PROCEEDINGS OF THE 1st MEDITERRANEAN SYMPOSIUM ON THE NON-INDIGENOUS SPECIES

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Proceedings of the 1st Mediterranean Symposium on the Non-Indigenous Species

FORWARD

Dear Friends and Colleagues,

Following the recommendations of:

- the **Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea** (adopted by the Contracting Parties to the Barcelona Convention in 1999 and updated in 2012)
- the **Action Plan for 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)
- the **Action Plan for 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
- the **Action Plan concerning Species Introduction and Invasive Species** (Adopted by the Contracting Parties to the Barcelona Convention in 2003 and updated in 2016)

a series of scientific symposia, dedicated to these habitats and NIS, was initiated in 2000 by organising the first Mediterranean Symposium on Marine vegetation. These initiatives aimed essentially to take stock of the recently available scientific data and to promote the cooperation between specialists and key actors working in the Mediterranean.

The Mediterranean Symposia on Marine Key Habitats are an important output, not only of the UNEP/MAP Mid-Term Strategy for the period 2016-2021 (Decision IG.22/1)[1], but also for MedKeyHabitats II Project[2] “Mapping of marine Key habitats and assessing their vulnerability to fishing activities in the Mediterranean” financed by MAVA foundation under its Mediterranean Strategy.

The "Mediterranean Symposia on Marine Key Habitats and NIS" will also provide an opportunity to discuss best practices in the monitoring of marine key habitats and non-indigenous species and provide elements to further improve the Integrated **Monitoring and Assessment Programme of the Mediterranean Sea and Coast and related Assessment Criteria (IMAP)**, of the Ecosystem Approach (EcAp) in the Mediterranean.

The organization of the "Mediterranean Symposia on Marine Key habitats and NIS" together back to back in Antalya from 14 to 18 January, is a joint collaboration among **UNEP/MAP-SPA/RAC** and the **Turkish Ministry of Environment and Urbanization**, as follows :

- 6th Mediterranean Symposium on Marine vegetation (from 14 to 15 January 2018)
- 3rd Mediterranean Symposium on the Conservation of Coralligenous and other Calcareous Bio-Concretions (From 15 to 16 January 2018)
- 2nd Mediterranean Symposium on the Conservation of the Dark Habitats (17 January 2018)
- 1st Mediterranean Symposium on the Non-Indigenous Species (From 17 to18 January 2018)

The Turkish Marine Research Foundation, **TUDAV**, as **SPA/RAC** partner will support the local organization of this event.

This edition will also be a good opportunity to discuss new topics such as monitoring and definition of Good Environmental Status (GES) in the Mediterranean and so strengthen links between scientists and scientific institutions.

Khalil ATTIA
SPA/RAC Director

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[1] https://wedocs.unep.org/bitstream/handle/20.500.11822/6071/16ig22_28_22_01_eng.pdf?sequence=1&isAllowed=y
[2] http://www.rac-spa.org/medkeyhabitats2

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9:15-9:45 Keynote conference: "**Advances in monitoring spatio-temporal trends for Mediterranean bioinvasion research and Marine Protected Areas**" by **Ernesto AZZURRO**, CERRI J. TESTAGROSSA A., LEK team

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15:00-15:15 "**Decline of *Caulerpa taxifolia* in the Adriatic Sea**" by **Ante ŽULJEVIĆ**, CVITKOVIĆ I., DESPALATOVIĆ M., LUČIĆ P., ŽUNEC A., LUŠIĆ J.

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KEYNOTE CONFERENCES

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ADVANCES IN MONITORING SPATIO-TEMPORAL TRENDS FOR MEDITERRANEAN BIOINVASION RESEARCH AND MARINE PROTECTED AREAS

Abstract
Determining the distribution of invasive species and tracing back their spatio-temporal dynamics, is crucial to feed early warning systems, risk assessments and to mitigate impacts through management actions. However, in the marine environment, this task can be particularly problematic because historical data are often missing and biological invasions can be neglected until they become sufficiently abundant and pose socio-economical concerns.
Here, drawing on recent experiences on Local Ecological Knowledge (LEK) and on its application through different Mediterranean countries, we report three different case studies to exemplify potential applications of eliciting LEK to retrace the dynamics of marine invaders. A growing interest on these methodologies is highlighted in either Mediterranean studies and worldwide, reflecting the need of broad ecological observations and by the LEK value as a mutually beneficial action to social and ecological systems. The application of standard LEK protocols across boundaries and jurisdictions can overcome some of the limits of bio-invasion research, providing a strategic and practical complement to traditional surveys and occasional species records. This practice is now being transferred to Mediterranean Marine Protected Areas and tested for the monitoring and management needs of small-scale fishery.

Key-words: Non-indigenous species, poleward range expansions, mapping, management.

Introduction

Tracing out the spatio-temporal dynamics of non-indigenous species is crucial for risk assessments, early warning systems and to unravel ecological factors underlying the emergence and spread of biological invasions. However, surveying marine environments is more challenging than for terrestrial ecosystems and researchers are seldom capable to monitor species distribution in real time or to fully understand historical changes, especially over large geographical scales. These difficulties ultimately lead to incomplete or missing information in existing datasets which might prevent us from reconstructing invasion dynamics back to their emergence. In the last two decades, as a partial solution to unsatisfactory ecological information, researchers, policy makers and Marine Protected Areas, became interested into the available ecological information that could be obtained from local communities living in close contact with nature. This knowledge, often termed "*Local Ecological Knowledge*" (LEK), can be defined as the 'information that a group of people has about local ecosystems'. We must consider that social groups such fishermen and divers rely on the marine ecosystem for their livelihood and gain ecological information through their everyday experience. Accessing this knowledge provides new opportunities for marine research, while achieving broader goals such as increased awareness and environmental literacy. In this paper, we will consider a few case studies to illustrate potential applications of LEK to study and monitoring biological invasions in the Mediterranean realm.

Materials and methods

LEK data are generated through a collective and coordinated effort based on the volunteer engagement of an international team of researchers and technicians (LEK team) well connected with local fishery communities. Small scale-fishermen were interviewed face-to-face, through a standard semi-structured interview protocol, developed and implemented by a number of Mediterranean research programs (see acknowledgements). Respondents were asked to reconstruct changes in both distribution and abundances of marine species, retrospectively. Fishermen ranked each species on a yearly timeline and through 6 classes of abundance: “Absent”, “Rare (observed once per year)”, “Occasional (observed sometimes in a fishing period)”, “Common (regularly observed in a fishing period)”, “Abundant” (regularly observed and abundant in a fishing period), “Dominant” (observed at every fishing session and with great abundances in a fishing period). Respondents draw a line on a pre-printed diagramming table, to express how these abundances had changed across time (see Annex 1). This graphical approach was adopted to facilitate memory retrieval, minimizing cognitive effort and recall bias. Moreover, interviews were supported by illustrated identification manuals, to help fishermen assigning each species correctly and to check for consistency and knowledge in respondents’ judgements. Examples provided here are extracted from a much larger dataset (Azzurro et al. *in prep*), which includes interviews realized in 95 locations, across 9 countries and 7 different subsectors in the Mediterranean. In the participating countries, specific trainings on the application of the interview protocol were provided through both, theoretical lessons and demonstrations of practical interviews made in collaboration with local fishermen. Participating researchers were guided in performing standardized interviews and advised on how to limit risks of potential biases, such as the ones related to taxonomical identification and ‘memory recall’ bias (Hassan, 2006). Data gathered from this collaborative effort was used to gain information on both indigenous and non-indigenous species, perceived as ‘new’ or increasing in each fishing area. In our first case (*Case I*), we extracted spatio-temporal data in perceived abundances of the indigenous range-expanding bluefish *Pomatomus saltatrix* from the Ligurian Sea and used breakpoint analysis to establish in which year respondents noted this species to have increased the most in abundance. Breakpoints, corresponding to years when the time series of perceived abundances differed from the overall trend, were assessed through changepoint detection based on the residual sum of squares (Zelleis *et al.,* 2015). We also explored how first records were associated with the longitude of the various fishing areas from which respondents came from. In our second case (*Case II*), we extracted the first sighting of the bluespotted cornetfish (*Fistularia commersonii*) (N = 154 interviewed people) filtering out those reported before the onset of the invasion in 2000 (N = 28). These georeferenced points were sorted along the temporal and latitudinal axes and compared with the known chronology of the species. In the third example (*Case III*) we show how to map the perceived abundances of an invader. We took the case of the Atlantic Blue crab (*Callinectes sapidus*), in the Lesina lagoon, a saltwater marsh located on the Adriatic coast, in Southern Italy. The interview protocol was here implemented with a section in which respondents were asked to draw how the perceived abundances varied in space. For this latter task, a participatory mapping methodology (Fagerholm *et al.,* 2012) was used and 25 fishermen participated to draw sectors with homogeneous abundances on a pre-printed map. Then abundances were imported by researchers on a vectorial grid on QGIS. Cohen's kappa was adopted to verify the agreement between respondents on how the Atlantic blue crab varied its abundances in the lagoon.

Results and discussion

Case I: The bluefish *Pomatomus saltatrix* is not an exotic species for the Mediterranean Sea, but its outbreak in the Ligurian sea is a good example to demonstrate how the arrival and subsequent increase in abundance of a species can be traced back to its emergence. Indeed, most of the interviewed fishermen had never seen this fish before the nineties. Fig. 1 shows a rapid increase in (perceived) densities, with significant breakpoints identified in three distinct occasions: 1996, 2003 and 2009. This means that in about 20 years, the bluefish jumped from the lowest levels of abundance (rare or absent) to the level of a common species, which much interacts with fishing activities and other native predators, such as *Dicentrarchus labrax*. This information is now used to feed vulnerability assessments and to support management plans of local Marine Protected Areas (*i.e.* Portofino MPA).

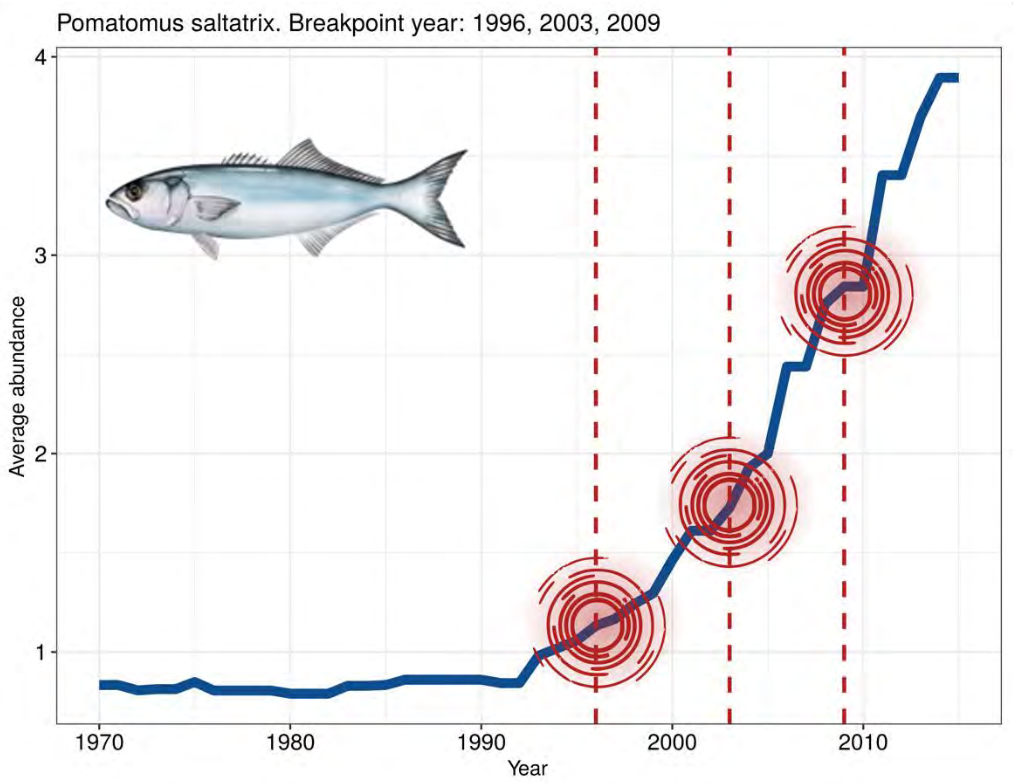

Fig.1: The bluefish *Pomatomus saltatrix* is indigenous for the Mediterranenan, but the temporal evolution of perceived abundances show as this species was formerly very rare in the Ligurian Sea (NW Mediterranean). Breakpoint analysis highlights three breakpoint years in 1996, 2003 and 2006. DATA extracted from the LEK team database: Query: *P. saltatrix*/Ligurian Sea; N = 52 interviewed fishermen.

Case II: LEK generated data are also coherent with the spatio-temporal dynamics of the invasion. By extracting the first LEK generated sightings of *F. commersonii* in the Mediterranen Sea, we can appreciate as the temporal evolution of this species reconstructed through fishermen's observations followed an East to West gradient, matching both, the direction and the spread rates reported by the scientific literature (Azzurro *et al.,* 2011). This exercise illustrates how the history of a marine bio-invasion can be reconstructed at the regional scale and through a coordinated observation system (Fig. 2).

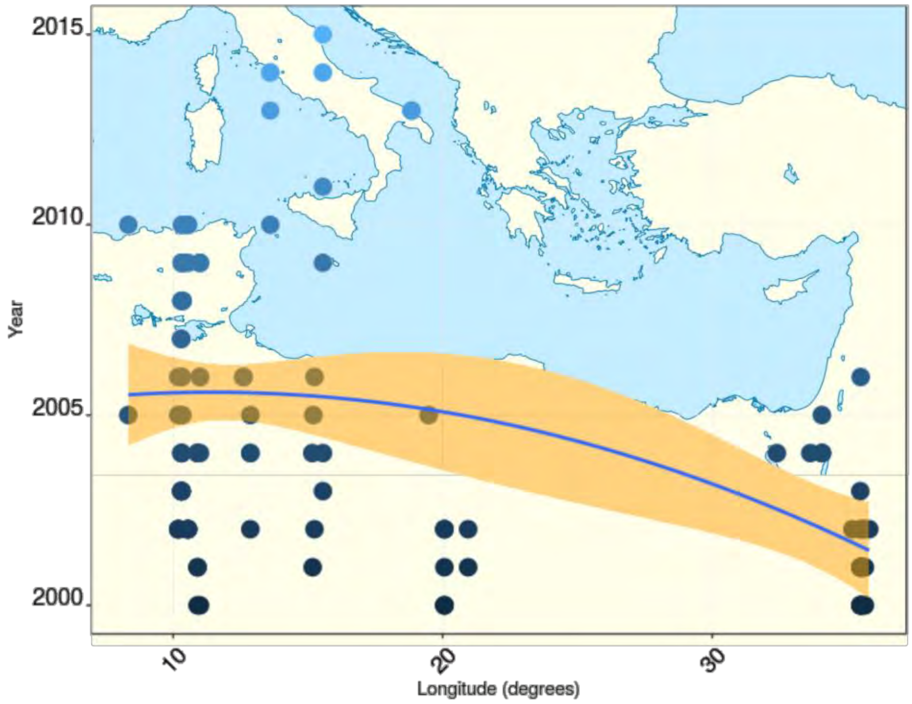

Fig. 2: Relationship between the latitude and the year of the first sightings of the bluestpotted cornetfish *Fistularia commersonii*, as reported by fishermen's interviews (N = 126)

Case III: In our third case we show LEK as a valuable tool for mapping the distribution of invasive species. Our experience with the fishermen of the Lesina Lagoon (Azzurro et al., *in prep*) proved that respondents had a relatively good level of agreement about the spatial distribution of the Atlantic blue crab *C. sapidus* (Cohen's kappa = 0.38; Fig. 3). Moreover, changepoint analysis demonstrates that this species increased its densities after 2010, becoming a dominant invader in the saltmarsh community after 2005. Looking at the participatory map (Fig. 3), it is clear that the invader is widely distributed in the lagoon, reaching its highest abundances in the North-Eastern sectors. The high level of agreement between respondents demonstrates the potential of LEK for mapping the abundance and distribution of invasive species and this low-cost approach could be particularly relevant whenever information from ecological surveys is scarce or not available (like in our case study). Moreover, LEK-based participatory mapping (see also Chambers *et al.,* 2006) might be extremely useful for monitoring species abundances during the application of control measures. In this context, rapid assessments made in collaboration with local communities can be very informative to understand how biological invaders respond to such initiatives, either in terms of their abundances and in their spatial distribution. A similar application of LEK for participatory mapping could also be extended to those contexts where a non-indigenous species are subjected to intensive commercial exploitation. These are just some examples to show the potential of monitoring marine bioinvasions by accessing LEK in a standard, structured and coordinated manner. A complementary monitoring approach, which empowers the observational potential and awareness of people living in intimate relationship with the natural environment.

