauna to a simulated vessel voyage, to gain insight into the factors facilitating or hampering this phase of the invasion process. We evaluated 1) the resistance of two species (one native and one invasive) to hydrodynamic stress; 2) whether the sessile component of the fouling (basibionts) enhances the chances of the invader overcoming the vessel trip; 3) the boldness behaviour of both species at zero-flow conditions.

Materials and methods
Fauna was collected from marina artificial structures in British Columbia, Canada; transported to the lab and acclimated. A total of 340 individuals were used for experiments (Fig 1).

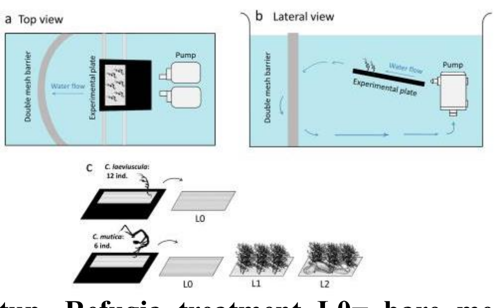
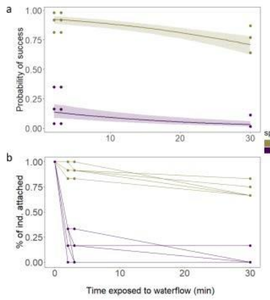


Fig. 1 abc: Aquarium setup. Refugia treatment L0= bare mesh; L1: mesh with the hydrozoan *Obelia dichotoma*; L2: mesh with *O. dichotoma* and the mussel *Mytilus trossolus*

Results and discussion

The invasive *C. mutica* does not bear an inherent advantage over its native congener in terms of resistance to hydrodynamic forces; instead, its chances of overcoming vessel transport highly depend on secondary substrate complexity, confirming that the basibiont component acts as a refugium for mobile invasive epifauna during vessel voyage.



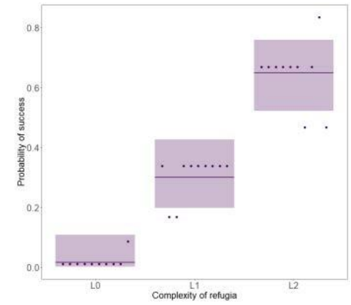


Fig. 2 a, b (left): Response to waterflow as a function of time. a) predicted probability of both species remaining attached to the mesh. b) observed individuals remaining attached. Fig. 3 (right): Predicted probability of *C. mutica* remaining attached to the mesh when offered different levels of basibiont complexity.

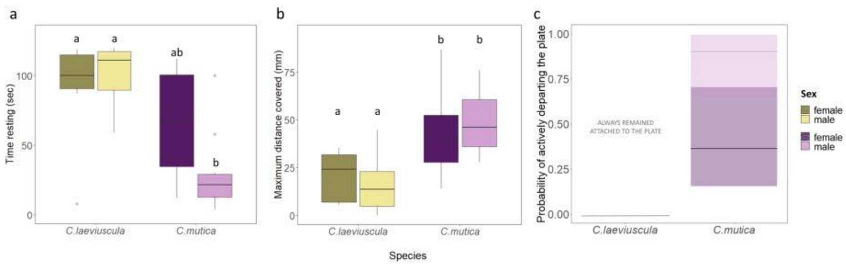


Fig. 4 a, b, c. Behaviour observations in no distress conditions. a) Time spent resting. b) Maximum distance covered when crawling. c) Predicted probability of actively departing the plate into the water column (ie swimming).

Besides, the invasive species also exhibited higher boldness than the native one despite being more vulnerable to hydrodynamic drag. This may confer an advantage at the post-arrival phases of the invasion process (colonization, establishment and spread). In the case of non-indigenous species with several invasiveness traits that may rely on fouling refugia to overcome translocation, the most effective management strategy is removal of biofouling prior to departure to a new destination. A rank 3 Level of Fouling (*sensu* Floerl *et al*., 2005) already incurs a biosecurity risk, as it most probably harbors non-indigenous mobile epifauna in transit, preventing natural loss of propagules during the transport phase.

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MONITORING MARINE ALIEN FAUNA IN MARINA OF GAMMARTH (BAY OF TUNIS, TUNISIA) WITH THE SERC PROTOCOL

Abstract
The introduction of Non-Indigenous Species (NIS) in marine ecosystems is considered one of the most important consequences of global warming, inducing both ecological and economic impacts. Indeed, the Mediterranean is the most invaded sea in the world. However, the most study on NIS are focused on new records and there are no standard tools of monitoring of NIS in Mediterranean Sea in general and in Tunisia in particular; In the last decades, it was been demonstrate that marinas are a hotspot of NIS, and the regular monitoring is essential to control the multiplication, spread and sprawl of NIS. Here we used the Protocol developed by Smithsonian Environmental Research Center (SERC) for sessile fouling species in *the southern Mediterranean Sea. In March 2019, 36 PVC plates (20×20 cm) were immersed in nine zones of the touristic port of Gammarth (North coast of Tunisia). Seasonal monitoring was conducted every three months and retrieved plates were observed under stereomicroscope for taxonomic identification of sessile invertebrates. A total of ten NIS was identified belonging to* Bryozoan: *Bugula stolonifera, Bugula neritina, Watersipora subtorquata, Watersipora arcuata; Ascidian: Clavelina lepadiformis, Botrylloides violaceus, Botryllus schlosseri, Ecteinascidia turbinata, Ciona robusta*; Polychaeta: *Hydroides elegans. five are reported for the first time in marinas of Tunisia. W. subtorquata and H. elegans were the most abundant species in our samples. However, NIS assemblages were quite similar whatever the season and zone.*

Key-words: Species richness, Fouling communities, PVC plates, Marina.

Introduction

Marinas have been classed one of the most vectors of introduction and a spread of NIS in the Mediterranean via recreation boats (Ulman *et al.*, 2019). In the last years, several methods have been developed for sampling marinas based on various techniques such as, rapid assessment survey, scraping, quadrat, and settlement plates. A combination of methods seems to be the best solution for monitoring biofouling. We assay here a recent protocol for monitoring NIS in marinas developed by Smithsonian Environmental Research Center (SERC protocol)

Materials and Method

Study was carried out during 1 year starting in March 2019 in Marina Gammarth (36.920158” N 10.306954” E). 36 PVC plates were emerged in 9 zones following the SERC protocol described in Jimenez *et al.* (2018). Each 3 months, 9 plates were retrieved and observed under stereomicroscope for taxonomic identification of sessile invertebrates. ANOVA two way was performed to test for differences in abundance of NIS across the sampling zone according to season variations and zone.