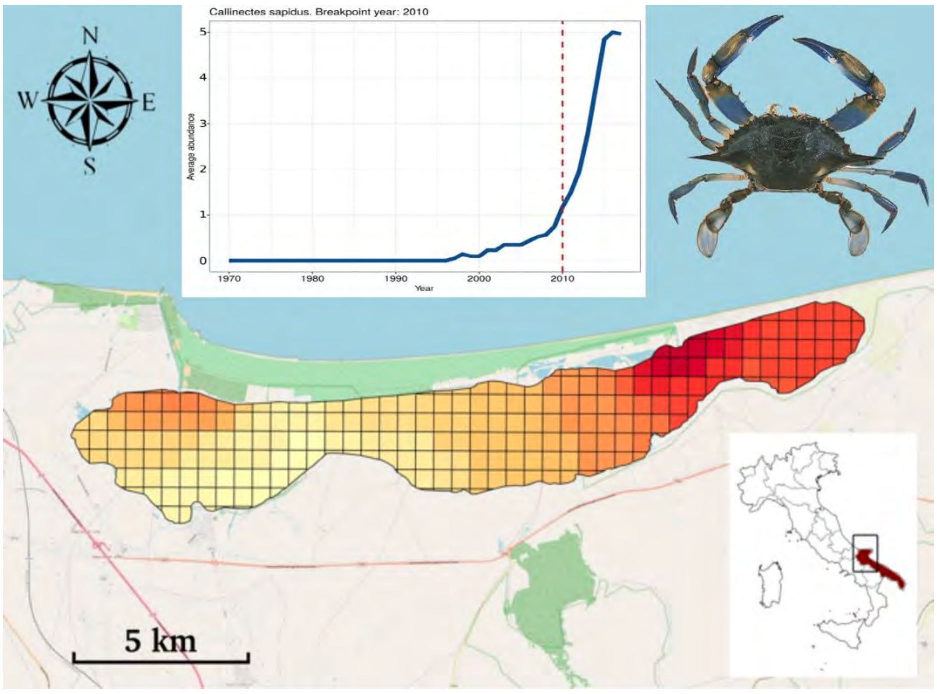

Fig. 3: Distribution of the Atlantic blue crab (*Callinectes sapidus*) in the Lesina lagoon, Italy as reconstructed by a participatory mapping exercise realized with (N = 25) small-scale fishermen during the period March-June 2008. Values ranged from "Rare" (light yellow) to "Dominant" (dark red). Cohen's kappa = 0.38. The upper graph shows the breakpoint analysis of the temporal evolution of average abundances of this species in the same area.

Conclusions

We are all living through an age when there is a sense and a reality of accelerating change, with dramatical ecological alterations often happening before our eyes. Hopefully our case studies can help to explain how the everyday experience of people living in close contact with these changes can be used to provide relevant information to contemporary ecologists and decision makers (see also Raymond *et al.,* 2010). In relation to the issue of Mediterranean bio-invasions, specific social groups such as small-scale fishermen and divers can be particularly informative and LEK actions can be effective in empowering them in detecting, monitoring, mapping and ultimately managing these species. If properly elicited, LEK can be adopted to integrate (or to replace if absent any other source of data), available ecological information from environmental surveys. Moreover, the use of semi-structured protocols for the elicitation of LEK could disclose further applications to understand how relevant stakeholders perceive biological invasions in relation to their ecological, cultural and economic dimension. We argue that a wide adoption of LEK might help researchers and policymakers both to better understand and to have more updated pictures about invasion dynamics at the local or even regional level. These processes need to be systematic and coordinated across boundaries and jurisdictions to ensure common responses to a common environmental problem.

Ethical statement

Data collection was confidential, as interviewers did not record any sensitive personal information about respondents. At the beginning of the interview, respondents were informed about the purposes of the study and gave informed consent to use the provided information for scientific purposes.

Acknowledgements

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MEDITERRANEAN SEA: 30 YEARS OF BIOLOGICAL INVASIONS (1988-2017)

Abstract
The number of new alien species invading the Mediterranean Sea, which peaked in the 2000-2005 period with more than 20 new species per year appears to be declining with 16 species on average introduced in the 2012-2017 period. This drop is documented for all taxonomic groups with the exception of fishes. While the number of new alien fishes introduced via the Suez Canal follows the same declining pattern, the number of intentionally released fish species, related to aquarium trade, is climbing up, particularly so in the central Mediterranean. At country level, the new proposed indicator (number of new alien species per 6 years period) is inconclusive, a fact attributed to lack of previous studies in high risk areas. At the same time number of new alien species per pathway highlights the role of aquarium trade as a pathway. Prioritization of alien species likely to arrive in an area, through horizon scanning, is necessary at country level as a precautionary measure towards. Finally, involvement of citizen scientists is encouraged at national, regional and Pan-European level.

Key-words: Trends indicator, pathways, Horizon scanning, Citizen scientists

Introduction

Pursuant to several decisions of the UNEP/MAP Contracting Parties, specific efforts were made during the past decade to implement the ecosystem approach (EcAp) with the objective to achieve the Good Environmental Status (GES) of the Mediterranean. The GES has been defined through eleven Ecological Objectives (EO) and their achievement is being monitored with the help of 27 indicators. These indicators are at the basis of EcAp's Integrated Monitoring and Assessment Program (IMAP). To enable the implementation of the EcAp process and in particular IMAP, the EcAp MED II project 2015-2018 supported by the European Union, focuses on the strengthening of the interface between science and policy. The overall objective of the EcAp-MED II project is to support the UNEP/MAP Barcelona Convention and its Southern Mediterranean Contracting Parties to implement the EcAp in synergy and coherence with the implementation of the European Union's Marine Strategy Framework Directive (MSFD). Among the proposed indicators, towards achievement of EO2, **Indicator 6** addresses "*Trends in abundance, temporal occurrence and spatial distribution of non- indigenous species, particularly invasive non-indigenous species, notably in risk areas in relation to the main vectors and pathways of spreading of such species"* and corresponds to descriptor 2 of the MSFD. MSFD (EU, 2008) is the environmental pillar of EU Integrated Maritime Policy that sets as an overall objective to reach or maintain "Good Environmental Status" (GES) in European marine waters by 2020. The MSFD update (COMMISSION DECISION (EU) 2017/848 of 17 May 2017) states that "The number of non-indigenous species which are **newly introduced via human activity** into the wild, per assessment period (**6 years**), measured from the reference year as reported for the initial assessment under Article 8(1) of Directive 2008/56/EC, is **minimised and where possible reduced to zero**". Furthermore, "Member States shall

establish the threshold value for the number of new introductions of non-indigenous species, through regional or subregional cooperation".

The EU policy which addresses exclusively invasive alien species (IAS) is the EU Regulation (No 1143/2014) establishes rules to prevent, minimise and mitigate the adverse impact on biodiversity of the intentional and unintentional introduction and spread within the EU. With regards to pathways, a number of International Policies address specific (shipping: IMO, 2017) or inclusively all pathways (CBD, 2014). The absence of marine invasive alien species (MIAS) from the list of the European Union Regulation 1143/2014 (IAS Regulation) does not match the magnitude of the threat they pose to the marine environment, particularly so to the Mediterranean

Horizon scanning (HS) is the systematic examination of future invasive alien species with the potential to threaten biodiversity and human health. The first Pan-European Horizon Scanning (HS) exercise focusing exclusively on marine alien species, aiming to deliver a ranked list of species that should be of high priority for risk assessment and possibly future inclusion in the EU IAS list was organized by JRC (4-5 October 2018, ISPRA).

Knowledge of the invasion process is essential in designing management plans to cope with the potential detrimental effects of invasive species, and to attempt to prevent their large-scale spread. The present work addresses trends in pathways/vectors for all Alien species (MAS) in the marine and estuarine environment.

Materials and methods

In the lack of data on impacts (as well as trends) of most of the Alien species in European Seas, the trends in MAS (all introduced species) is used as a proxy of the trends of the most invasive marine alien species.

Considering that the status of 2011 will be the baseline for the assessment of any D2 related indicator, trends were calculated as number of New MAS per 6-year intervals at Mediterranean, subregional and country level, the latest period being 2012-2017. A simple information system herein called HCMR/EEA database has been developed in HCMR since 2002 to serve as a resource in developing a trends indicator and for reporting to EEA. The Mediterranean component of it has been transferred to EASIN. The list is under constant update considering the new findings and a degree of uncertainty in the alien status of some species. Miscategorising alien species as native is not rare. Many pseudoindigenous species occur in the Mediterranean, precisely because many old taxonomic works originated in the Mediterranean. Phylogenetic studies have revealed that some records of cryptogenic species belong to true aliens.

The list of alien species in the Mediterranean has undergone a major revision and cleared out (Zenetos et al., 2017). The list for Mediterranean alien species is still an underestimate as it does not include monocellular algae while the status of foraminiferans is under revision.

In addition, there is always uncertainty in the true year of introduction. Even if the first detection date was the true introduction time, the trends in introduction suffer because of the time lapse between observation and publication. Species collected in the 2016-17 period are expected to be published in the next 2 years (Azzurro et al., 2016; Zenetos, 2017).

Pathways describe the processes that result in the introduction of alien species from one location to another. Identification and categorization of pathways follows the CBD classification scheme (CBD, 2014). Information on vectors are mostly derived from the authors' speculations, since specific research projects aimed at identifying vectors and occurrences are complicated and demanding large resources. Unless found when deliberately moved, evidence of actual transfer is seldom known. In a large number of cases, likely pathways are merely inferred, for example considering the most common activity occurring in a specific location (shipping, aquaculture), but no scientific evidence is provided.

Results

All analyses are based on 491 taxa observed for the first time since 1988. Figure 1 shows the contribution of marina taxa in the MAS composition in the Mediterranean. The trend in introduction of MAS in the Mediterranean which culminated in the 2000-2005 period with more than 20 new species per year (122 in total), appears to be **decreasing** over the last 6-year (96 species) (Fig. 1). As opposed to Invertebrates and Primary Producers, Vertebrates appear to increase with 34 species detected in the 2012-2017 period *vs* 26 species in the period 2006-2011. The decreasing rate in MAS is evident across the Mediterranean MSFD areas with the exception of the Central Mediterranean where an increase is observed attributed to Vertebrates (Fig. 2). Indeed, 17 new fish alien species were detected in the central Mediterranean in the period 2012-2017 vs 8 fishes in the period 2006-2011. These are MAS either spreading from the eastern Mediterranean to the central or newly introduced species in the area.

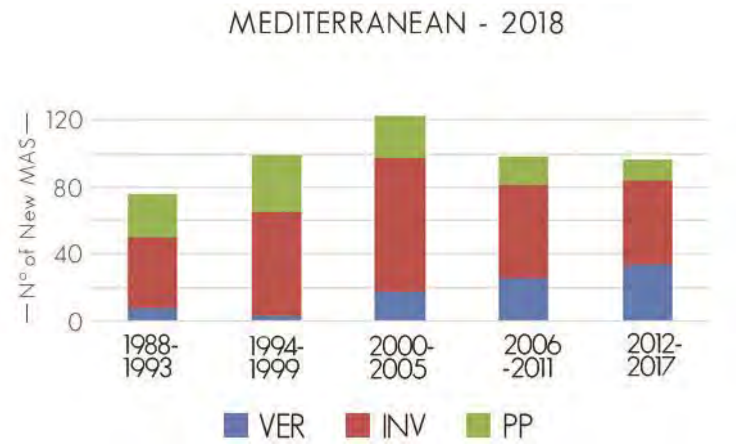

Fig. 1: Number of New MAS per 6 years in the Mediterranean since 1988. VER=Vertebrates, INV=Invertebrates, PP=Primary producers.

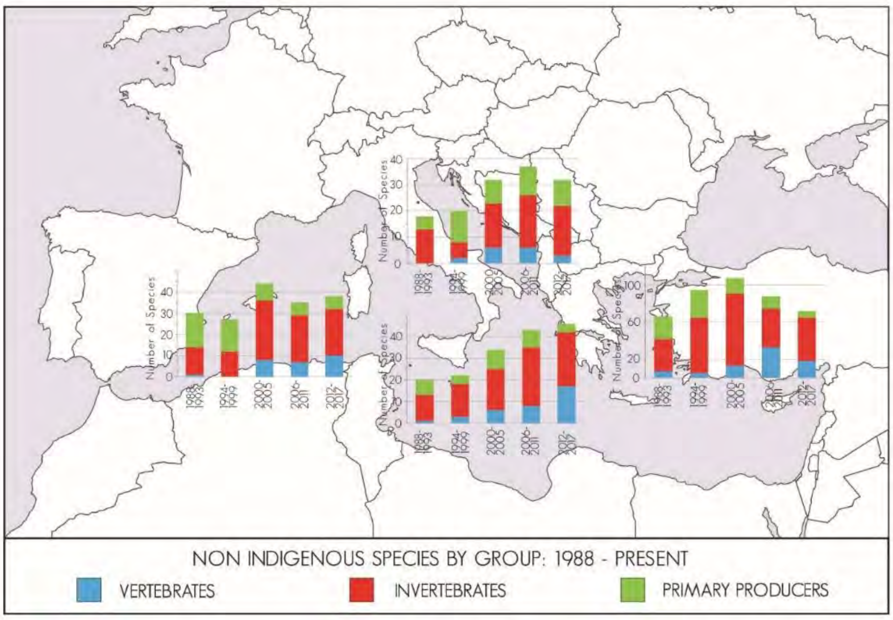

Fig. 2. Number of new MAS per 6 years in the Mediterranean MSFD regions since 1988

At national scale, trends of alien taxa per EU country since 1988 is depicted in figure 3. Greece has received the highest number of MAS over the last 30 years, followed by Italy, and Cyprus. However, an increasing trend is evidenced in all countries except Greece, where a slight decline is observed. The peak in new MAS was observed in Cyprus (45 species) in the period 1994-1999, whereas the number of biological invasions is far less in the east Adriatic Sea countries (Fig. 4).