Results

A total of 10 NIS was identified belonging to Bryozoan, Ascidian and Polychaeta (Tab.1). Five species are reported for the first time in the marinas of Tunisia (*Bugula stolonifera, Watersipora subtorquata, Watersipora arcuata, Botrylloides violaceus, Ciona robusta).* The result of the ANOVA test showed no differences among seasons and zones in NIS abundance (F=14.008, p=1.91). The highest number of NIS observed during September (37.29%) compared to the other period of monitoring. *W. subtorquata* and *Hydroides elegans* were the most abundant species in our samples. Some species have been absent during December such as *Clavelina lepadiformis, W. arcuata. C. robusta* and appear in September. The abundance of this last species grows up in the next period of sampling. However, *Botryllus schlosseri* and *Ecteinascidia turbinata* have been detected only during September (Tab.1).

Tab. 1: Abundance variation and status of NIS found during monitoring period in marina of Gammarth

	Status	March	June	September	December
Bryozoan					
Bugula stolonifera	Cryptogenic	20	10	25	4
Bugula neritina	Cryptogenic	6	3	3	11
Watersipora subtorquata	Cryptogenic	127	148	235	92
Watersipora arcuata	Non indigenious	3	61	32	
Ascidian					
Clavelina lepadiformis	Cryptogenic	60	38	62	
Botrylloides violaceus	non indigenious	3		1	42
Botryllus schlosseri	Cryptogenic			36	
Ecteinascidia turbinate	Non indigenious			4	
Ciona robusta	Non indigenious			1	4
Polychaeta					
Hydroides elegans	Non indigenious	144	210	248	103
	Total	**363 (20.92%)**	**470 (27.09%)**	**647 (37.29%)**	**255 (14.70%)**

Conclusion

Seasonal sampling and monitoring of NIS showed the similar phenomenon of abundance of one or two species in all plates (*Watersipora subtorquata* and *Hydroides elegans*). This result was observed in the study of Tamburini *et al.* (2021) in Italy. This could be related to the ecology of species. SERC protocol seems like a good method for sampling NIS. It's rapid for early detection, quantitative and specific techniques.

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ALIENS ON OUR DOORSTEP: THE CASE OF THE LIGURIAN MACROFAUNA

Abstract
In the last decades, the Mediterranean Sea has been particularly affected by invasion of non-indigenous species (NIS), whose monitoring represents one of the main topics included in the European Marine Strategy Framework Directive (2008). Since 2015, the Ligurian Regional Agency for the Environment Protection (ARPAL), under the coordination of ISPRA-SNPA, manages the monitoring activities in the Italian part of the Ligurian Sea (Genoa and La Spezia harbours), with the aid of the Genoa University. Samples are collected twice a year, both on soft and hard bottoms. So far 3 alien species have been recorded: i) the isopod Paranthura japonica*, native of the Japan Sea, reaching a maximum density of 760 individuals* m^{-2} *in La Spezia, ii) the sabellid fan worm* Branchiomma luctuosum*, showing a maximum density of 510 individuals* m^{-2}*, and iii) the small calcareous sponge* Paraleucilla magna*, collected on Genoa panels. All those species are, at this point, considered established in the Ligurian Sea. These activities are crucial to monitor the presence, abundance and distribution of NIS, in order to optimize the management of coastal environments.*

Key-words: non- indigenous species; isopoda; polychaeta; porifera; migrations

Introduction

The invasions of non- indigenous species (NIS) represent a very contemporary worldwide topic and is therefore an integral part of the EU Marine Strategy Framework Directive (2008). In the Ligurian Sea, the NIS monitoring activities are carried out by ARPAL (Regional Agency of Environment Protection for Liguria), under the coordination of ISPRA-SNPA.

Materials and methods

NIS monitoring activities in the Ligurian Sea are performed in the harbours of Genoa (44° 25’11’’N; 008°48’51’’E) and La Spezia (44,0709°N; 9,8569°E). Macrofauna was collected from soft bottoms using a 15l Van Veen grab while epilithic communities of hard bottoms are scraped on 30x30 cm^2 squares. In addition, 6 plastic panels (15x15 cm^2) have been placed in the Genoa harbour to assess the alien species colonization process. Collected samples are sorted and identified to the lowest taxonomic level. All activities are conducted twice a year, to monitor seasonal pattern variations in the settlement of alien species.

Results

During the analysis of macrofaunal samples obtained from 2021 campaign in harbours, both on soft and hard bottoms, 3 NIS has been recorded.

- ***Paranthura japonica***

P. japonica Richardson 1909 is a marine isopod currently established in the whole Mediterranean basin (Goulletquer, 2016). Most individuals were recorded on shallow water (3-6 m) hard bottoms, with a maximum density of 180 specimens m^{-2} in Genoa, and 760 specimens m^{-2} in La Spezia; in addition, few specimens were recorded in the soft bottom cores performed at 16-35 m depth in different localities of the central-western Ligurian Sea. More than 1/3 of the collected individuals were eggs-carrying females; this fact, together with the elevated density of specimens in the harbour and the new records along the coasts, suggest an ongoing and quick colonization of the Mediterranean basin by this recently arrived invader.

- ***Branchiomma luctuosum***

B. luctuosum (Grube 1870) is a tube-building polychaete entered from the Red Sea. A maximum density of 320 individuals m^{-2} was recorded in the Genoa harbour, and 280 individuals m^{-2} in La Spezia at the end of the summer period. The species is therefore extremely common in port environments, and can be found also in natural bays, representing a successful NIS in the Ligurian basin.

- ***Paraleucilla magna***

The calcareous sponge *P. magna* Klautau, Monteiro & Borojevic, 2004, native from the Brazilian coast, is common in the lower infralittoral communities, and mainly occurs on artificial hard substrata (floats) of port ecosystems, where the water movements are reduced, or in mussels and oysters farms. In 2021 in Genoa harbour, this species has been recorded on plastic panels with a maximum coverage percentage of 2,39%.

Discussion

The Ligurian Sea, thanks to its geographical, morphological and hydrographical features, represents one of the coldest portions of the Mediterranean basin, thus it is relatively preserved by NIS invasions; nevertheless, the increasing diversity, frequency and spatial distribution of alien species recently observed in the Ligurian basin indicate a rapidly progressing invasion, which ecosystemic consequence are yet to be fully understood.

Acknowledgements

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UNCOMMON RECORD OF THE NON-INDIGENOUS BARNACLE *AMPHIBALANUS EBURNEUS* ASSOCIATED WITH THE LOGGERHEAD SEA TURTLE, *CARETTA CARETTA* IN TUNISIAN WATERS

Abstract
The ivory barnacle Amphibalanus eburneus (Gould, 1841) (Arthropoda: Hexanauplia: Sessilia) is, native to the Western Atlantic, has been widely introduced worldwide through shipping and has been found throughout the Mediterranean and the Black Sea since the 19th century, where it is now well established with multiple records from several countries. In a prospecting campaign within the framework of a project on "Medbycatch", adult and juvenile's barnacles were found among biofouling fixed to a carapace of female of loggerhead sea turtle Caretta caretta, accidentally captured a live by a trawler in Mahdia (the Central East Tunisia Region) in September 2021. Among them twenty specimens of A. eburneus were identified and clearly differentiated from the native species Chelonibia testudinaria (Linnaeus, 1758). The present record constitutes the first sighting of Amphibalanus eburneus on turtles in Tunisia waters and elsewhere. Most occurrences of this barnacle are associated with mussel shells. Amphibalanus eburneus, in particular, is a fouling organism common to molluscs. The presence of A. eburneus juveniles suggests the successful establishment of the species in the area.