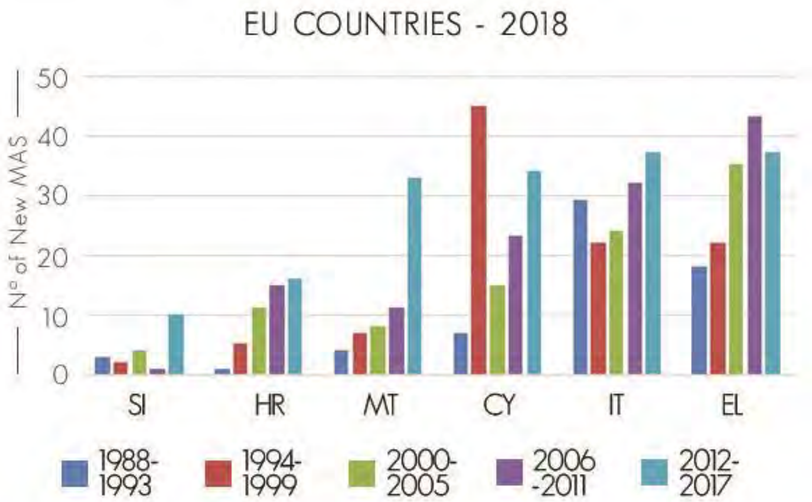

Fig. 3: Number of new MAS in EU Mediterranean countries since 1988 per 6 years. France and Spain are not presented here as they have also Atlantic borders.

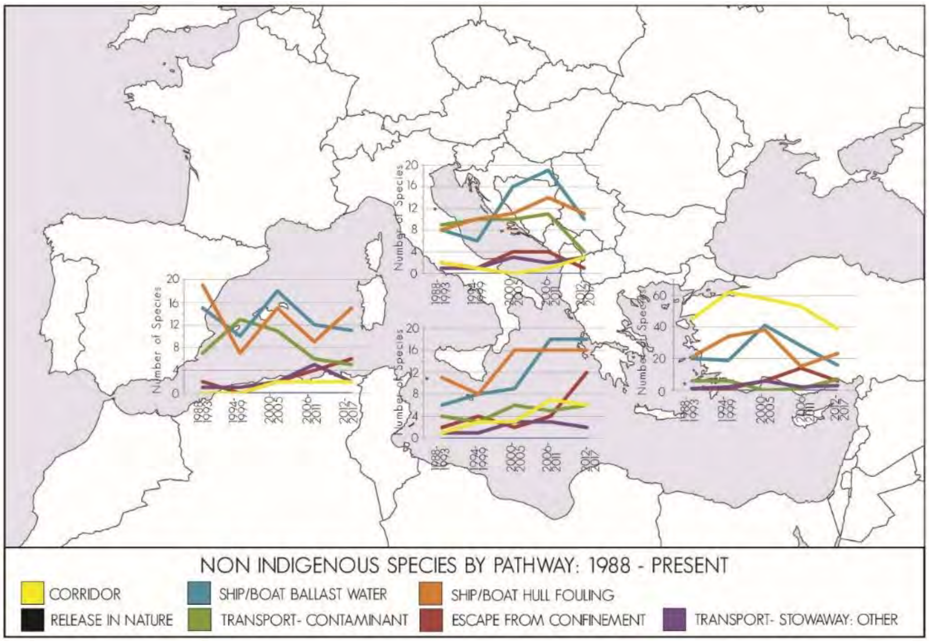

Fig. 4. Role of pathways in the introduction of MAS **in the Mediterranean MSFD regions since 1988**

Discussion

The updated Mediterranean MAS list which counted 821 multicellular species in December 2016 (Zenetos et al., 2017), counts to date (November 2018) 957 species including Foraminifera. Among the introduced MAS during the last 30 years (491 taxa), Invertebrates dominate with >58% (287 species) represented mostly by molluscan and decapods. Primary producers follow with approximately 114 species among which macroalge and in particular rhodophytes prevail. Vertebrates (mostly fishes) follow closely with 90 species. The trend in introduction of MAS in the Mediterranean appears to be decreasing after 2005. Interesting is the relative decrease in introductions in the eastern Mediterranean (Zenetos, 2017), where, the enlargement of the Suez Canal was perceived as a double trouble (Galil et al. 2015). Vertebrates (fish only) are dominated by Lessepsian immigrants but over the last decade the number of fish species related to aquarium trade which have been intentionally released in the wild (classified as escapees from confinement) is increasing (Zenetos et al., 2016; Marcelli et al., 2017; Deidun et al., 2018 etc).

Analysis of the initial reporting lists of MAS revealed important knowledge and data gaps, as well as vague definitions and significant differences on the level of detail and focus of the approach followed by the MS, pointing the need for common standards (Palialexis *et al*. 2015). Besides monitoring, all Mediterranean EU countries are currently validating their check lists for the needs of D2 (e.g Greece: Zenetos et al., 2018) and the output is stored in EASIN, but non EU countries are also carrying out monitoring of MAS and publish their results [Tunisia: Sghaier et al., 2016; Ben Amor et al., 2016 - Lebanon: Bitar et al. 2017 - Algeria: Grimes et al., 2018- Libya: Shakman et al., 2017].

The increasing rates in the 2012-2017 period at national level are mostly the result of a study of NIS focused on marinas in Spain, France, Italy, Malta, Greece, and Cyprus, during which many new species were reported (Ulman et al, 2017). However, the findings of Ulman et al. (2017), should be attributed to lack or previous studies in the studied areas than to recent introductions since the reported MAS are mostly widespread alien species whose introduction is related to vessels. Thus, presently the results are inconclusive. Increasing trend at national level does not necessarily imply BAD GES. At national level further studies are needed in high risk areas including MPAs.

A preliminary list to species to be evaluated by means of HS in the Mediterranean is reported in Zenetos (2016). In addition to the first HS at national level (Cyprus) in the Mediterranean in the framework of a research project, HS has been conducted for marine species in Croatia, Slovenia and Greece in the framework of the EU MEDCIS project (*"MEDCIS" No. 11.0661/2016/748067/SUB/ENV.C2)*- results not presented here.

Engagement of citizen scientist is crucial and has proven to be a valuable tool towards a) enriching the country lists of MAS (Giovos et al., 2018; Crocetta et al., 2017). and b) monitoring the invasive MAS (Azzurro et al., 2018). What figure 3 shows clearly is that Italy, Cyprus, Malta, Slovenia host many MAS which are yet unreported. The check list of Cyprus is expected to increase significantly due to concerted actions by the national authorities' especially in Marine protected areas.

Actions to be considered

- ✓ Updating the distribution lists based on data to be collected/reported in the course of future monitoring projects in risk areas at national level.
- ✓ Considering the difficulties of reporting new alien species and monitoring the invasive ones, engage citizens and NGOs into reporting selected target species through national/international networks such as the jellyfish watch, eye on earth, spot the alien fish, etc.

- ✓ Developing national networks, depository systems, databases on MAS at national level.
- ✓ Expanding the existing system by incorporating info on impacts of as many invasive MAS as possible.

Acknowledgements
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ORAL COMMUNICATIONS

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THE CORSICA ALIEN NETWORK: A TOOL FOR MONITORING AND TRACKING MARINE EXOTIC SPECIES

Abstract
In the marine environment, the introduction of alien species, and the biological invasions that can result, constitute one of the main ecological, socio-economic and health threats. The identification of new marine exotic species in Corsica has encouraged regional partners (Office de l'Environnement de la Corse, Direction Régionale de l'Environnement, de l'Aménagement et du Logement) to extend the approach, initiated in 2003 for Caulerpale, to all alien species, by setting up, in 2015, the Corsica Alien Network (RAC). The aim of this network is to identify as early as possible any arrival of new species along the coasts of Corsica, and to monitor their spread, in order to propose appropriate management measures. A census of the available data has shown that about fifty alien species have already been reported in Corsica. Several communication tools have been developed (e.g. species descriptions, sighting report forms, etc.) and made available to inform RAC partners and the public of the presence and potential threats of these species, and to facilitate their identification. 50 observations of introduced marine species were recorded in 2017. While these observations concern mainly Caulerpa cylindracea*, which is present along almost the entire coastline, we note an increase in the presence of the invasive crab* Percnon gibbesi. *By means of regular monitoring of the areas likely to shelter it, 43 reported sightings were recorded and 124 individuals counted during the year 2017 (no individual was observed between December 2016 and May 2017), and weekly monitoring was introduced in June 2018. To facilitate the dissemination of information, the results are communicated at the end of the season to IUCN Mediterranean for integration within the MedMIS platform.*

Key-words: Monitoring, exotic species, invasive species, Corsica, *Percnon gibbesi*

Introduction

Marine biological invasions became more frequent and more widespread during the twentieth century with the increase in human activities (Streftaris *et al.*, 2005; UNEP-MAP-RAC/SPA, 2011). These alien species, also known as "introduced, non-native or allochthonous species" (Otero *et al.*, 2013), can cause the disappearance of native species, leading to the reduction and extinction of populations. These invasions are considered as the second leading cause of biodiversity loss after habitat destruction, and are one of the top four threats to the world's seas (PNUE-PAM-Plan Bleu, 2009).

The introduction of exotic species is a permanent phenomenon throughout the Mediterranean, although it appears to be more prevalent in the Eastern Basin, due to the proximity of the Suez Canal. In 2012, 986 exotic species were recorded in the Mediterranean sea (775 in the Eastern Basin, 249 in the Central Zone, 190 in the Adriatic Sea and 308 in the Western Basin; Zenetos *et al.*, 2012). All exotic species are not problematic, but can become so when they establish populations and propagate in a new environment. In 2002, the Convention of Biological Diversity (CBD, 2002) defined an invasive alien species as "an alien species whose introduction and / or spread threatens biological diversity". This highlights, inter alia, the need to prioritize prevention to fight the introduction of invasive alien species, both within and between countries. To this end, it is essential to detect invasive alien species as early as possible in order to take early action to prevent them from becoming permanently established.

Corsica's coastline is no exception to this problem, and for this reason in 2003, the *Office de l'Environnement de la Corse* (OEC) launched the Corsica Caulerpa Network, specifically dedicated to the monitoring of the introduced species *Caulerpa taxifolia* and *Caulerpa cylindracea*. In 2015, the observation of new alien species prompted the OEC to extend monitoring to all exotic marine species, setting up the Corsica Alien Network (RAC). This network is an original association of scientists (University of Corsica), divers (*Fédération Française d'Etudes et de Sports Sous-Marins*), trainers in environmental education (U Marinu) and Corsican stakeholders (*Direction Régionale de l'Environnement, de l'Aménagement et du Logement* & OEC). The purpose of the RAC is thus to identify as early as possible any arrival of new species along the coast of Corsica in order to alert the public to the potential threats related to these species, and to implement, if possible, management measures to restrict their negative impact on the environment or on human activities.

Materials and method

Among the numerous exotic species recorded in the Mediterranean, a first selection has been made on the basis of blacklists and available inventories, prioritizing species already observed in the Western basin, and then the focus was narrowed to species sighted along the Corsican coasts. Several communication tools have been made available for different audiences (e.g. public, recreational fishermen, divers, etc.) to facilitate the data collection, transmission of information and identification of species sighted.

A standardized form for sightings was produced for the purpose of collecting the data. This form presents essential information, such as (i) the observer's coordinates, (ii) the location of the observation, (iii) the species observed, and the manner of transmitting the data. To simplify the identification of exotic species, around twenty species identification sheets were produced and distributed in the various diving clubs of the island. Organized on the basis of the main taxonomic groups (Microalgae, Macroalgae, Ctenophora, Cnidaria, Bryozoa, Mollusca, Annelida, Crustacea, Tunicata and Pisces), they are identified by a color code and an icon. They include the scientific and common names of the species, its geographical origin and a summary of the important features: (i) identification of the species, (ii) its environment and / or way of life (substrate, depth, eating habits, behavior), (iii) invasiveness (mode of spread, hazards, potential risk), as well as significant differences from similar species (Monnier *et al.*, 2017).

After receiving the forms, scientists and specialists registered within the RAC are responsible for confirming the authenticity of the sightings on the basis of photographs or samples sent in by the observers. If necessary, and if possible, the sighting is verified *in situ*. After this step, the sighting is indexed in a geographic information system (GIS), so that it can be viewed online or via Google Earth©, and shared by all the partners of the RAC. At the end of the year, all the validated data are sent to International Union for Conservation of Nature and uploaded in the Mediterranean Marine Invasive Species platform (MedMIS).

Following the increase in reported sightings of the invasive crab *Percnon gibbesi*, in 2016 (Monnier *et al.*, 2017), active monitoring has been initiated, in the shallow rocky areas that constitute favorable habitats for this species. A visual inspection of these favorable areas is performed, either with a bathyscope, or by observation by swimmers or divers (snorkeling or scuba-diving). For each sighting of *Percnon gibbesi*, the GPS coordinates of the observation, the depth, the temperature of the water, the characteristics of the specimen (size, sex) and of the habitat, are noted. In addition, following regular sightings of this crab on the East coast, monitoring has been initiated on the seawall of the harbour of Taverna, between June to October 2017, and weekly since June 2018.

Results

The sightings collected to date indicate that there are around 50 introduced marine species in Corsica. However, some old bibliographical sightings (*e.g. Sargassum muticum,* mentioned in 1992 in Diana lagoon) require confirmation. Most of the sightings are from scientists, due to the difficulty of identification of several taxonomic groups (*e.g.* micro-algae). In contrast, the sightings made by divers, in the framework of the RAC (50 reports collected in 2017), concern less than ten species, usually easy to recognise. Only 2% of these observations are not been validated due to a lack of information, 70% were validated from photographs and 20% by experts. The species sighted are: *Caulerpa cylindracea* (48%), *Percnon gibbesi* (22%), *Asparagopsis taxiformis* (16%), *Callinectes sapidus* (8%), and *Parabennius pilicornis*, *Codium fragile* & *Womersleyella setacea* (2% each).
Concerning the monitoring of *Percnon gibbesi,* 12 sites were surveyed in Corsica in 2017. No crabs were sighted until mid-May 2017, despite active surveys, notably since the end of March 2017. In the same way, in certain areas, favorable to the species and visited several times between mid-May and early October, no crabs were sighted. Overall, 43 reported sightings resulted in the observation of 124 individuals over the whole period. Most crabs are observed between the surface and 1 m depth, although some of them are found at down to 4 m depth. Each crab was sighted in a rocky environment, with dense cover of algae in the vicinity, although they are themselves positioned in unvegetated fissures. Moreover, the presence of the sea urchin *Arbacia lixula* is often noted. However, crabs seem also to be very common on the artificial substrate (concretes) of seawalls. Among the various methods of observation, the bathyscope was ineffective, but no significant differences were observed between snorkeling and scuba diving.
In 2017, the Taverna harbour seawall was noted for its impressive population of *Percnon gibbesi*, with a total of 63 individuals sighted between June and October 2017, and a maximum of 31 individuals in one survey, at the end of August. We observed an increase in the size of individuals during the observation period, and at the end of August, some juveniles are detected. In 2018, the frequency of monitoring was increased to once a week, in order to better monitor the presence of crabs along the seawall. Thus, 140 individuals, of ever-greater size, were observed between June and September 2018, with a maximum of 14 individuals in one survey at the end of July (Fig. 1). This size pattern trend is consistent with those observed the previous year, with the identification of the first juveniles at the beginning of September. Firstly, higher concentration of crabs is noted in July and August 2018, with respectively 33% and 34% of the individuals found in this season, with a peak of observations in the last two weeks of July (14 individuals observed by prospecting), while 19% of *Percnon gibbesi* were observed in September 2018, and 15% in June. Over the entire period, 53% of the individuals observed were females, and 36% were males (the sex of 11% of the specimens could not be determined). Furthermore, 82 % of the individuals observed were medium or large sized (between 2 to 3.5 cm), 8% small sized (between 1.5 and 2 cm), 6% of juveniles (< 1.5 cm) and 4% of very big (> 3.5 cm).

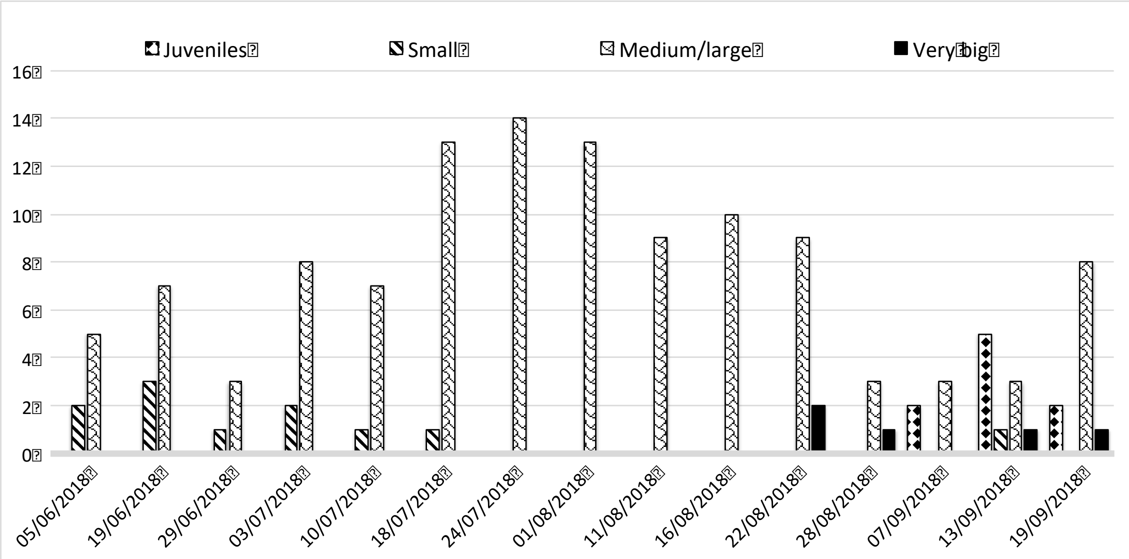

Fig. 1: Reported size / number of *Percnon gibbesi* by prospection

Discussion and conclusions

Compared to previous results (Monnier & Pergent-Martini, 2016), the number of reported sightings is rather similar in 2017. On the other hand, the species sighted differ somewhat. There was no sighting concerning *Fistularia commersonii* in 2017, although the species seems to be present along the entire coastline according to Bodilis *et al*. (2011). It is possible that the main partners of the RAC (FFESSM diving clubs) are not the best observers for this species; moreover, its resemblance to the Orphie *Belone belone* can lead observers to confuse them. The same remark is probably true for most of the fish species. For the species *Caulerpa cylindracea*, as in 2016 (Monnier *et al*., 2017), new sectors were surveyed, resulting in new reported sightings, which confirms the presence of the species on the whole of the Corsican coastline. The second most frequently reported species is the Crustacean *Percnon gibbesi*. While in 2015, the species was reported only at Balagne and the Gulf of Ajaccio (Monnier *et al*., 2017), it is present today at Cap Corse, on the east coast (Bastia, Taverna harbour) and in the south-east of Corsica (from Favona to Santa Gjulia), demonstrating a rapid spread along the Corsican coasts. It would seem, therefore, that the spread of the species along the Corsican coasts has been rapid. Sightings doubled in 2016 compared to previous years, and increased fourfold in 2017. Finally, the geographical distribution of these sightings proves that *Percnon gibbesi* has already colonized much of the coastline. The attempt to monitor this crab in 2017 resulted in a lack of sightings when the water temperature was less than 20°C. Landeira & Lozano-Soldevilla (2018) underline in their study the absence of larvae in February in the Canary Islands. It is likely that climate change plays an important role in the dispersal of the species (Felix Hackradt *et al*., 2010). We noted an increase in the number and size of crabs in phase with an increase in the temperature of the water. Similarly, an increase in the number of individuals was demonstrated during the summer of 2005 in the Gulf of Messiniakos (Greece), as well as a significant decline during the winter of 2005-2006 (Thessalou-Legaki *et al*., 2006). This decrease in the winter population may support the hypothesis that water too cold for *Percnon gibbesi* would influence its development. Moreover, before spreading throughout the Mediterranean, this species extended preferentially to areas with the warmest temperatures, and was therefore absent from colder areas, which may indicate a need for higher temperatures in order to survive (Katsanevakis *et al*., 2011). We also note the absence of *Percnon gibbesi* at certain sites with conditions favorable to its presence. Knowing that a large number of species have developed behavioral strategies to minimize the exposure of larval stages to stressful conditions (Ji *et al*., 2010), and that *Percnon gibbesi* shows a preference for the high temperatures of subtropical seas (Manning & Holthuis, 1981), it is possible that the cold water in Corsica in winter prevents its total establishment. In 2018, monitoring revealed a decline in the number of individuals per survey, with a maximum reached in 2018 of 14 crabs, compared to 31 in 2017. This difference observed in the same area could be explained by the peak of very late cold conditions, observed in Corsica on 27 February 2018, as well as the lack of sunshine and snowfalls in the plains (Météo-France, 2018). Since this crustacean prefers waters between 16°C and 31°C (Galil, 2011), it is possible that the late increase of temperatures that year caused a delay in its propagation and a limited colonization. In general, the annual cycle of temperate decapod larvae has two peaks in abundance, one in spring and the other in summer (Pan *et al*., 2011). If we were not able to observe the juveniles in spring, the second breeding season was not however delayed because in both cases, juveniles were reported over the same periods (28 August 2017 and 7 September 2018). The only difference concerns the presence of very large crabs in September 2018 that were not be detected in 2017. Nevertheless, several other factors may be involved in larval release, such as temperature, phytoplankton proliferation, daylight duration and tides (Shirley & Shirley, 1989; Starr *et al*., 1990; Anastasia, 2008), and the little data concerning this species in its new biotope does not offer a sufficient basis for drawing any firm conclusions. It would be necessary to carry out more precise monitoring of both the patterns of change in the water temperature and the presence of crabs in order to verify these hypotheses.

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RELEASE FROM PARASITE INCREASED THE SUCCESS OF INTRODUCTION OF INVASIVE FISH IN THE MEDITERRANEAN SEA?

Abstract
Biological invasions are considered a major threat to biodiversity around the world, but the role of parasites in this process is still understudied in marine ecosystems. This also applies to invasions from the Red Sea to the Mediterranean Sea via the Suez Canal, the so-called Lessepsian migration. Here, we studied the parasitofauna of Fistularia commersonii *collected in both its donor area (Red Sea from off Gulf of Aqaba) and in the Mediterranean (off the Gulf of Gabès, Tunisia). To compare parasite richness, prevalence and intensity, 43 individuals of* F. commersonii *were sampled from the Gulf of Gabès and 30 from the Gulf of Aqaba. Additionally, we reviewed the literature to identify native and invasive parasite species recorded. The composition of the parasite community in the two regions showed low similarity. Our results suggest the loss of at least four parasite species of the Lessepsian fish during the migration to the Mediterranean Sea. Simultaneously, it has co-introduced seven parasite species that are assumed to be originate from the Red Sea. In addition, we found that the invasive fish has acquired eighteen parasite species that are native from the Mediterranean Sea. However, parasite richness, prevalence and intensity were overall much higher in the invasive range compared to the native range. These results suggest that the Lessepsian migrant may potentially altering the dynamics of native and invasive parasite-host interactions via parasite release, parasite co-introduction and parasite acquisition. The higher infection levels of* F. commersonii *in its invaded range also agree with the theoretical predictions of the 'enemy release hypothesis'. These findings demonstrate that community studies or cross-localities comparisons such as the one presented here, are valuable tools to identify the role of parasites in Lessepsian migration.*

Key-words: Lessepsian migration, *Fistularia commersonii*, Enemy release hypothesis; parasite co-introduction; parasite spillover/spillback, Mediterranean Sea

Introduction
Biological invasions are considered a major threat to biodiversity around the world, but the role of parasites in this process is still understudied in marine ecosystems. Knowing how the parasite community of non-native hosts varies between the different regions into which it is introduced is essential for understanding their distribution of parasite fauna. The enemy release hypothesis (Elton, 1958) states that invasive species may gain a competitive advantage over native species by losing all or part of their natural parasites, during the invasion process (Blakeslee *et al.,* 2013). However, invasive hosts can often co-introduce parasites to their non-indigenous range (Lymbery *et al.,* 2014). This co-introduction depends on the host specificity and life cycle of the parasite species. In the new range, invasive species can infect native hosts (parasite spillover; Kelly *et al.,* 2009), or acquire native parasites from native host species (parasite spillback; Kelly *et al.,* 2009). This also applies to species invasions in the Mediterranean Sea, an ecosystem with an extraordinarily high rate of species introductions, with more than 821 alien species

(Zenetos *et al.,* 2017) mainly introduced from the Red Sea via the Suez Canal, called "Lessepsian migrants" (Sensu Por, 1978). Despite this high migration rate, parasitological studies on exotic species have been surprisingly rare in this region (Pérez-del-Olmo *et al.,* 2016). Here we investigated metazoan parasite infections in the Lessepsian fish *Fistularia commersonii* Rüppel, 1838, along the Tunisian coast in the central Mediterranean Sea. This species has spread rapidly throughout the Mediterranean. Genetic studies have shown that this successful invasion was the result of a single introduction episode that resulted in a serious bottleneck of the Mediterranean population (Golani *et al.,* 2007). By sampling the invasive fish *Fistularia commersonii* along the Gulf of Gabès (Mediterranean Sea) and the Gulf of Aqaba (Red Sea), and by conducting an additional parasitological literature survey, we aimed to answer the following specific research questions: (i) Is there evidence that *F. commersonii* experienced a release from its native parasites from the Red Sea?, (ii) did this fish co-introduce parasites from the Red Sea and/or did it acquire native parasites from the Mediterranean Sea?

Materials and methods

Between February 2014 and Mai 2016, 43 specimens of *Fistularia commersonii* were collected from the Gulf of Gabès and 30 specimens were collected from the Gulf of Aqaba. Fish skin, fins, nasal pits, eyes and buccal cavities were thoroughly examined for the presence of ectoparasites. Gill arches were separated by incision, removed, rinsed and examined individually. Internal organs (stomach, pyloric caeca, intestines, heart, liver, spleen, gall bladder and gonads) were separated and individually examined for the presence of endoparasites. 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. Prevalence and mean intensity were determined for each parasite species.

Results

Twelve different parasite species infecting *Fistularia commersonii* from the Mediterranean sea were found. While five species were found from Red Sea. Our additional literature survey added other parasite species records from the Mediterranean or Red Sea to the total parasite species list of this fish. Parasite richness and infection levels of *F. commersonii* from the Mediterranean Sea were overall much higher compared to the native region (Red Sea) (Fig. 1, Tab. 1).

Our results suggest the loss of at least four parasite species of the invasive fish. At the same time, the Lessepsian migrant has co-introduced seven parasite species to the Mediterranean Sea, that are assumed to be originate from the Red Sea. In addition, we found that the invasive fish has acquired eighteen parasite species that are native in the Mediterranean Sea. Five of those species were found in our survey in Tunisian coasts and thirteen species had been noted in *F. commersonii* elsewhere in the Mediterranean Sea. However, parasite richness, prevalence and intensity were overall much higher in the invasive range compared to the native range of the fish host.

Tab. 1: Parasite species of *F. commersonii* from the Mediterranean Sea and the Red Sea found in this study and recorded in the literature. For each species, the type of life cycle, the inhabitant status in the Mediterranean Sea and the occurrence in the two region (Mediterranean Sea and Red Sea) are given. If quantitative data were available, mean prevalence and intensity in the host species and region are given. + denotes published records of specific parasite species in the host species in a region.

Parasite taxa	Parasite species	Life cycle	Status Med Sea	***Fistularia commersonii*** (invasive range - Med Sea)		***Fistularia commersonii*** (native range - Red Sea)		Reference
				P **(%)**	***MI***	***P*** **(%)**	***MI***	
Acanthocephala	*Sclerocollum* sp.	complex	invasive	4.65	1.5	13.33	2.25	This study
	Breizacanthus ligur	complex	native	17.6	1.5			Merella *et al.*, 2016
Cestoda	Phyllobothriidae	complex	native	39.53	73.11			This study
	Nybelinia africana	complex	native	27.90	54.83			This study
	Pseudogrillotia sp.	complex	native	23.5	1.9			Merella *et al.*, 2016
	Dasyrhynchus basipunctatus	complex	not present			+		Beveridge *et al.*, 2014
Copepoda	*Caligus fistulariae*	complex	not present			100	110.83	This study
	Caligus elongatus	complex	native	5.9	1			Merella *et al.*, 2016
	Caligus minimus	complex	native	2.9	1			Merella *et al.*, 2016
	Caligus flexispina	complex	present			+		Rigby *et al.*, 1999
Digenea	*Allolepidapedon petimba*	complex	invasive	100%	341	68.75	1	This study
	Neoallolepidapedon hawaiiense	complex	invasive	69.76	3.8	3.33	1	This study
	Ectenurus virgulus	complex	native	16.27	1	+		This study
	Lecithochirium sp.	complex	present	23.25	1.5			This study
	Stephanostomum fijiensi	complex	invasive	13.95	1.16	+		This study
	Allolepidapedon fistulariae	complex	invasive	73.5	11.6	+		Merella *et al.*, 2016
				66.7	7.5			, Merella *et al.*, 2016
	Bucephalidae	complex	native	2.9	3			Merella *et al.*, 2016
	Lepocreadiidae larva	complex	invasive	16.27	3.42			This study
	Hemiuridae	complex	native	2.9	1			Merella *et al.*, 2016
	Trematoda (juvenile)	complex	native	2.9	1			Merella *et al.*, 2016
	Trematoda (metacercaria)	complex	native	8.8	1.3			Merella *et al.*, 2016
	Lecithochirium microstomum	complex	present			+		Rigby *et al.*, 1999
Hirudinea	*Trachelobdella lubrica*	complex	native	20.93	1			This study
Isopoda	*Gnathia* sp.	complex	native	11.62	3.8			This study
Nematoda	Anisakidae	complex	invasive	69.76	8.43	10	1.33	This study
	Hysterothylacium aduncum	complex	native	8.8	2.3			Merella *et al.*, 2016
	Hysterothylacium fabr	complex	native	41.2	5.1			Merella *et al.*, 2016
	Spinitectus sp.	complex	native	8.8	1.3			Merella *et al.*, 2016
	Cystidicolidae	complex	native	2.9	1			Merella *et al.*, 2016
	Philometridae	complex	native	2.9	1			Merella *et al.*, 2016
Total species richness				**26**		**11**		

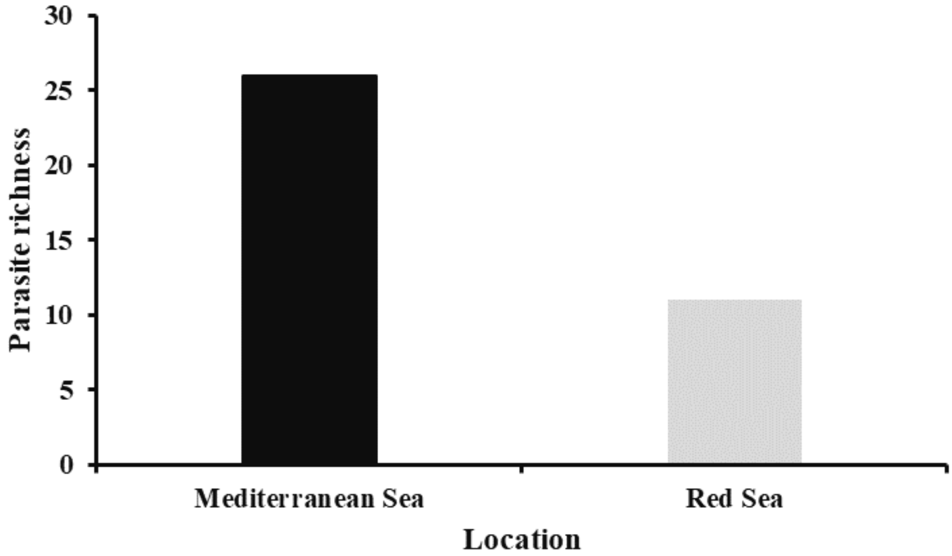

Fig. 1: Parasite species richness of *Fistularia commersonii* per Location. Values are given at the Mediterranean Sea (n=43) and at the Red Sea (n=30).