Key-words: Cirripedia , bioinvasions, establishment, competition, expansion

Introduction

The ivory barnacle *Amphibalanus eburneus* (Gould, 1841), a cirriped species of the family Balanidae Leach, 1817, is one such marine species transported globally via anthropogenic means. This taxon is usually found in estuarine habitats, tolerates considerable salinity variations extending from near full-salinity waters to nearly fresh waters, and avoids strong currents, living mainly attached to immobile objects (e.g. mollusk shells, rocks, harbor installations, and artificial substrates) in areas protected from wave action (Dineen & Hines, 1994). *Amphibalanus eburneus*, native to the Western Atlantic (Fofonoff *et al*., 2018), has been widely introduced worldwide through shipping (Carlton *et al.,* 2011) and has been found throughout the Mediterranean and the Black Sea since the 19th century, where it is now well established with multiple records from several countries (Zenetos *et al*., 2017). However, its distribution in the Mediterranean basin remains patchy, presumably due to the absence of field research and taxonomic experts. Since its first occurrence in Tunisia waters in 2012 (El Lakhrach *et al*., 2012), the species has been reported occasionally and mainly in brackish waters associated with mussel shells. The present record constitutes the first sighting of *Amphibalanus eburneus* on turtles in Tunisia waters and elsewhere.

Materials and methods

In a prospecting campaign within the framework of a project on "Medbycatch" (a project that develops a standardized multi-taxa approach to study and mitigate bycatch in the

Mediterranean) in September 2021, a loggerhead sea turtle *Caretta caretta* were accidentally captured a live by a trawler in Mahdia tunisian waters (35° 32’ 19.909’’; 11° 12’ 37.79’’) at a depth of 140 to 200 m.

The turtle was disentangled, measured and photographed. All barnacles were carefully and quickly scraped with a knife from the carapace and then fixed and preserved in 70% ethanol. Then the animal released alive to the sea water. All Cirripedia were later counted and identified according to the Relini (1980).

Results

On the female of loggerhead sea turtle *Caretta caretta* (Weight = 70 kg; Curved carapace length= 58cm; curved carapace width= 53cm), accidentally captured a live by a trawler in Mahdia, fifty specimens of barnacles are mainly associated on the carapace. These barnacles belong to two different species, the native species *Chelonibia testudinaria (Linnaeus, 1758*) and the non-indigenous species *Amphibalanus eburneus* with 20 specimens of which 15 individuals are adults. The species is characterized by a shell with a conic/cylindrical aspect, variable in shape according to the amount of crowding, with a toothed and almost pentagonal orifice. The diameter, taken from the base of the rostrum to the base of the hull, varies from 10 to 20 mm. The heights vary between 7.5 and 20 mm.

Conclusion

The presence of *A. eburneus* juveniles suggests the successful establishment of the species in the area. Several hypotheses concerning the possible vector transporting *A. eburneus* to Tunisian waters will be discussed. Indeed, the invasive potential of the family Balanidae is well known, with a substantial number of alien species found globally, several of which have adverse effects on native ecosystems by competing for space and food and contributing to biofouling on ships and anthropogenic structures. Therefore, future studies may focus on the possible impacts of this invader on native biota.

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MARINE INVASIVE SPECIES AND FISHERIS IN THE VENICE LAGOON AND NORTHERN ADRIATIC SEA: a PhD project

Abstract
During the last decade, two main invasions have spread in the Venice Lagoon and Adriatic Sea: the sea walnut, Mnemiopsis leidyi, and the Atlantic blue crab, Callinectes sapidus. Both species originated from American coasts and invaded a great number of the Earth's Seas. Nowadays those two invaders arrived in the Venice Lagoon and in the northern Adriatic Sea, but their socio-economic and ecological impacts have been scarcely studied, not only in lagoon waters but also in the bordering northern Adriatic Sea. Here we present the structure and methodologies of a project aiming to investigate the biology of M. leidyi and C. sapidus in these waters, their impacts on the nektonic biodiversity of the study areas and the fisheries, as well as to explore management solutions, with the final goal to develop with local fishers a participatory strategy to mitigate the impacts of these invasive species.

Key-words: Invasive species, *Mnemiopsis leidyi*, *Callinectes sapidus*, fisheries, Venice Lagoon

Introduction

Invasive Alien Species (IAS) can have large ecological and socio-economic impacts: they modify the local food webs and the diversity of the invaded ecosystems, and they can also negatively affect human activities (Tsirintanis *et al.*, 2022). During the last decade, two main invasions have spread in the Venice Lagoon and Adriatic Sea: one carried out by the sea walnut, *Mnemiopsis leidyi*, and the second one carried out by the Atlantic blue crab, *Callinectes sapidus*. Both species are native to the American coasts, and, through ballast waters, they have invaded a great number of the Earth's Seas (Shiganova *et al.*, 2019; Mancinelli *et al.*, 2021). In most of the invaded seas they have had great impacts on fisheries and environments. The Venice Lagoon and northern Adriatic Sea are invaded by those two animals. Lagoon artisanal fishers assert an impact of these species on their activities. Specifically, they state a loss in captures and biomass of local fishery products caused by *M. leidyi* (locally called "acqua grossa") and a loss in the biomass of the local and commercially important green crab, *Carcinus aestuarii*, caused by *C. sapidus.* They also express a widespread concern that the artisanal fishery will be lost if nothing is done to mitigate the impacts of these species. On the other hand, the blue crab is entering in the economy of the fish markets of the Venice Lagoon. Here we outline the structure and methodologies used, in the framework of a PhD project, to assess the impact of these invaders on local biodiversity and ecosystem services, as well as to explore management solutions.

Objectives

The final objective of this project is to develop together with fishers, and so in a participatory fashion, a shared strategy to mitigate the effects caused by *M. leidyi* and *C. sapidus*. Investigating both species will provide us with a comprehensive picture of the Venice Lagoon and northern Adriatic Sea conditions allowing to identify the most sustainable strategies to mitigate this urgent problem for fisheries as well as biodiversity and ecosystems of these coastal ecosystems.