Discussion and conclusions

Based on samples from the Tunisian coasts, Red Sea and additional literature data, our analyses indicate that the invasive Lessepsian migrant *F. commersonii* lost seven parasite species during its introduction to the Mediterranean Sea. This loss of several natural parasite species is consistent with the enemy release hypothesis (Torchin *et al.,* 2001; Blakeslee *et al.,* 2013). In fact, parasites may not be able to cope with the environmental conditions in the canal or in the Mediterranean Sea and do not survive to the migration the new ecosystem.

The invasive fish also co-introduced six parasites from the Red Sea (4 Digenea, 1 Acanthocephala, 1 Nematoda). In spite of their heteroxenous life cycle, these parasites seems to be co-introduced in the Mediterranean Sea. This successful introduction can be explained that parasites had found suitable hosts and environmental conditions to complete the life cycle in the new habitat or their intermediate hosts are already co-introduced in the Mediterranean Sea and their life cycle has been achieved (Merella *et al.,* 2016). However, the fact that all the specimens of these parasites were retrieved at the adult stage could also suggest a residual infection that took place before of the host migration. *F. commersonii* acquired eighteen parasites in the Mediterranean Sea. This is can be explained by the fact that introduced species are naïve hosts to native parasites, which can cause novel and sometimes profound instances of pathology (Colautti *et al.,* 2004). Therefore, native parasites could represent an important source of selection against migrants (MacColl & Chapman, 2010). However, parasite richness and infection levels were overall much lower in the native range compared to the invaded range, suggesting a potential competitive advantage for the Lessepsian migrant and confirm that this species is today one of the most successful invaders of the Mediterranean Sea. Indeed, all the Lessepsian migrant fish populations display a high genetic similarity with the Red Sea/Indo-Pacific ones, with no evidence of genetic bottlenecks (Bernardi *et al.,* 2010). *F. commersonii* is the only Lessepsian fish that shows a severe bottleneck which offer in the new habitat a rich parasite assemblage that potentially would threaten it more than in its native range.

Our study suggest that the Lessepsian migrant has the potential to affect native fish hosts by altering the population dynamics of native parasite species via parasite release, parasite co-introduction and acquisition of native parasites, resulting in increased infection levels in invaded regions. This study demonstrated that community studies or cross-localities comparisons such as the one presented here, are valuable tools to identify the role of metazoan parasites in Lessepsian migration.

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CONTRIBUTION TO THE SETTING UP OF A MONITORING PROGRAMME FOR NON-INDIGENOUS SPECIES IN MONASTIR BAY, TUNISIA

Abstract
Rapid identification of new invasive marine species is a critical element of prevention their dispersal and to promote an effective surveillance procedure for ecological monitoring. Within this context, Tunisia has developed a holistic and comprehensive national monitoring programme for biodiversity and non-indigenous species (NIS) with the support of the Specially Protected Areas Regional Activity center (SPA/RAC). The objective of this work is to elaborate a first checklist of Non-Indigenous Species (NIS) in the risk areas with a spotlight on the "worst" invasive species in Monastir Bay. It is also aimed to identify Risk Areas in Monastir Bay. Distribution maps are already drawn to visualize hotspots and to prevents the future spread of those invaders. During the summer of 2018, six ports, two aquaculture farms and Kuriat islands (future Marine and coastal Protected Areas MCPA) were surveyed for the presence of NIS. Diverse survey methods were employed. These included, Rapid Assessment Surveys of epibiota on artificial structures in harbors, a standardized one-hour transects by snorkeling and diving of infra-littoral species. A total of 14 alien species were recorded: 36% Mollusca, 22% chlorophyta 7% Polychaeta, 7% Magnoliopsida, 7% Rhodophyta, 7%Asidiacea, 7% Crustacea and 7% Porifera. Most of these aliens are represented by warm-water species.

Key-words: Non-Indigenous Species, Monitoring, Rapid Assessment Survey, Monastir Bay, Tunisia

Introduction
Increasingly, Non-Indigenous Species (NIS) represent a catastrophic impact on the recipient communities of the Mediterranean Sea (Parker *et al.*, 1999; Boudouresque &Verlaque, 2002). An extreme examples can be given like the case of the indo-pacific lionfishe *Pterois milesin* in the eastern Mediterranean (Özbek *et al.,* 2017) and the blue swimming crab *Portunus segnis* (Forskål, 1775) in the Gulf of Gabes, Tunisia (Rabaoui et *al.,* 2015). Eradication of invasive species is challenging non-realistic task in marine ecosystems, especially when a species is already established in its new environment (Lodge *et al.,* 1998; Parker *et al.,* 1999). Therefore, the best option is prevention and early detection of these species (Leung *et al.,* 2002). In July 2017, SPA/RAC has elaborated a national monitoring programme for biodiversity and non-indigenous species (hereafter NIS) in line with the Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast (IMAP) requirements. Regarding NIS, the national monitoring program proposes to monitor the trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly the ones with the 'Invasive' criteria. This should be done especially in areas with high risk (such as Marinas, harbours and aquaculture farms) close to areas of special conservation areas such as Zembra and Zembretta National Park and Kuriat archipelago. Kuriat archipelago

is located in the eastern part of Monastir Bay and so far, all the reported observations of alien marine species consist of occasional or incidental findings. In this study, we identified eight potential risk areas within Monastir Bay and assessed the occurrence of NIS in these areas and in the Kuriat archipelago, providing a basic inventory of NIS.

Materials and methods

This study was conducted in Monastir Bay off the central Tunisian coast (35°46′ N, 10°49′ E) (Figure 1). The identification and localisation of the potential risk areas within Monastir Bay was based on data collection from the field work and investigation done in collaboration with the Tourism and fishing administration. This data was then mapped using an open source software (Quantum GIS or QGIS) (Figure 1). Eight risk areas were identified, out of these there were five fishing ports and one Marina in the Cape of Monastir (Marina Cap Monastir). Two aquaculture farms were also selected for the study since they represent a general hotspot areas. The field survey has been carried out in only one area of special interest which is the Kuriat archipelago (Tab. 1). Two different survey methods have been adopted, these are Rapid Assessment Survey (RAS) (Campbell, Gould & Hewitt, 2007) and line transects (one-hour preidentified snorkelling transect while recoding encounters).

Tab. 1: Sampling areas and methods (RAS: Rapid Assessment Survey and Line Transect)

Prospected Areas	**Coordinates (deg.min.sec)**	**Date**	**survey method**	**Substrat/ bottom**
Fishing ports				
Bkalata,	35°37'26.53"N - 11° 2'54.87"E	July 2018	RAS	Ropes, Rocky
Teboulba,	35°39'35.15"N - 10°57'29.17"E	July 2018	RAS	Ropes
Sayada,	35°40'27.66"N - 10°53'30.52"E	July 2018	RAS	Ropes
Ksibet El Madiouni,	35°41'28.75"N - 10°50'45.77"E	July 2018	RAS	Algae, Ropes, Rocky, Docks
Monastir	35°45'22.84"N - 10°50'10.29"E	July 2018	RAS	Docks, Ropes
Marinas				
Marina Cap Monastir	35°46'45.25"N - 10°49'58.36"E	July 2018	RAS	Docks, Ropes
aquaculture farms				
Hanchia Fish farm	35°44'830"N - 11°05'166"E	July 2018	RAS	Fish farming nets
Prima Fish farm	35°47'500"N - 10°56'700"E	July 2018	RAS	Fish farming nets
Kuriat archipelago				
K1	35°45'36.54"N - 11° 0'6.83"E	August 2018	snorkelling	Seagrass
	35°45'48.87"N - 11° 0'43.41"E	August 2018	Snorkelling	Rocky, Seagrass
	35°46'05.95"N - 11° 0'37.82"E	August 2018	Snorkelling	Sand, Rocky
	35°46'17.89"N - 11° 0'22.06"E	August 2018	Snorkelling	Rocky, Seagrass
	35°46'10.19"N - 11° 0'8.44"E	August 2018	Snorkelling	Rocky, Seagrass
	35°45'56.19"N - 11° 0'32.87"E	August 2018	snorkelling	Seagrass, Sand
K2	35°47'23.62"N - 11° 1'32.13"E	August 2018	snorkelling	Rocky, Sand
	35°47'35.60"N - 11° 2'25.88"E	August 2018	Snorkelling	Algae, Seagrass, Sand
	35°48'17.99"N - 11° 2'11.77"E	August 2018	Snorkelling	Seagrass, Rocky
	35°47'54.20"N - 11° 1'30.52"E	August 2018	snorkelling	Algae, Seagrass, Rocky

Results and discussion

A total of 14 NIS species were sighted and identified during the survey (Tab. 2) with 36% Mollusca, 22% Chlorophyta 7% Polychaeta, 7% Magnoliopsida, 7% Rhadophyta, 7%Asidiacea, 7% Crustacea and 7% Porifera. The highest number of NIS was found in the Marina with 64% of the total number of NIS found in other area, followed by Monastir port and port of Bekalta, Ksibet El Madiouni with 36%. in the rest of the ports seems to hold a lower percentage of 27% in port of Sayada and 14% in the port of Teboulba. Moreover, in the Kuriat islands (MCPA), 36% of NIS species were found which might be an alarming indication in a designated Marine Protected Area. As expected from the anthropogenic uses and management policies, the intensity of NIS varied between sites of the two aquaculture farms. The rate of NIS was higher in front of Monastir (Prima Fish farm) (21%), than in Teboulba (Hanchia Fish farm) (14%). This maybe related to the closeness and distance of each site to the other more contaminated areas.

The vast majority of NIS occurring in this study (79%) are warm-water species, mainly of Red Sea/Indo-Pacific origin (Tab. 2).

Tab. 2: Occurrence of NIS found per prospected area (Be: Bekalta, Te: Teboulba, Sa: Sayada, Ks: Ksibet El Madiouni, Mo: Monastir, Ma: Marina Cap Monastir, Ha: Hanchia, Pr: Prima Fish, Ku: Kuriat archipelago); Origins IP: Indo-Pacific, RS: Red Sea, IO: Indian Ocean, PO: Pacific Ocean, Ci: Circumtropical, T: tropical, IWP: Indo-West Pacific)

Taxonomic	Non-Indigenous Specie	Origin	Be	Te	Sa	Ks	Mo	Ma	Ha	Pr	Ku
Ascidiacea	*Symplegma brakenhielmi* (Michaelsen, 1904)	T					×	×			
Polychaeta	*Branchiomma luctuosum* (Grube, 1870)	IP			×		×	×			
Crustacea	*Portunus segnis* (Forskål, 1775)	IO	×								
Porifera	*Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004	IP					×	×		×	
Mollusca	*Brachidontes pharaonis* (P. Fischer, 1870)	IO-RS			×		×	×			
	Pinctada imbricata radiata (Leach, 1814)	IP-RS	×				×	×	×	×	×
	Cerithium scabridum Philippi, 1848	IO-RS			×	×	×	×			×
	Melibe viridis (Kelaart, 1858)	IWP				×					
	Bursatella leachii Blainville, 1817	Ci		×							×
Magnoliophyta	*Halophila stipulacea* (Forsskål) Ascherson, 1867	IO -RS		×			×	×			
Rhodophyta	*Lophocladia lallemandii* (Montagne) F.Schmitz, 1893	RS-IP	×		×	×	×	×	×	×	×
Chlorophyta	*Caulerpa chemnitzia* (Esper) J.V.Lamououx, 1809	RS- T	×								
	Caulerpa cylindracea (Forsskål) J.Agardh, 1873	IP	×			×	×	×			×
	Codium fragile (Suringar) Hariot, 1889	PO				×					
	Total		5	2	4	5	9	9	2	3	5

Identification and localisation of the potential risk areas

In Monastir Bay, one marina, five fishing ports and 11 offshore fish farms were identified as risk areas for NIS Introductions (Figure 1). All these areas might have been under the influence of human assisted introductions of NIS and, hence, act as stepping stones to facilitate their dispersal along the Monastir Bay. The bay is also characterized by intense fishing activities, which provide up to 44% of the national fish production of Tunisia (Ali Ben Smida *et al.,* 2014). Eleven active offshore fish farms are set up inside the bay, five fishing ports and one Marina, which are also constitute risk areas in terms of the number of introduced species (Fig. 1).

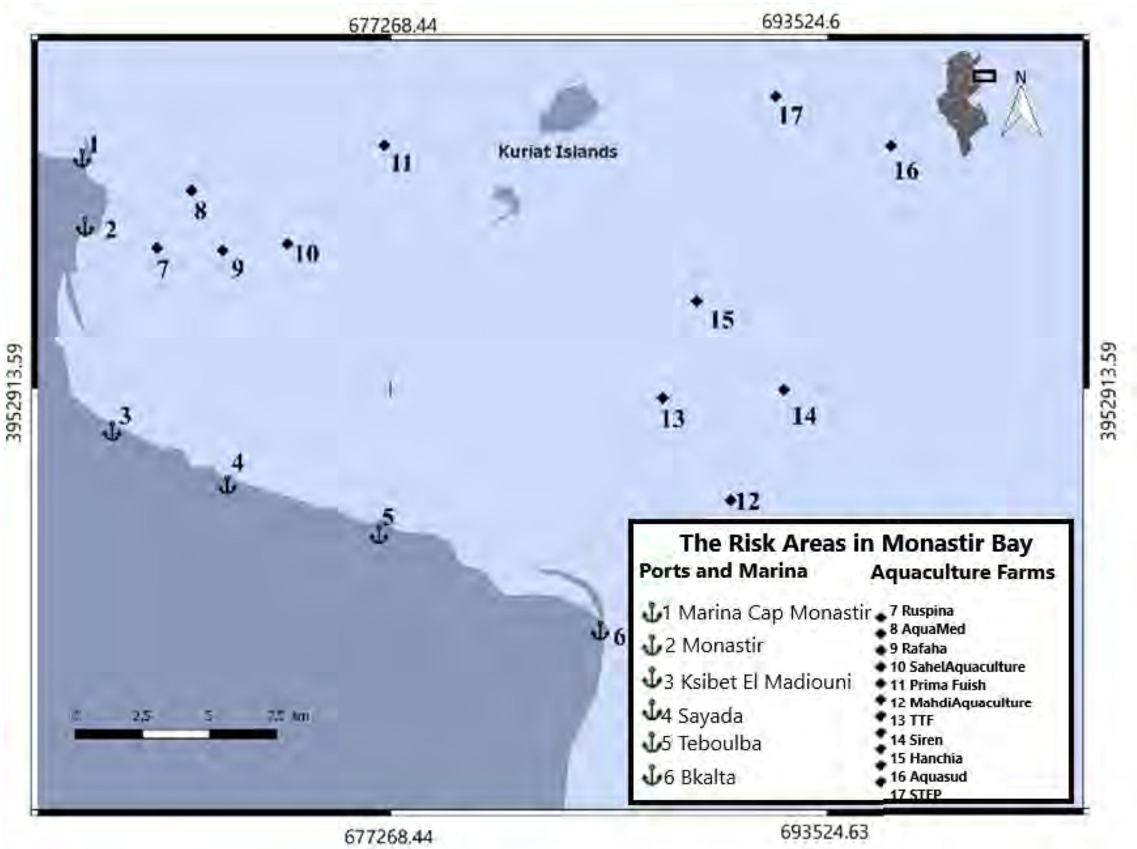

Fig. 1: Risk areas for NIS introduction in Monastir Bay

Conclusions

The large number of aquaculture farms and the intense maritime traffic between the different hotspot areas of Monastir bay (Harbours, Marina and Aquaculture farms) can facilitate the spread of NIS. We might also argue that the proximity of floating cages From Kuriat Island (about 4 km from the Kuriat islands) does increase the risk of NIS transportation into the Islands area. However, Kuriat Islands has a variety of habitats, such as the Maerl beds and also the barrier reefs of Posidonia which has a reparable natural landmarks of significant heritage value. Therefore, preventing the introduction of NIS in this area is an imperative and crucial step to protect and conserve the islands. This inventory and risk identification can be considered as a first step to protect the MCPA of Kuriat Islands from the threat posed by biological invaders. Management measures should seriously consider following the early detection programme of a new potential invader in these areas.