Study approach

A multidisciplinary approach is often necessary to investigate the complex ecological and socio-economic impacts of invasive species. In this project, we integrate information on the biology and ecology of *M. leidyi* and *C. sapidus* in the Venice Lagoon and in the northern Adriatic Sea. This information is collected from field monitoring and aquarium experiments, with time series analyses, fishery sampling and local ecological knowledge (Tsirintanis *et al*., 2022). This integrated approach is employed to clarify the impacts of these invaders on the nektonic biodiversity of the study areas and the ecosystems services they provide, allowing us to explore management solutions with the final goal of contributing to the sustainability of the traditional fisheries. Several methods will be integrated to collect biological and ecological data and assess impacts on local biodiversity and fisheries. Field samplings of *M. leidyi* and *C. sapidus* will be carried out in all seasons to understand the spatial distribution of the species and to assess which life cycle stages are present, where and when in these waters. Seasonal samples are expected to provide a better understanding of the distribution of the species in different areas for their reproduction and overwintering, and in refugium areas in the case of *M. leidyi*. To evaluate the influence of abiotic factors on the two species, aquarium experiments will be made by maintaining the animals at different temperatures and salinities and, for the blue crab, with different sediment types. To assess the interactions between the invaders and their possible competitors, behavioural experiments will be performed. To evaluate the ecological and economic impacts of the invaders, the statistical analyses of landing time series in two local fish markets, Burano and Chioggia (covering both the lagoon and the sea), will be carried out. An analysis of landing composition in terms of species will also be carried out to evaluate which of the local species have been most affected by the arrival and the subsequent invasion of *M. leidyi* and *C. sapidus*. Moreover, samplings of fisher's captures will be carried out on board of fishing boats of local fishers. Local Ecological Knowledge (LEK) of fishers will be a complementary tool to investigate the evolution over time of the invasions, and to assess their socio-economic consequences. A local fisher cooperative, "Società Cooperativa San Marco - Pescatori di Burano", mainly operating in the lagoon is involved in the project to plan research and discuss results and management options, assist in field sampling, and provide data on landing records, occurrence, and economic impact of invasive species in the past years (via questionnaires, logbooks and interviews).

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PRELIMINARY RESULTS OF THE EFFECTS OF *MNEMIOPSIS LEIDYI* A. AGASSIZ 1865 ON A SMALL-SCALE FISHERY IN THE VENICE LAGOON

Abstract
Mnemiopsis leidyi (Agassiz, 1865) is a highly invasive ctenophore, native to the West Atlantic Sea, that is currently spreading in the Mediterranean Sea. Blooms were reported also in the Venice lagoon, a hotspot of habitat richness and biodiversity. However, no studies have been published about the impacts of this ctenophore on the lagoon ecosystem and its services, as traditional small-scale fisheries. Combining local ecological knowledge (questionnaires), statistical modelling (time-series analysis) and field sampling (boardings) we investigated whether the presence of M. leidyi is affecting artisanal fisheries. Preliminary results indicate that i) overall the landings of the lagoon fishers strongly declined with the blooms and ii) the mechanical clogging of the nets by the ctenophore severely impedes fishing activities. Our results call for a better understanding of M. leidyi impact on both lagoon ecosystems and the socio-economic consequences.

Key-words: invasive species, local ecological knowledge, small-scale fisheries, time series analysis, *M. leidyi*

Introduction
In marine habitats, invasive alien species (IAS) are difficult to monitor, hence the environmental and economic damages they cause are challenging to predict and contrast (Molnar *et al.*, 2008). Transitional environments, such as lagoons, are highly productive but delicate environments that provide a wide range of ecosystem services (i.e., biodiversity support, nursery areas, feeding grounds, small-scale fishery etc.) (Newton *et al.*, 2018). Being highly productive refugia areas compared to the open sea, lagoons in the Mediterranean Sea have been recently suggested to be vulnerable to the invasion of *Mnemiopsis leidyi* Agassiz, 1865 (Delpy *et al.*, 2016; Marchessaux & Belloni, 2021) a ctenophore listed among the 100 most invasive species worldwide (IUCN Lowe *et al.*, 2000). The Venice lagoon is the largest Mediterranean lagoon, located along the Northern Adriatic Sea coast. Despite the first bloom of *M. leidyi* was registered in 2016 (Malej *et al.*, 2017), nowadays no assessment of its presence and impacts exists.

Materials and Methods
To fill this gap, we dated the appearance, spread and bloom of *M. leidyi* using structured questionnaires to recover the ecological knowledge of traditional fishers and the consequences on their fishing activities. Then, time series (1997-2019) of lagoon fishery landings (kg/day) of the most valuable species targeted by the local small-scale fishery were extracted using official data of the Chioggia Fish Market. We included in the analyses the lagoon daily water temperature, a major driver of both environmental changes and the spread of *M. leyidi* (Delpy *et al.*, 2016). We boarded with lagoon fishers to assess *M. leyidi* biomass over the total net content.

Results

Statistically derived clusters of years based on landings from the Chioggia fish market highly matched the time frames identified by fishers in the questionnaires. We grouped *M. leidyi* occurrence as Absence (A; 1997-2009), Presence (P; 2010-2013), and Bloom (B; 2014-2019) (nMDS, stress 0.1). Since the P period landings started to decrease, markedly between the A and B periods (linear model: B vs A; t=-2.849, p-value=0.010; ANOVA *M. leidyi*: F=3.856; p-value=0.039). We also found that winter (of the previous year), summer and autumn lagoon temperature had a statistically significant effect on blooms of *M. leidyi* (tab.1). Boarding with fishers, we found that during summer, all nets were filled for more than the 50% of their volume and often entirely clogged by the ctenophore in the central and the southern lagoon basins, while in the northern lagoon the occurrence of *M. leidyi* was lower.

Tab. 1: Lagoon water temperature (1997-2019) related to *M. leyidi* A, P, B periods.Multinomial Logistic regression model, p-values < 0.05 are in bold.

Season	p-value
Spring (Mar-May)	P 0.142 B 0.068
Summer (Jun-Aug)	P 0.107 **B 0.022** (+)
Autumn (Sep-Nov)	P 0.061 **B 0.025** (+)
Winter (Dec-Feb)	P 0.161 **B 0.024** (+)

Discussion

Our preliminary results indicate that *M. leidyi* started to massively bloom in 2014 in the Venice lagoon likely contributing to a decline in lagoon fishery landings. High temperature was confirmed to be a major driver of blooms. Data from the field showed that *M. leidyi* mechanically impedes fishing activities by clogging the nets. The robustness of our findings on the impacts of *M. leidyi* is supported by the combination of multiple data sources and approaches. Further investigation is needed of the direct (as predator of eggs and larvae) or indirect (as competitor for zooplankton) impacts *M. leidyi* can have on the lagoon food web and ecosystem functioning.

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TESTING EFFECTIVENESS OF DIFFERENT ANTIFOULING AND BOAT MAINTENANCE PRACTICES IN THE CONTEXT OF BIOINVASIONS

Abstract
Biofouling, known as the settlement and growth of organisms on wetted surfaces, is one of the major challenges faced by the boating sector, since it can compromise the hydrodynamics and efficiency of boats, while contributing to the spread of non-indigenous species (NIS). In this context, different antifouling solutions have been developed, being the most common ones biocide-based. Although highly effective, some of these compounds have proven to have adverse effects on the environment. Thus, new commercially available alternatives have been developed, including non-toxic solutions focused on altering the surface characteristics of the ship hull. This research aims to test in field conditions the effectiveness of different antifouling solutions, including paint type (biocide-based; non-toxic; no paint) and maintenance practices (regular hull cleaning; no cleaning). For this purpose, 48 PVC panels with different treatments were submerged in two marinas located in the Gulf of La Spezia (Italy) and sampled at two different periods. Panels were analysed in the lab to determine how community structure and composition differed according to the treatment and time, giving special attention to NIS and initial colonizers. Preliminary results show a significant difference according to treatment, both at community level and total biomass. Besides, Watersipora subtorquata has been identified as a biocide resistant organism that facilitates the attachment of others. Further analysis of the samples will enable to: 1) determine the extent of effects of each of the factors, 2) assess differences in NIS presence and abundance among factors and 3) suggest best practices for boat maintenance and point out priorities for biofouling management.