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NON INDIGENOUS FISH SPECIES IN NORTH OF CYPRUS: ECOLOGICAL STATUS AND IMPACTS ON KEY HABITATS

Abstract
Located in the middle of the Eastern Mediterranean, Cyprus is one of the hotspots for invasive/non indigenous species with an increasing rate of new records. Evaluation of fisheries statistics, professional and amateur fishermen catch records and monitoring with underwater visual census (UVC) technique around the waters of Cyprus between 2002 and 2017 showed that the population traits of recorded invasive fish species demonstrates different patterns. In this study, invasive species' ecological responses and adaptation to marine key habitats, as well as their impacts, are being investigated. Depending on the data of occurrences, 34 non indigenous species recorded during the study. In addition to well established species; Lagocephalus sceleratus *(Gmelin, 1789),* Siganus luridus *(Rüppell, 1829) and* Siganus rivulatus *Forsskål & Nieburh, 1775 are considered to be invasive and have high impact on key habitats of all sites; where* Pterois miles *(Bennett, 1803) and* Torquigener flavimaculosus *Hardy & Randall, 1983 have increasing populations and become invasive in certain locations.*

Keywords: *non-indigenous, marine key habitats, ecological status, environmental impacts, Eastern Mediterranean*

Introduction

In a recent study, non indigenous fish fauna listed with 35 species in the waters of Cyprus (Iglésias and Frotté, 2015). The concept of biological invasion is a ecologically, socially and economically growing concern and accepted as a pervasive component of global change (Simberloff *et al*., 2013). Understanding and evaluation of impacts of non indigenous species gains importance in creating response and put in place mitigation measures. Impacts of non indigenous species can be various, EICAT lists impacts as competition, predation, hybridization, disease transmission, parazitism, poisoning/toxicity, bio-fouling, grazing/herbivory/browsing, rooting/digging, tramping, flammability, interaction with other invasives, and chemical, physical, or structural impact on ecosystem with impact classes as massive, major, moderate, minor and minimal (Hawkins *et al*., 2015) and it was applied to certain species with its socio-economic counterpart SEICAT Protocol depending on reported/demonstrated impacts (Galanidi *et al.,* 2018). In this study, it is aimed to evaluate impacts and impact classes of 34 non indigenous fish species observed during field surveys and fisheries statistics provided.

Material and Methods

The occurence and abundance of non indigenous fish species were recorded depending on the fisheries statistics, professional and amateur fishermen catch records and monitoring with underwater visual census (UVC) technique between 2002 and 2017. In

UVC surveys, in addition to abundance data, ecological information like seasonality, behaviour, relationships with other species, habitat (biotope) and habitat related data were collected (Data not presented here) where fisheries statistics and fishermen catch records remains limited or unable to provide. On the other hand, UVC has weaknesses when depth, visibility and time is considered. Instead of difficulties in sampling in extreme or inappropiate conditions, UVC can be applied successfully in clear and calm marine environments like seen in the most of the Mediterranean coastal zone (Bellwood, 1988; Bibby *et al.*, 1998; Cappo and Brown, 1996; De Girolamo and Mazzoldi, 2001). In this study, UVC, was applied either SCUBA or snorkelling on the transects on different habitat types in selected 7 sites with different habitat types of north of Cyprus (Fig. 1).

Impacts of the well established non indigenous and invasive taxa estimated according to their relative abundance in time, dominance in the specific habitat and population trends of competing and prey species. Although fisheries and fishermen data is not efficient in evaluating habitat related information and direct observation/counting of floral and faunal components but used to evaluate the relative abundance in time for especially pelagic and deep water species (Data not presented here).

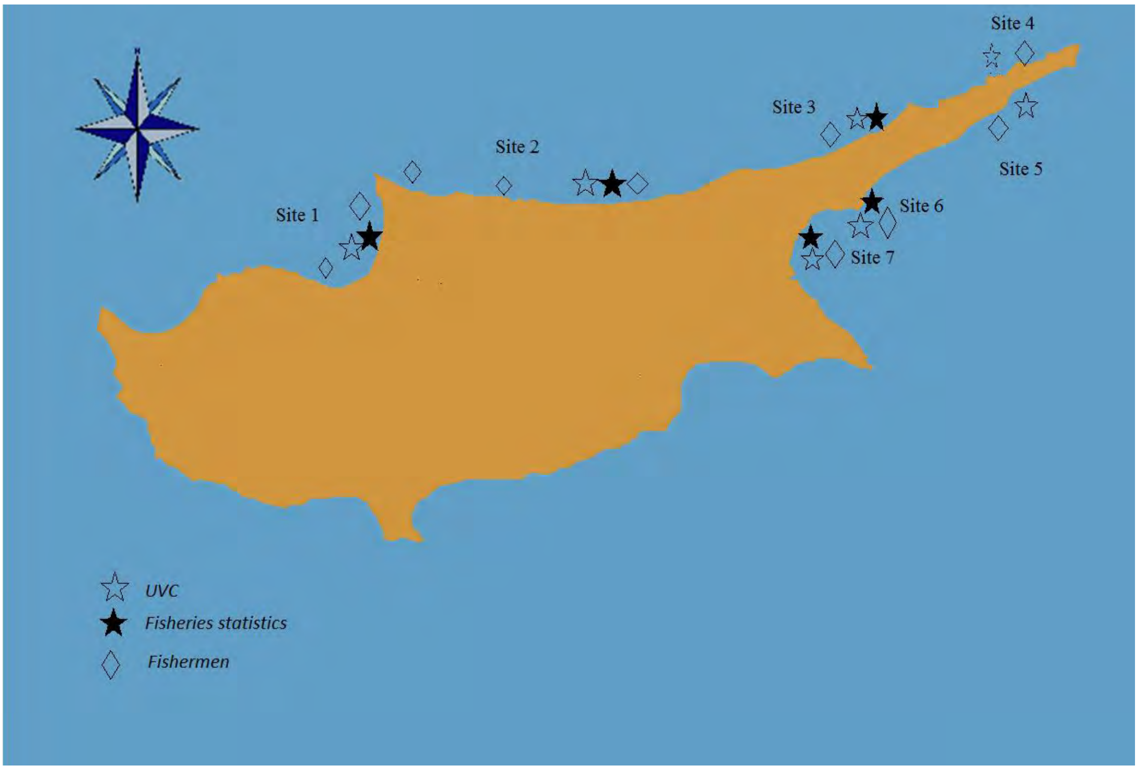

Fig. 1: Sites of data collected from north of Cyprus (details are given in Table 1).

Tab. 1: Coordinates of sites (Boundaries determined on fishermen and UVC samplings. UVC data gathered between 2002 and 2017; fishermen data and fisheries statistics between 2009 and 2017).

		Coordinates			
		Start		End	
Site No	Area	Latitude N	Longitude E	Latitude N	Longitude E
Site 1	Morphou Bay	35°12'7.14"	32°42'28.58"	35°24'19.05"	32°55'20.81"
Site 2	Kyrenia	35°24'19.05"	32°55'20.81"	35°25'22.59"	33°45'19.62"
Site 3	Yeni Erenköy	35°25'22.59"	33°45'19.62"	35°37'24.84"	34°20'47.95"
Site 4	Karpaz N	35°37'24.84"	34°20'47.95"	35°42'32.30"	34°35'46.22"
Site 5	Karpaz S	35°42'32.30"	34°35'46.22"	35°28'5.35"	34°15'53.65"
Site 6	İskele	35°28'5.35"	34°15'53.65"	35°13'5.58"	33°54'19.22"
Site 7	Famagusta	35°13'5.58"	33°54'19.22"	35° 4'25.08"	34° 0'40.40"

Results

Abundance of a total of 34 non indigenous fish were recorded in this study (Tab. 2). *Atherinomorus forskalii (Rüppell, 1838), Sargocentron rubrum (Forsskål, 1775), Scomberomorus commerson (Lacepède, 1800), Upeneus spp., Sphyraena spp., Apogonichthyoides pharaonis (Bellotti, 1874), Pempheris rhomboidea Kossmann & Räuber, 1877, Upeneus pori Ben-Tuvia & Golani, 1989, Torquigener flavimaculosus Hardy & Randall, 1983, Nemipterus randalli Russell, 1986, Siganus luridus (Rüppell, 1829), Siganus rivulatus Forsskål & Nieburh, 1775 and Lagocephalus sceleratus (Gmelin, 1789)* are found to have well adapted to the habitat concerned and established stable populations where the last three are considered as invasive with high negative impact on the indigenous populations and on the ecosystem. In addition, decrease in population traits of *Fistularia commersonii (Rüppell, 1835) and Alepes djedaba (Forsskål, 1775);* where a rapid increase in *Pterois miles (Bennett, 1803)* are found.
Evaluation of impacts of non indigenous species on habitats is constructed on Hawkins *et al.* (2015), depending on the population traits and relations with native species and adaptation to marine key habitats (Tab. 3).

Discussion and Conclusions

It was argued that efforts should be spend to understand organisms impact on the ecosystem rather than their origin (Davis *et al.,* 2011). Continious observation, research and statistics may reveal some sightings although it is not easy to eliminate the impacts of other factors like climate change, overfishing, pollution or synergy created with other invasive or native species. As an example, during the UVC surveys, decline of echinoid *Paracentrotus lividus* populations were observed in the field since 2010 in the waters of north Cyprus and the populations collapsed at 2014 in all stations. *Lagocephalus sceleratus* (Gmelin, 1789) and *Torquigener flavimaculosus* Hardy & Randall, 1983 were suspected of this situation in this study, but later, collapse of the echinoid *Paracentrotus lividus* population in the Eastern Mediterranean was reported due to rising seawater temperatures (Yeruham *et al*., 2015). On the other hand, in such cases, impacts can be tested in an controlled environment or the impact by it self is quite apparent (Sala *et al.*, 2011).

Tab. 2: Distribution and population status of alien fish in north of Cyprus. Population status: vr=very rare, ra=rare, ca=casual, pr=prevalent, ab=abundant, in=invasive

Species	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Nemipterus randalli Russell, 1986	ca	ca	ca	ra	ca	pr	ca
Ostorhinchus fasciatus (Shaw, 1790)		vr	ra			vr	ra
Parupeneus forsskali (Fourmanoir & Guézé, 1976)	vr					vr	
Pomadasys stridens (Forsskål, 1775)	vr						
Sphyraena obtusata Cuvier, 1829	ca	ca	ra	ra	ra	ra	ca
Spratelloides delicatulus (Bennett, 1832)	ra	ca	ra	ra	ra	ca	ca
Pterois miles (Bennett, 1803)	ab	ab/in	ab	ab	ab	ab/in	ab/in
Acanthurus coeruleus Bloch & Schneider, 1810	vr						
Scarus ghobban Forsskål, 1775	vr	ra	vr		vr	ra	
Torquigener flavimaculosus Hardy & Randall, 1983	ab	ab/in	ab/in	ab	ab	ab/in	ab/in
Sillago suezensis Golani, Fricke & Tikochinski, 2014	vr		ra			vr	vr
Scomberomorus commerson (Lacepède, 1800)	pr	ab	pr	pr	pr	ab	ab
Lagocephalus suezensis Clark & Gohar, 1953	ca	ca	ca	ca	ca	ca	ca
Lagocephalus guentheri Miranda Ribeiro, 1915	ca	ca	ca	ca	ca	ca	ca
Dussumieria elopsoides Bleeker, 1849	ca	ca	ca	ca	ca	ca	ca
Lagocephalus sceleratus (Gmelin, 1789)	in	in	in	in	in	in	in
Upeneus pori Ben-Tuvia & Golani, 1989	pr	pr	pr	pr	pr	ab	ab
Parexocoetus mento (Valenciennes, 1846)	ra	vr		vr		vr	ra
Etrumeus golanii DiBatistta, Randall & Bowen, 2012	ra	vr	vr	ra	vr	vr	ra
Fistularia commersonii (Rüppell, 1835)	ca	ca	ca	ca	ca	ca	ca
Pteragogus trispilus Randall, 2013	ca	ca	ca	ca	ra	ca	ca
Pempheris rhomboidea Kossmann & Räuber, 1877	ab	ab	ab	ab	ab	ab	ab
Alepes djedaba (Forsskål, 1775)	ca	ca	ca	ca	ca	ca	ca
Apogonichthyoides pharaonis (Bellotti, 1874)	ca	pr	ca	ca	ca	pr	pr
Hemiramphus far (Forsskål, 1775)		vr				ra	vr
Siganus luridus (Rüppell, 1829)	in	in	in	in	in	in	in
Sphyraena pinguis Günther, 1874	ca	ca	ca	ca	ca	ca	ca
Upeneus moluccensis (Bleeker, 1855)	pr	ab	pr	ab	pr	ab	pr
Equulites klunzingeri (Steindachner, 1898)	ca	ca	ca	ca	ca	ca	ca
Sargocentron rubrum (Forsskål, 1775)	in	in	in	in	in	in	in
Saurida lessepsianus Russell, Golani & Tikochinski, 2015	ra	ra	ca	ca	ra	ca	ra
Stephanolepis diaspros Fraser-Brunner, 1940	ca	ca	ca	ca	ca	ca	ca
Atherinomorus forskalii (Rüppell, 1838)	pr	ab	pr	ab	pr	pr	ab
Siganus rivulatus Forsskål & Nieburh, 1775	in	in	in	in	in	in	in

Tab. 3: Non indigenous fish species with well-established populations on marine key habitats

Species	Impact mechanism	Impact class	Habitat
Pterois miles (Bennett, 1803)	Competition, predation	Moderate	Rocky
Torquigener flavimaculosus Hardy & Randall, 1983	Competition, predation	Moderate	Rocky, *Posidonia oceanica* , sandy/muddy
Lagocephalus sceleratus (Gmelin, 1789)	Competition, predation	Major	Rocky, *Posidonia oceanica* , sandy/muddy
Siganus luridus (Rüppell, 1829)	Competition, grazing	Major	Rocky, *Posidonia oceanica*
Sargocentron rubrum (Forsskål, 1775)	Competition, predation	Moderate	Rocky
Siganus rivulatus Forsskål & Nieburh, 1775	Competition, grazing	Major	Rocky, *Posidonia oceanica* , sandy/muddy
Upeneus spp.	Competition, predation	Moderate	Sandy/muddy

Galil (2007), discussed in detail the impacts of non indigenous species and ask the question if the new guests are making Mediterranean rich in biodiversity. Open niches may wellcome new species but consequences alter genetic diversity and habitat structure and functioning. On the other hand, environmental conditions are changing globally and the new conditions may not appropriate for native species. Marras *et al.* (2015) show that, changing thermal conditions effects competition between native and non indigenous fish species by means of better adaptation of indigenous species' methabolism to the environment. Depending on the seawater temparature increase, changes in water quality parameters are also expected.

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EU RISK ASSESSMENT OF *LAGOCEPHALUS SCELERATUS*. STATE OF KNOWLEDGE, EVALUATION AND CRITERIA, DATA NEEDS / FORMATS AND MANAGEMENT

Abstract
Lagocephalus sceleratus *is undoubtedly one of the most aggressive and harmful invasive fish species in the Mediterranean and has raised significant concerns at the stakeholder, scientific and policy/management level. Following repeated queries to the European Commission (EC), a thorough Risk Assessment (RA) study of the species was recently undertaken under the auspices of the EC. In this talk we will present the main findings of this RA and discuss how the specific requirements and scoring criteria of the RA protocol guide the collation and detailed evaluation of the existing data. Furthermore, we will highlight data needs and ensuing data formats that can improve the confidence of the assessment. Particular data gaps include the paucity and poor documentation of environmental impacts and the lack of information on the eco-physiological requirements of* L. sceleratus. *A climate-matching approach based on the distribution of spawning aggregations in Cyprus and information on spawning temperatures of a congeneric species was employed for the identification of the potential distribution and the areas under risk of impact by* L. sceleratus. *Finally, the management options that were submitted with the Risk Assessment will be briefly outlined and their cost-effectiveness discussed. Co-ordinated monitoring, awareness raising and damage control are the priority measures, while subsidized removal programs can offer some compensation for financial damages. Long-term population control and mitigation may be achieved with a commercial fishery to supply pharmaceutical research, pending a feasibility study of such an undertaking.*

Key-words: *Lagocephalus sceleratus*; invasive; risk assessment; management measures

Introduction

Recognizing that invasive alien species (IAS) pose one of the biggest threats to biodiversity and associated ecosystem services, the EU in its Biodiversity Strategy, Target 5, committed to taking specific actions to combat IAS (EC, 2011). One of these actions was the adoption of legislation, i.e. EU Regulation (No.1143/2014), which establishes rules to prevent, minimize and mitigate the adverse impact on biodiversity of IAS (EU, 2014). The Regulation introduces a priority list of IAS of Union concern, selected on the basis of specified inclusion criteria (outlined in Article 4), compliance with which is ascertained by detailed Risk Assessment (RA), according to provisions made in Article 5. Thus, a Risk Assessment is an absolute requirement for any species to be considered for management at the EU or the regional level.
The silver-cheeked toadfish, *L. sceleratus* is a species of Indo-pacific origin, first reported in the Mediterranean in 2003 from Turkey (Akyol *et al*., 2005). Since then it has exhibited a remarkable westward and northward expansion establishing populations throughout the Aegean-Levantine Sea, the Ionian Sea and the Adriatic and all along the north African coast until Algeria, while casual records are reported from Spain and the Sea of Marmara (for a distribution review see Guardone et al., 2018). It is a large

voracious predator that has been implicated in native species population declines and is acknowledged as a fisheries pest as it attacks and damages fishing gear but also the target species caught by fishermen (Nader et al., 2012). It is also a poisonous species and has already caused numerous severe poisoning and fatal intoxications in both its native and invaded range (reviewed by Galanidi et al., 2018). Raising public concerns have reached the European Parliament; as a result, the European Commission, DG-ENV requested a thorough Risk Assessment of *L. sceleratus* to evaluate the impacts and the potential for management of the species at the EU level. The results of this RA are summarized in the present study.

Materials and methods

In this study we employed a Risk Assessment protocol developed in the framework of the EU commissioned program ENV.B2.ETU/2016/0013, which explicitly addresses all the requirements set out in the EU Regulation (No.1143/2014) (EU, 2018). The scheme comprises different sections, presenting basic organism information, describing the distribution of the organism, current and potential (under current and future climate conditions) and addressing the four main aspects of the invasion process (Introduction, Establishment, Spread and Impacts). The RA area covers all European Seas excluding the outermost regions. The description of pathways of Introduction and Spread follows the CBD classification proposed in (CBD, 2014). The main factors considered for the assessment of the likelihood of introduction include the reproductive strategy of the species (i.e. number of propagules that can travel along each pathway in one year), its ability to survive passage along the all pathways examined and transfer to suitable habitats in the recipient areas and the effectiveness of existing management practices to detect it or affect its survival. Regarding the potential for establishment, particular emphasis is given to the physiological requirements of the species and its reproductive strategy in relation to the conditions encountered in the RA area. Literature on the con-generic *Lagocephalus lunaris spadiceus* (Fujita, 1966) and the distribution of spawning aggregations of *L. sceleratus* in southern Cyprus (Rousou *et al*., 2014) indicate that there might a thermal limit for spawning at around 21-22 °C SST in June (peak spawning month). A threshold of 21.7 °C SST for the month of June was tentatively applied as a limiting factor for spawning (value taken from Fujita, 1966). Possible biotic interactions in the form of predation, competition and parasitism are also considered. Regarding the Impacts section, authors are asked to assess separately the demonstrated impacts inside and outside the risk assessment area and further provide an expert opinion on the potential future impacts. This distinction has to be presented as clearly as possible in order to aid Member States to arrive at their decision regarding the possibility of listing with all its implications. The scoring guidelines of the Impacts Section are largely based on the EICAT (Blackburn et al., 2014) and SEICAT (Bacher et al., 2017) impacts assessment schemes for environmental and socio-economic impacts respectively and are governed by the same principles. Additionally, a Management Annex describes and evaluates a suite of possible management measures, ranging from pre-border stage (i.e. prevention) measures to population control and mitigation of impacts for already widely established species, including information on their feasibility, cost and cost-effectiveness.

Results

Introduction via the Suez Canal is the primary pathway of introduction into the Mediterranean Sea - recipient countries are located in the Levantine basin and continuous introductions are considered very likely (Tab. 1). This is followed by natural dispersal (Unaided pathway), as indicated by the pattern of spread of *L. sceleratus* in a gradual progression from the Suez Canal towards the north-east Mediterranean and along the north coast of Africa simultaneously. This pattern of spread, often against the prevailing currents along the Suez Canal and the southern Mediterranean coast, suggests that spread by adult migration/movement plays a strong role in the dispersal of the species. An additional pathway we considered was "Escape from Confinement" but introduction via this pathway is not expected to be significant as the species is currently displayed in a small number of public aquaria in countries where it is already widely (European Union Aquarium Curators, pers. comm.).

Table 1: Risk of Introduction

Pathway of Introduction	Response	Confidence level
CORRIDOR (Suez Canal)	Very likely	High
UNAIDED	Very likely	High
ESCAPE (aquaria)	Unlikely	High

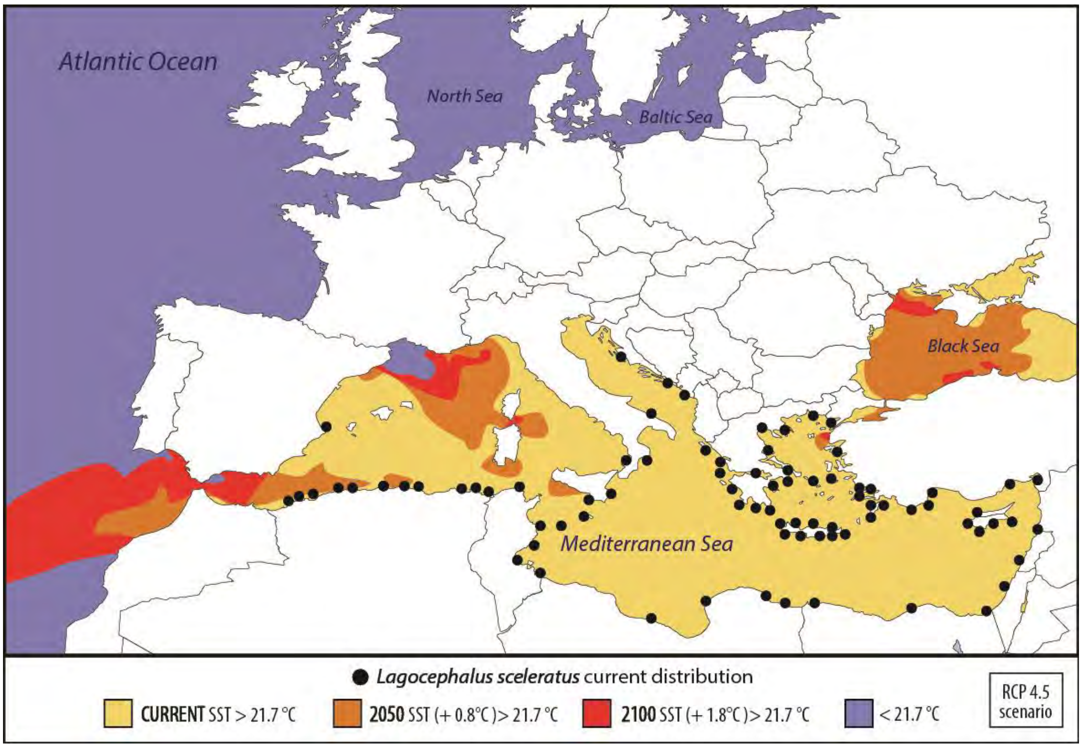

Figure 1. Risk of Establishment & Spread based on a thermal limit for spawning of 21.7 ºC in June under current climatic conditions and the RCP 4.5 scenario. SST values represent monthly means for June averaged over a 5-year period (2012-2016).

The map produced based on the tentatively identified limit for spawning shows a remarkable agreement with the current distribution of *L. sceleratus* in the RA area and indicates limited further spread outside the Mediterranean Sea, even under potential future climate conditions (Fig.1). While temperature was used as a limiting factor in Figure 1 (notice also that no depth limit is imposed), low salinities in the Black Sea are also expected to restrict the establishment and spread of the species beyond the Sea of Marmara (Tab. 2); *L. sceleratus* has rarely been recorded at salinities lower than 30 psu (OBIS, 2018). The assessment of Impacts is summarized in Tab. 3, where it is evident that socio-economic impacts are currently more severe and better documented.

Table 2: Risk of Establishment and spread

	Response	Confidence level
Mediterranean Sea	Very likely	High
Bay of Biscay and the Iberian Coast, Black Sea (Sea of Marmara)	Moderately likely	Low
Baltic Sea, Greater North Sea, Celtic Seas	Unlikely	High
SPREAD	Rapid	High

Table 3. Assessment of Impacts Sc=Score, Cl=Confidence level

ENVIRONMENTAL	Current	Future
• **Biodiversity** Predation (cephalopods, crustacea, fish) Competition for resources	Sc: Moderate Cl: Low	Sc: Major Cl: Low
• **Ecosystem services** Food provisioning (prey species populations) Regulating (nursery value of *Posidonia oceanica* meadows) Cultural (recreation)	Sc: Major Cl: Low	Sc: Major Cl: Low
• **Conservation value** Soft-sediment habitats Seagrass meadows 38 Natura sites (GR 29, CY 2, HR 2, IT 2, MT 2, ES 1)	Sc: Moderate Cl: Low	Sc: Moderate Cl: Low
SOCIO-ECONOMIC		
• **Economic impacts** Damage fishing gear & catch Extrapolated from ≈ 4.5million € in Turkey in gear & labour losses (Ünal et al., 2015; Ünal & Bodur, 2017) Depletes stocks of commercial species ?	Sc: Major Cl: Medium	Sc: Massive Cl: Medium
• **Social impacts** Fishermen withdraw their vessels Recreational fisheries Sense of safety for beach users	Sc: Major Cl: Medium	Sc: Major Cl: Medium
• **Health impacts** Fatalities (outside the RAA) Severe poisoning (invaded range, GR, CY)	Sc: Major Cl: Medium	Sc: Major Cl: Medium

With respect to management, prevention of further introductions and eradication are considered unrealistic for *L. sceleratus* due to the active primary pathway of introduction, secondary dispersal by the already widespread and abundant populations, high fecundity, mobility and long pelagic duration of its early life stages. Early detection through scientific/stakeholder/citizen scientist networks, awareness raising by competent authorities and coordinated monitoring with FAO/GFCM activities is already taking place and damage control with fishing gear modifications is being explored. Subsidized removal programs can offer some compensation to fishermen for financial damages but have not proven very effective in suppressing populations in Cyprus (N. Michailidis, pers. comm.). Identifying spawning grounds and targeting spawning aggregations should be a priority. Harvesting for commercial/pharmaceutical purposes is a possibility for long-term population control but would need to be implemented very carefully as it could introduce conflicting management objectives.

Discussion

L. sceleratus is already present in the RA area, with well-established populations that are continuously spreading and steadily increasing to devastating densities at some locations. With respect to further establishment, some uncertainty is introduced by the lack of information on the eco-physiological requirements of the species and its tolerance thresholds to temperature and salinity at different life stages. Nevertheless, our approach is in relatively good agreement with a recent habitat suitability model for *L. sceleratus* (Coro *et al*., 2018) and captures remarkably well its current Mediterranean distribution. Tolerance and/or adaptability to lower temperatures (Nader *et al*., 2012) may enhance the potential for spread in cooler waters. Concerning the assessment of impacts, population declines of native cephalopod species can only be classified as a moderate impact, while no major impact due to local population extinction has been recorded so far. In a similar manner, the local disappearance of an activity from at least a part of the invaded range, such as the withdrawal by Cretan fishermen of their vessels from the small-scale local fishery, constitutes a major socio-economic impact. Concerning the strength of evidence for environmental impacts, our inference was based on fisheries landings data, which represent catch declines and may not necessarily reflect actual population declines. Furthermore, such correlative data do not permit the assigning of causation of these declines to *L. sceleratus*; this is reflected in the low confidence rating of our assessment (Tab. 3). Different types of data that may provide suitable information for a better characterization of the impacts and quantify the presence of the species include different census methods (e.g. underwater surveys) looking at *L. sceleratus* concurrently with potential prey species (see also GFCM-UNEP/MAP, 2018) and indicators derived from food web modelling (Michailidis *et al*, in prep). Finally, we urge scientists to use standardized metrics when reporting abundances of *L. sceleratus* (and other NIS) such as CPUE, CPUA that allow comparisons across studies. In conclusion, despite the lack of strong evidence, there are indications for potentially major environmental impacts by *L. sceleratus* through predation, while the demonstrated socio-economic impacts are severe both in terms of economic losses and with regards to human health risk. The Risk Assessment thus concluded that *L. sceleratus* is a high-risk invasive species for the EU such that concerted action is needed to effectively prevent, minimize or mitigate its adverse impacts.