Key-words: biofouling, antifouling paints, bioinvasions, non-indigenous species, recreational boats

Introduction

Fouling communities of recreational boats have proven to be one of the major vectors of introduction and dispersion of non-indigenous species (NIS) (Ulman *et al*., 2019). To avoid the settlement and growth of foulers, different antifouling coatings have been developed (Wezembeek *et al*., 2018), being biocide-based coatings (BC) the most widespread ones. However, many of these substances have proven to have adverse effects in the environment (Miller *et al*., 2020) and unclear effectiveness (Floerl *et al*., 2005). Thus, 'non-toxic' substitutes, such as foul-release (FR) coatings that minimise the adhesion strength, are being developed. This study aims to assess the effectiveness of two antifouling paint typologies, simulating in a manipulative experiment the maintenance practices followed by boaters. To do so, we combined the application of antifouling coatings with maintenance practices, resulting in 6 types of antifouling strategies.

Materials and methods

The selected study site was the Gulf of La Spezia, on the eastern side of the Ligurian coast. Following the protocol developed by the Smithsonian Environmental Research Center

(SERC) (Marraffini *et al*., 2017), PVC plates with a standardized surface area were deployed in the two study sites. Two commercially available paints were selected: a traditional BC and an alternative FR. In total, 48 PCV panels were deployed: 16 plates were coated with BC, 16 with FR and 16 uncoated. Plates were sanded and painted accordingly on one side, attached to a brick ensuring downward orientation of the coated/sanded side and secured to docks, submerged at 1 m depth. Half of the plates were periodically cleaned with sponges, simulating in-water manual cleaning by boat owners; the remaining plates, instead, were left untouched. The plates were retrieved after 3 and 9 months (T1 and T2, respectively) of submersion and preserved in ethanol until their analysis in the lab. Presence or absence of organisms was recorded to obtain values of coverage and species were identified to the lowest taxonomic level possible.

Results

A total of 52 species were identified, being 43 native and 9 NIS. Regardless of the antifouling treatment, all plates exhibited at least a few fouling species. The community structure showed changes in its composition through time and among treatments. So does the coverage, being control panels and maintenance panels completely covered already in T1, while all BC panels show significantly lower coverage for that season. However, the coverage increases significantly in all BC coated panels in T2. FR panels follow a similar trend to those uncoated. In general, the abundance of NIS present in samples not subjected to maintenance was greater than in samples that had been periodically cleaned, regardless of the coating.

Discussion and conclusion

This study shows that different antifouling management strategies have different effects in terms of protection from biofouling and NIS. BC coating confirms effective in preventing attachment more efficiently in short term, but fails to do so for longer submersion periods. Although maintenance alone does not reduce biofouling, it does affect the NIS community, being the only factor that reduced the number of NIS, on long term as well. Although FR coating showed a similar trend to the uncoated panels, it must be highlighted that the fouling community was weakly attached and easily removed. We here identified some species exhibiting resistance to cleaning procedures and BC paint that facilitate fouling development by providing substrate for secondary colonisers.

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POPULATION DYNAMICS OF THE PACIFIC SPECIES *PARANTHURA JAPONICA* (CRUSTACEA: ISOPODA) IN A MEDITERRANEAN SITE

Abstract
Our knowledge of marine Non-Indigenous Species (NIS) biology and ecology suffers from several gaps, including data on their most basic life history traits. Determining the characteristics of successful invaders, identifying the traits directly or indirectly related to invasiveness, while predicting the profiles that are more likely to cause future invasions, remain major goals in marine invasion biology. The present study aims to fill the knowledge gap related to the biology and ecology of the Pacific isopod Paranthura japonica Richardson, 1909 in a newly invaded site, Marina di Ragusa (Sicily) touristic harbour, focusing on: (I) its population dynamics, (II) gathering biological and ecological data on this species, and (III) testing the effectiveness of an Artificial Substrate Unit for this type of investigations. Our study took place from December 2017 to December 2018; we collected three artificial sponges every two weeks, after they had been deployed underwater and kept there for two months. Sex class, length, number of eggs per female and egg developmental stage were registered for more than 1400 individuals collected throughout the year. We conclude that P. japonica, native to the cold Okhotsk Sea, has well adapted to the warm Mediterranean conditions. Its population has been found well-established and persistent throughout the year, while its numeric positive response to increasing temperature was one of our most surprising findings. In fact, reproductive events occur throughout the year, but peak during summer. Finally, we show that nylon bath sponges represent an efficient artificial substrate for obtaining quantitative samples of peracarid crustaceans.

Key-words: Alien species, bioinvasion, population dynamics, Isopod, artificial substrate units

Introduction

The pacific isopod *Paranthura japonica* Richardson, 1909 currently represents one of the most common alien species in Mediterranean marinas (Ulman *et al*., 2019); however, very little is known about its biological and ecological traits. In fact, besides taxonomic descriptions and notes on its geographic distribution, the only additional knowledge regards its habitat preferences and feeding strategy, common to other members of the family Paranthuridae. Other traits, especially those related to life cycle, have never been investigated so far. The poor knowledge regarding this species is to be attributed to the difficulties in spotting specimens in the wild and collecting standardised, quantitative data. Therefore, studying the biology and ecology of the non-indigenous *P. japonica* requires appropriate and specific methods. The present study addresses the aforementioned problems and aims to study the population dynamics of this species in a newly invaded site.

Materials and methods

The study occurred in Marina di Ragusa (southern Sicily), a tourist port in the Central Mediterranean Sea. Its fouling community had already been studied in 2016 by Ulman *et al*. (2019), showing a high richness of sessile fauna, including several NIS. Our study took place for the duration of one year, from December 2017 to December 2018. We deployed three artificial sponges fortnightly at 30 cm depth, in direct contact with the quay, and collected them after two months, a time considered suitable for fouling colonisation at these latitudes (Ros *et al*., 2020). During every sampling event, we used a digital thermometer to measure water temperature. A total of 72 sponges were collected and stored in plastic containers filled with 90% ethanol. The fouling component was sorted under a stereomicroscope; all *P. japonica* individuals were measured, and the total and mean abundance along with standard deviations (SD) were determined for every sampling event. Mean length and SD were plotted, for the entire population as well as separately for males, females, and juveniles alone.

Results

Over 1400 individuals of *P. japonica* in the Marina di Ragusa port were found across the 12 months of study, sexed, and measured for total length; 63% were juveniles, 28.5% were females and 8.5% were males. The species presented a strong seasonal pattern, surviving at very low densities during winter (minimum values observed in February), while reaching its higher densities in summer (maximum values observed in August). Size ranged between 1.172 mm and 11.884 mm. Both smaller and larger size classes occurred through all the year, although with variable abundance.

Discussion and conclusions

The population of *P. japonica* in the Marina di Ragusa touristic harbour was well established and persistent throughout the year. An interesting result of our study regards the positive response of the population to increasing temperature: despite its native origin in the cold Okhotsk Sea, (Richardson, 1909; Nunomura, 1975), it appears that *P. japonica* has shown to have well adapted to the warm conditions of Mediterranean summer. All artificial sponges were successfully colonised by mobile and sessile fauna, and by *P. japonica* individuals, hence they can be considered a useful and reliable substrate for quantitative mid/long-term studies on population dynamics of small marine invertebrates.

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PARASITES OF ALIEN FISHES *SPHYRAENA FLAVICUDA* RUPPLELL, 1838 AND *SPHYRAENA CHRYSOTAENIA* KLUNZINGER, 1884 IN WESTERN COAST OF LIBYA

Abstract
Fish parasites are considered as a part of marine biodiversity worldwide and it is very important to know the native and invasive species in Libyan waters to be included in the Libyan marine biodiversity database. Here we investigate fish parasites in alien barracudas, Sphyraena flavicauda and S. chrysotaenia, which entered the Mediterranean through the Suez Canal. A total of 46 and 10 individuals of S. flavicauda and S. chrysotaenia, respectively were collected along the western coast of Libya. A total of seven parasites were identified. The rates of infection in S. flavicauda and S. chrysotaenia were 46% and 32% respectively, the highest prevalence was 95.7 % for the D. cazauxi and the lowest prevalence was 4.35% for the Contracaecum type III in S. flavicauda., Moreover, the highest prevalence in S. chrysotaenia was 70% for Halacarus sp. and the lowest prevalence was 30% for Diplectanum dunanchae. D. cazauxi and D. dunanchae are monogeneans, belonging to the family Diplectanidae. These results provide a first assessment of the parasitofauna of alien barracudas in the Mediterranean Sea, filling a gap of knowledge on the biological and ecological trait of these Lessepsian fishes.

Key-words: classification, fish parasites, Lessepsian fish and Libya

Introduction

Marine ecosystems of the Mediterranean have changed at an alarming rate over the past two centuries, due to the human-mediated arrival of new species (Rilov and Galil, 2009). Marine organisms serve as hosts for various parasites and other pathogens, which can negatively affect the host population but also the food chain, with socio-economic implications (Lessios, 1988). This study aimed to investigate parasites associated to alien barracudas and assess their origin (donor region -Red Sea vs Mediterranean).

Material and methods: A total of 56 fishes were collected by local fishers along the western coast of Libya and the study was focused on metazoan parasites. The parasites examination was carried out according to Heil (2009).

Results

The rates of infection in *S. flavicauda* and *S. chrysotaenia* were 46% and 32% respectively. A total of seven parasites was identified. Their prevalence, intensity and abundance are given in Table. 1.

Tab. 2: The prevalence, intensity and abundance of parasites in *S. flavicauda* and *S. chrysotaenia* collected along the western coast of Libya

Host	*S. flavicauda*			*S. chrysotaenia*		
	Prevalence	Intensity	Abundance	Prevalence	Intensity	Abundance
Hysterothylacium aduncum	6.52	1	0.06	-	-	-
Contracaecum type III	4.35	3	0.04	-	-	-
Diplectanum cazauxi	95.7	1.36	0.95	-	-	-
Diplectanum dunanchae	85.1	1.48	0.85	30	0.30	0.30
Gnathia sp .	30.4	2	0.30	-	-	-
halacarus sp.				70	0.70	0.70
Paraclanus paravus	21.2	10	0.21	-	-	-

Discussion

Fish hosts can display a different parasitofauna when comparing native and invasive populations (e.g. Mele *et al*., 2012). In this study we provide new data on the parasitofauna of alien barracudas highlighting relatively high levels of infection. Gnathiid were recorded in all examined fishes. These parasites have probably infested the exotic barracudas after their introduction in the Mediterranean Sea. Other species of parasites are known in both the Mediterranean and the Red Sea, but their short lifespan would indicate that they were acquired in the newly invaded habitat (Mackenzie and Abaunza, 2014).

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SPATIAL EXPANSION OF THE ALIEN INVASIVE BIVALVIA *BRACHIDONTES PHARAONIS* AROUND TUNIS SOUTHERN LAGOON AND ITS ECOLOGICAL AND SOCIO-ECONOMIC IMPACT

Abstract
Recently mussel beds of the Lessepsian Mytilid Brachidontes pharaonis (Fisher P. 1870), were observed all around the Tunis Southern lagoon (TSL) located near commercial ports. This study was carried out in order to collect qualitative and quantitative data about B. pharaonis versus Mytilus galloprovincialis and to estimate their densities. It also aims to assess the potential of non-indigenous mussels to spread and colonize new areas. The socio-economic impacts of the B. pharaonis invasion were identified on the basis of local fishermen interviews. This work highlights that this invasive species occupied most of rocky shores of the lagoon reaching a density exceeding 18500 ind.m^{-2}. B. pharaonis competes with the native mussel and has probably displaced it from the lagoon, since no M. galloprovincialis were found, with negative impacts on the local fishery sistem.

Key-words: *Mytilus galloprovincialis*, competition, habitat, mapping, fishermen income

Introduction

The Red Sea mussel *Brachidontes pharaonis* (Fischer, 1870) was one of the first NIS to invade the Mediterranean Sea in 1869. Since its first occurrence in Tunisia in 2013, *B. pharaonis* had spread substantially throughout most of the country with a potential threat for native species (Sarà & De Pirro, 2011). After the ecological restoration of the lagoon in 2001, *Mytilus galloprovincialis* colonized all artificial rocky shores and was exploited for commercial purpose.

Material and methods

This survey was carried out along the shores of TSL in order to estimate the level of expansion of *B. pharaonis* in 20 stations, a distance of 1.5 km, during two field campaigns in 2022. Percentage of coverage and densities were calculated using a quadrat (50 x 50 cm). Images of each quadrat were treated by the ENVI software. For the socio-economic study, 32 face-to-face interviews were conducted with local fishermen.

Results and discussion

This study highlights the large expansion of *B. pharaonis* in all the rocky shores of the lagoon. The extreme densities vary between 300 to 19000 ind.m^{-2} with a mean average of 7400 ind.m^{-2} .The highest values of densities were observed near harbours suggesting a shipping vector of introduction. The size structure of the population of *B. pharaonis* revealed that all cohorts (from 2 to 35 mm) were present with a dominance of large individuals (>20 mm total shell length). This class-size represents 33% of the total number of individuals. The estimation of biofouling of artificial rocky substrate via image treatment using the software ENVI revealed a percentage of coverage by the alien invasive mussel oscillating between less than 10 to 90 % (Figure 1). The invasion of *B. pharaonis* reasonabily displaced the native mussel *M. galloprovincialis*. Indeed, this species occurred in the past with high densities, whilst no *M. galloprovincialis* were found during the present study. All the respondents (32 fishers) reported a decline of about 30 % in their income as a consequence of the disappearance of *M. galloprovincialis*. Such cases of displacement of native Mediterranean Mytilid had already been reported with *Mytilaster minimus* as a consequence of the invasion of *B. pharaonis* (Safriel & Sasson-Frostig, 1988; Rilov et *al.*, 2004). We conclude that the invasivion of *B. pharaonis* has dramatically changed the structure of native rocky shore assemblages in the Tunis Southern Lagoon.

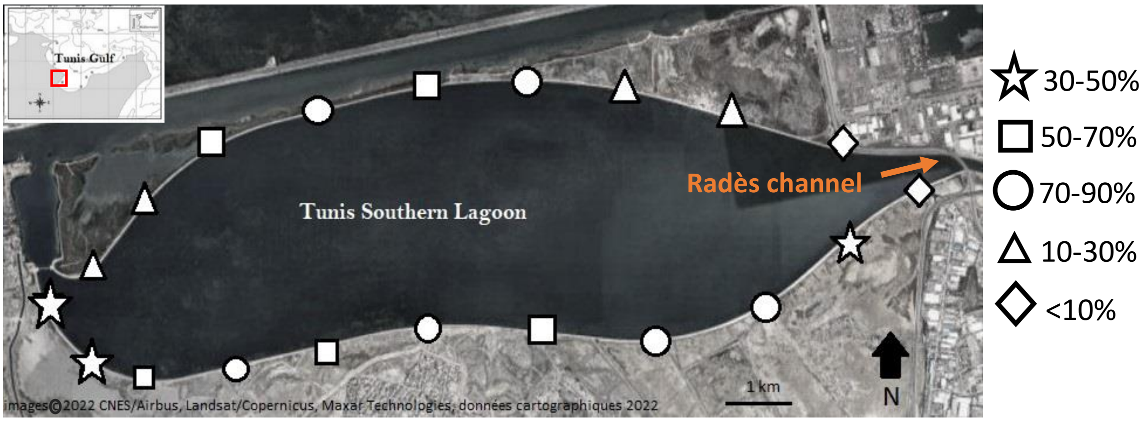

Fig. 1: Spatial coverage (%) of rocky artificial shores of Tunis Southern Lagoon by *B. pharaonis* (April 2022)

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PORT ENVIRONMENTAL GRADIENTS AND NON-INDIGENOUS SPECIES

Abstract
Ports are among the main transit points for non-indigenous species around the world. The large availability of artificial substrates and the reduced water quality usually lead to simplified benthic communities that offer limited biotic resistance toward non-indigenous species. The aim of this study is to highlight the role of environmental gradients within ports in favouring the settlement, permanence, and diffusion of non-indigenous species. The canal-port of Ravenna (northern Adriatic Sea), which extends for 11 km inland, and the high traffic of commercial ships, represents an ideal case study. This canal-port generates a water pollution and confinement gradient, which is reflected in a characteristic substitution of intertidal assemblages along the concrete quays. The outer harbour and the first stretch of the canal are characterized by beds of the native mussel, Mytilus galloprovincialis*, which assemblages are rich in native species. Towards the innermost areas of the port, the native mussel is replaced by the invasive mussel* Xenostrobus securis*. After a short transition zone characterized by the coexistence of both species,* X. securis *monopolizes the substrate accompanied by an increase in non-indigenous species.*

Key-words: Alien species, Marine traffic, Harbour, Mediterranean Sea, Padstow mussel

Introduction
The Ravenna harbour has a main canal-port, called Candiano, where water quality is affected by urban surface water drainage, civil and industrial wastewater discharges, and relatively strong tides (up to 80 cm). All these elements generate an evident environmental gradient by modifying the salinity and pollutant concentration, which are relevant in the NIS settlement (Airoldi *et al.*, 2015; Lopez-Legentil *et al.*, 2015). The aim of the study was to analyse how this gradient influences the non-indigenous species (NIS) abundance along the Candiano canal.

Materials and methods
Samples of intertidal benthic assemblages were taken by scraping the concrete quays of the Candiano canal at 6 sites over 3 years, from 2016 to 2018, to evaluate the influence of the environmental gradient. Collected specimens were counted and identified at best possible taxonomic level. Since the quantity of sample varied with the thickness of the mussel layer, the abundance of organisms of the different species was standardized and expressed as a percentage.

Results
Overall, 67 taxa were found, 56 classified at the species level, including 11 NIS (Zenetos *et al.*, 2017). Among them the most abundant NIS were the barnacle

Amphibalanus eburneus (Gould, 1841), the serpulid polychaete *Ficopomatus enigmaticus* (Fauvel, 1923), and the mussels *Xenostrobus securis* (Lamarck, 1819) and *Arcuatula senhousia* (Benson, 1842). *X. securis* form dense beds in the innermost part of the port, where an increase in NIS number and abundance were found (Fig. 1).

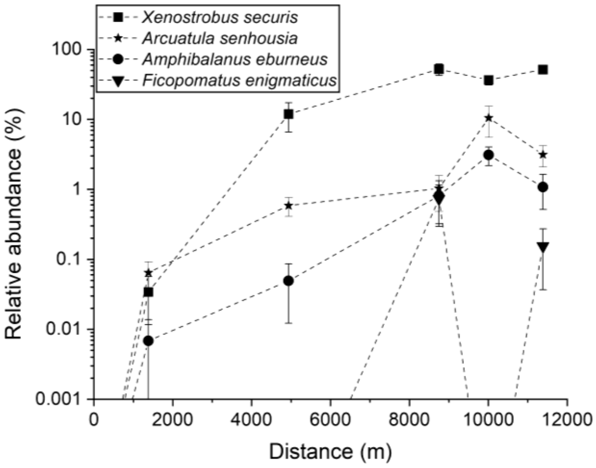

Fig. 1: Relative abundance (mean ± standard error) of the NIS *X. securis*, *A. senhousia*, *A. eburneus*, and *F. enigmaticus* along the canal-port. The distance was calculated starting from the outermost site (0=1st sampling site).

Discussion and conclusion

Most of the NIS found cope well with water quality degradation in the port's inner part. In turn, beds of *X. securis* could provide ideal conditions for other NIS by creating some invasional meltdown (*sensu* Braga *et al.*, 2018). While most of the practices to contain the spread of NIS are aimed at limiting the transport of individuals and propagules (e.g., cleaning hulls, developing better antifouling paints, regulating ballast waters), greater attention should be paid to understanding the ecological processes that favour the settlement and long stay of NIS within ports to develop preventing strategies and technologies around the world. Maintaining favourable environmental conditions and substrates for native mussels could be the first step in this direction.

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THE TOP THREE ALIEN MACROALGAE INVADERS IN CROATIA IN 2022

Abstract
Based on regular monitoring of benthic non-native species, we discuss the three most invasive macroalgae in the Croatian part of the Adriatic Sea in 2022. Our assessment is based on the algae's spreading dynamics, opportunistic behavior, impact on native species and habitats, and on expert judgment regarding the species' potential for further expansion and global influence. As the most invasive alga, we nominate Caulerpa cylindracea Sonder, 1845, which is a species with an exceptional and demonstrated impact on native benthic species and habitats. This is a fast-spreading alga present almost everywhere in the Croatian sea and developing on all types of bottoms between 0 and 55 m depth. The second most invasive species is Acrothamnion preissii (Sonder) E. M. Wollaston, 1968. This alga is recorded in two distinct areas. Around Dubrovnik (south Adriatic), it affects almost every rhizome of Posidonia oceanica (Linnaeus) Delile, 1813, forming dense cotton-like coverage and probably drastically displacing native epiphytic species. The third most invasive alga is Stypopodium schimperi (Kützing) Verlaque & Boudouresque, 1991. It was recorded for the first time in 2020, but in two years it has demonstrated an exceptional increase in coverage, tending to affect 100% of the rocky bottom between 5 and 15 m depth with total displacement of native erect species. Based on its expansion and impact in just two years, we believe that this alga might soon become the no. 1 invader.

Key-words: *Caulerpa cylindracea*, *Acrothamnion preissii*, *Stypopodium schimperi*, Adriatic Sea, invasive species.

Introduction
The Croatian part of the Adriatic Sea has its biological and ecological specificity, which has consequences not only in its native benthic assemblages but also in the spread and impact of alien species. Approximately 20 non-native macroalgae have been recorded in Croatia (Tsiamis *et al*., 2019). Some of them (e.g., *Caulerpa taxifolia*) declined after rapid expansion (Žuljević *et al*., 2019). Based on extensive fieldwork, we present the three most invasive alien macroalgae in Croatia in 2022.

Materials and methods
To identify the three most invasive non-native algae in 2022, we consider the most current information collected from the field during monitoring for the MSFD D2 and Benthic NIS scientific projects. Field monitoring was done through scuba diving and drop-down cameras. This assessment was based on spreading dynamics, opportunistic behavior, impact on species and habitats, and expert judgment regarding the species' potential for further expansion and global influence.

Results and discussion
On the basis of collected information, *Caulerpa cylindracea* resulted to be the most invasive macroalgae in Croatia. First recorded in 2000, this species is now present almost everywhere. It develops dense settlements that might cover 100% of the different types of sea bottoms, from a depth of 0 to a maximum of 55 m, in pristine and eutrophic areas. This alga can develop more

than 1491 m m^{-2} of stolon with 16125 fronds m^{-2} (Žuljević *et al*., 2003). It drastically displaces native species, especially in photophilic macroalgae habitat and coralligenous assemblages. Dense colonies develop in meadows of *Cymodocea nodosa* and in sparse meadows of *Posidonia oceanica*, where it drastically reduces epiphytes on rhizomes (Antolić *et al*., 2008). It overgrows and displaces sessile invertebrates, particularly sponges. The alga was observed growing on the bivalve mollusk noble pen shell, *Pinna nobilis*, and on colonial coral, *Cladocora caespitosa*. On circalittoral sandy bottoms, it develops an extensive and dense network of thalii, changing the bottom into a meadow of *C. cylindracea*. Below its dense matte, the increased accumulation of sediment with organic detritus is usually accompanied by anoxic conditions and the development of *Beggiatoa* spp. bacteria (Matijević *et al*., 2013). The alga probably reproduces only by fragmentation and spreads primarily using currents. It can be consumed by *Paracentotus lividus*, but this sea urchin does not have a significant impact on algal colonies. The second most invasive macroalga is *Acrothamnion preissii*. It has been detected in two distant areas, around the city of Dubrovnik in 2008 and later in the Kornati National Park. In 2022 in Dubrovnik, it developed extremely dense settlements, mainly affecting the rhizomes of *Posidonia oceanica* along > 30 km of coastline, from a depth of 5 to 30 meters. Almost no rhizomes free of *A. preissii* now exist in the area of Dubrovnik. Its development has dramatically changed the biodiversity of the Posidonia meadows, though concrete measurements of its impact are still missing. The current situation indicates its potential further expansion and high impact on the southern part of the Adriatic. The third most invasive macroalga is *Stypopodium schimperi*. Discovered sporadically in 2020 in Vis Island in the central Adriatic, it still has a limited spatial distribution. However, it has exhibited exceptional local spreading capabilities. The number of thalii on monitored transects increased from 10 to several thousands in two years. This has resulted in the predominance of *S. schimperi* on rocky bottoms between 8 and 15 m depth. With 40 cm in length and covering 100% of the bottom, this species totally displaces erect native macroalgae. Considering documented fast spread, increase in coverage and impact on macroalgae, *S. schimperi* might soon become the worst invasive benthic organism in the central and southern parts of the Adriatic.

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CONCLUSIONS AND RECOMMENDATIONS OF THE 2ND MEDITERRANEAN SYMPOSIUM ON THE NON-INDIGENOUS SPECIES

1. The number of Mediterranean NIS is increasing with no signs of saturation and the meeting highlighted the need of reinforcement of sub-regional and regional cooperation and harmonize the strategies toward NIS monitoring and managing.

2. Prioritization in monitoring should be structured in order to take into account the severity of impacts on threatened species and habitats. Marine Protected Areas can act as sentinel sites where to monitor the arrival and subsequent impact of NIS, as well as other biotic responses to anthropogenic pressures and Climate Change.

3. Prioritization in management should consider both negative and positive outcome of species invasions and both ecological and socio-economic implications. Concrete actions are needed to help the adaptation/mitigation processes. These actions should be guided by an informed management strategy, in collaboration with the relevant stakeholders. This process should be implemented at either the local, national and subregional scale, promoting regional cooperation at all levels.

4. Integrative methods are welcome to support the monitoring of NIS and their taxonomical identification. Despite its current limitations, new technological development (such us eDNA metabarcoding) could improve early detection, monitoring NIS and can be used as complementary tool to traditional taxonomic analyses.

5. Local Ecological Knowledge (LEK) approach can greatly contribute to the collection of historical and spatially explicit information. In particular, the use of participatory mapping has the potential of providing new insights for spatially informed management of NIS and strengthens the involvement and participation of stakeholders, especially fishers.

6. A large number of studies highlighted the importance of investigating fouling communities in harbours and marinas for the early detection of NIS and for exploring both theoretical and applied aspects of marine bioinvasions, including the assessment of related vectors.

7. The rapid change of Mediterranean biota under an accelerating climate change reality, stresses the need of adopting strategies for adaptive management of NIS, promoting cooperation between research bodies and involving all the relevant sectors of civil society.

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