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NO MPA IS AN ISLAND-CONSERVATION IN A THE WORLD'S MOST INVADED SEA

Abstract
Marine protected areas, created to conserve the indigenous diversity, are meant to offer an ecosystem-based approach to conservation, and to provide protection to species, habitats, ecosystems, and insurance against environmental or management uncertainty. The prevailing theory holds that MPAs, owing to their high species diversity and putative abundance of indigenous predators/competitors/parasites, are resistant to bioinvasions. Aiming to fulfill Aichi Target 11, some Mediterranean countries have been planning to increase marine areas under protection, though most plans fail to account for bioinvasions. It is likely the risk of bioinvasion will increase in future driven by trends in vectors and the alteration of propagule pools and conditions in recipient destinations arising from climate change. It is questionable whether MPAs, or even networks of MPAs, are able to conserve the indigenous biota under increased NIS load. We present examples which suggest that some Levantine MPAs have already been overwhelmed by NIS, nullifying conservation aims. It is essential to assess whether MPAs with large NIS populations serve as 'seed banks' inducing 'spill-over effect' to adjacent areas. *Planned MPAs should be located away from the regional hubs of vectors and pathways of introduction, whereas NIS populations in established MPAs should be monitored and effective options for their long term control assessed. Unless active bioinvasion management is urgently incorporated in* spatial marine planning*, we endanger the conservation of the indigenous biota.*

Key-words: bioinvasions, Marine Protected Areas, conservation planning, Levantine Sea, Mediterranean Sea

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INVASIVE BIOTA IN THE DEEP-SEA MEDITERRANEAN: AN EMERGING ISSUE IN MARINE CONSERVATION AND MANAGEMENT

Abstract
Although the ecological importance and impact of non-indigenous species is increasingly recognised and documented in shallow water ecosystems, their presence beyond the shelf has scarcely been documented. A survey of the upper slope biota of the Mediterranean coast of Israel revealed the presence at 200-m depth of individuals of three Erythraean species, the crocodile toothfish Champsodon nudivittus*, Golani's round herring* Etrumeus golanii*, and the burrowing goby,* Trypauchen vagina*. In the past decade several Erythraean species, some newly arrived, some well-established, have been collected on the Levantine lower shelf and upper slope. The species invasion revealed that thermal niche estimations based on the species' native environment may have underestimated their ability to tolerate lower temperatures. The results reported here suggest that the wide thermal tolerance of some Erythraean species may facilitate their bathymetric and geographic expansion. Their spread to the depths where the unique, diverse and fragile mesophotic 'animal forests' occur, bodes ill to these beleaguered communities.*

Key-words: bioinvasion, upper slope, conservation, Levant Sea, Mediterranean Sea

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ASSESSING THE STATE OF INVASIVE FISHES IN TWO MEDITERRANEAN MARINE PROTECTED AREAS AND ADJACENT UNPROTECTED AREAS

Abstract

The Mediterranean Sea is the most invaded marine region in the world. Besides their numerous ecological impacts, invasive species also affect the provision of ecosystem services with severe socio-economic consequences for coastal communities. In this study, we conducted underwater visual census inside and outside two Eastern Mediterranean Marine Protected Areas (MPAs), Zakynthos MPA in the Ionian Sea and Kaş-Kekova MPA in the Levantine Sea, to estimate the density and biomass of native and alien fishes. Additionally, we conducted interviews with local fishers to better assess the state of fish populations in the study regions. Fishers were asked about the presence of alien species in their catches, the change observed over the years, and the general state of fish stocks in their area. According to the fish surveys the biomass of alien species corresponded to 19% of total fish biomass in Zakynthos MPA, and 42% in Kaş MPA. In Zakynthos, the species Siganus luridus *was the only alien visually recorded, whereas in Kaş we recorded six alien species. Interviews revealed that fishers in Kaş perceived biological invasions as the most important reason for the current fish stock depletion whereas overfishing was identified as the most important reason in Zakynthos. The abundance of most invasive fishes caught by fishers in Kaş decreased since the year 2000 whereas in Zakynthos the abundance of invasives increased. In Zakynthos, none of the invasive fishes had a commercial value; conversely, the commercial value of all invasives increased in Kaş, with* Upeneus moluccensis *presenting a 4-fold increase in its price since 2000. In both regions, nearly all fishers stated that the current situation could be improved if overfishing is stopped through the enforcement of existing regulations.*

Key-words: fishers, impacts, invasive fish, Marine Protected Areas, Mediterranean

Introduction

Biological invasions are one of the main components of global change and the second most important driver of biodiversity loss (Bellard *et al.,* 2016). Invasive alien species cause disturbances to native communities in the recipient ecosystem through competition, predation, hybridization, and the displacement of native species (Molnar *et al.,* 2008).

A massive introduction of alien marine organisms into the Mediterranean Sea, known as Lessepsian migration (Por, 1978), occurred after the opening of the Suez Canal in 1869 which eliminated the natural geographical barrier that separated the Indo-Pacific ecosystem from the Mediterranean Sea. Some of these species have become invasive causing severe negative ecological and socio-economic impacts (Katsanevakis *et al.,* 2014). Up to date, more than 100 Lessepsian fishes have been recorded in the Mediterranean Sea (Golani *et al.,* 2013) both in unprotected and protected areas (Galil *et al.*, 2017).

In theory, Marine Protected Areas (MPAs) could host lower abundances of alien/invasive species based on major hypotheses in invasion biology and mechanisms related to the

effects of MPAs on ecosystems. For example, the restoration of top-down regulation processes (e.g., restoration of top predators' populations) in MPAs could help control the population of some alien species inside MPAs. However, relevant evidence is very limited and should be considered with caution (Giakoumi & Pey, 2017).
Herein, we assessed the state of invasive alien fishes in two Mediterranean MPAs, Zakynthos MPA in the Greek Ionian Sea and Kaş-Kekova MPA in the Turkish Levantine Sea, and adjacent unprotected areas by conducting underwater surveys. To have a broader view of the presence and impact of invasive alien fishes in the regions, we complemented the data from fish surveys with local ecological knowledge by performing interviews with local fishers.

Material and methods
Rocky habitats at 6-10 m depth were sampled at 3 replicate stations within each MPA and at another 3 replicate stations in adjacent unprotected areas. We conducted SCUBA surveys of the abundance and size of fishes along 6 replicate 25 m-long and 5 m-wide transects at each station. The diver swam at constant speed, identifying, counting and estimating the size of all individuals within 2.5 m on either side of the transect line. Length estimates of fish from surveys were converted to wet weight by using the length-weight relationship: $W = aL^b$, where W is weight in grams and L is total length in cm. Parameters a and b were obtained from Fishbase (Froese & Pauly, 2016). Alien species were classified according to Zenetos *et al.* (2010).
We then performed a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) to detect the effects of protection on the structure of alien species. "Protection" was considered a fixed factor (2 levels: protected vs unprotected), "Site" was a random factor orthogonal to "Protection", and "Station" was a random factor nested in "Site" and "Protection".
A total of 21 small-scale fishers (n = 13 in Kaş and n = 8 in Zakynthos) were interviewed based on semi-structured questionnaires. All fishers used mainly gill nets and long lines. Fishers were asked about the presence of alien species in their catches, the changes in their abundance observed over the years, and the commercial value (if any) of alien species. They were also questioned on the general state of fish stocks in their area and the reasons associated to the decline or increase of fish stocks. If fishers stated that they have observed decline of fish stocks, they were asked to suggest measures to reverse this negative trend.

Results
During the visual surveys in Kaş, we recorded six alien species: *Fistularia commersonii*, *Pempheris rhomboidea, Sargocentron rubrum, Siganus luridus*, *S. rivulatus*, and *Torquigener flavimaculosus* (Tab. 1). In Zakynthos, the only alien species recorded was *S. luridus* (Tab. 1). The mean biomass of alien species in the Kaş and Zakynthos MPAs was 501 (± 87) and 292 (± 41) gr/125 m^2 respectively (Fig. 1a), corresponding to 42% and 19% of total fish biomass, respectively. In terms of density, the average values were 21 (± 4) in the Kaş-Kekova MPA and 7 (± 1) individuals/125 m^2 in the Zakynthos MPA (Fig. 1b).
The effect of protection on the overall alien species biomass was significantly different between the protected and unprotected sites only in Zakynthos (pseudo-F = 6.5302, p = 0.043). In this MPA, *S. luridus* biomass was significantly higher than in unprotected adjacent areas (see further information in Giakoumi *et al.,* In Press).

Tab. 1: List of alien fishes recorded during visual census surveys in Kaş and Zakynthos and/or present in fishers' catches as reported by small-scale fishers.

Alien fish species	Kaş visual census	Kaş fishers' catches	Zakynthos visual census	Zakynthos fishers' catches
Fistularia commersonii	✓	✓		✓
Lagocephalus sceleratus		✓		✓
Pempheris rhomboidea	✓			
Sargocentron rubrum	✓	✓		
Siganus luridus	✓	✓	✓	✓
Siganus rivulatus	✓	✓		✓
Stephanolepis diaspros		✓		✓
Torquigener flavimaculosus	✓			
Upeneus moluccensis		✓		

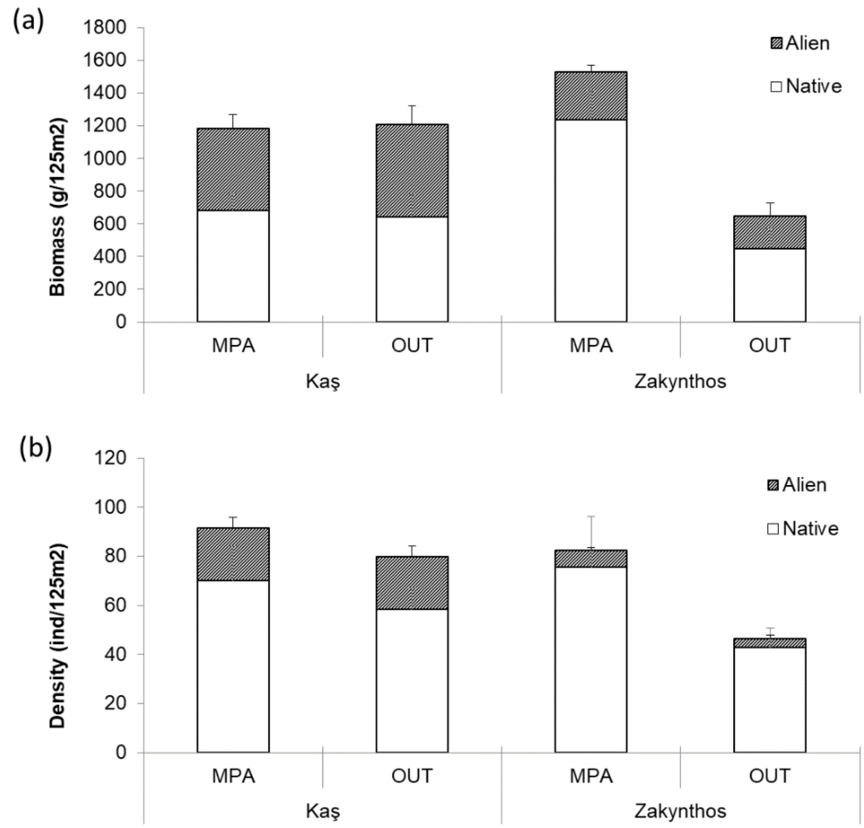

Fig. 1: Composition of native and alien fishes in and out of the Kaş and Zakynthos MPAs in terms of (a) biomass (g/125 m^2) and (b) density (individuals/125 m^2). The white part of the bar corresponds to native fish and the stripped part to aliens.

In Kaş, fishers reported that the following alien species have occurred in their catches: *Lagocephalus sceleratus*, *F. commersonii*, *Stephanolepis diaspros*, *S. rubrum*, *S. luridus*, *S. rivulatus* and *Upeneus moluccensis* (Tab. 1). When fishers were asked about the change (if any) of alien species abundance in their catches since the year 2000 (year of reference), the majority responded that the abundance of most species decreased except for the abundance of *L. sceleratus* and *U. moluccensis* which increased. Conversely, in

Zakynthos, the abundance of all alien species present in fishers' catches (*L. sceleratus*, *F. commersonii*, *S. diaspros*, *S. luridus* and *S. rivulatus*; Tab. 1) has increased since 2000. In Kaş, all fishers stated that the only discarded alien fish was *L. sceleratus*. Conversely, in Zakynthos all alien fishes were discarded; only one fisher (out of eight) stated that he eats *S. rivulatus* and two that ate *S. diaspros*. In Kaş, all alien species were commercially exploited except for *L. sceleratus*, *F. commersonii*, and *S. diaspros*. In fact, the value of the commercially exploited alien species increased in the past decade with *U. moluccensis* presenting a 4-fold increase in its price since 2000. In Zakyntos, none of the alien species caught had a commercial value.
When fishers were asked about the general state of fish stocks, all fishers in Zakynthos (n=8) stated that fish stocks have been declining whereas in Kaş the opinions were divided, 54% stating that the fish stocks are stable while the rest stating decreasing fish stocks. In Kaş, biological invasions were reported as the main reason for their catches decline whereas in Zakynthos overfishing was identified as the principal cause (Tab. 2). Stopping overfishing and illegal fishing activities by enforcing existing laws was identified as the most important measure to help the recovery of fish stocks by all fishers in both regions. In Kaş, the vast majority of fishers (92%) thought it was equally important to manage and reduce the impacts of invasive alien fishes.

Tab. 2: Reasons explaining the depletion of fish stocks according to fishers' perceptions. Some fishers gave more than one answer.

Reasons for fish stock depletion	Zakynthos (fishers No)	Kaş (fishers No)
Overfishing	6	4
The use of illegal fishing tools by professional fishers (e.g. use of dynamite)	0	2
Recreational fishing - spearfishing	1	4
Illegal fishing practices by recreational fishers	0	0
Large scale fishing (trawlers, purse seiners)	1	0
Pollution	2	4
Climate change	4	1
Lack of law enforcement	2	9
Dolphins, seals, or turtles	1	6
Invasive species	0	13

Discussion and conclusions

Invasive alien species are a major threat for Mediterranean marine ecosystems which based on our evidence present equal or greater biomass in MPAs than in unprotected areas. This evidence suggests that additional management actions are required in MPAs for the control of invasive alien fish populations (Giakoumi *et al.,* In Press).
In both MPAs, the biomass of the herbivore alien fish *S. luridus* was particularly high. In the Kaş-Kekova MPA, *S. rivulatus* was also present. Evidence shows that the combined grazing effect of these two herbivore fishes can create barrens (rocky reefs deprived of vegetation) and consequently lead to biodiversity impoverishment (Vergés *et al.,* 2014). Although other factors (abiotic and biotic) can contribute to the creation of barrens,

limiting the grazing activity of these alien fishes in Mediterranean MPAs by controlling their populations should be a management priority.

The region of Kaş in the Levantine Sea has been exposed to biological invasions for a longer period than Zakynthos in the Ionian Sea. This fact has affected the way local fishers perceive the impact of invasive species on marine ecosystems. In highly impacted Mediterranean areas, such as the Levantine and South Aegean Seas, fishers perceive biological invasions as a major reason for the decrease in their catches (see also Panagopoulou *et al.,* 2017). More specifically, the high level predator fish *L. sceleratus* is perceived to cause serious damages to the small-scale fishing gears. At the same time, this fish being extremely poisonous cannot be commercially exploited.

The period of invasion exposure is also related to the commercial value attributed to alien fishes. In Kaş, where currently almost half of the fish biomass corresponds to alien species, most of these species are consumed and their commercial value has been increasing. Conversely, in Zakynthos, where fish invasion is relatively recent, alien species are discarded from fishers' catches and they are not commercially exploited. Increasing the consumption of alien fishes, and particularly of *S. luridus*, through marketing and targeted removal, could benefit substantially native marine communities and increase fishers' income.

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OUT OF SIGHT, OUT OF REACH, OUT OF MIND: INVASIVE LIONFISH *PTEROIS MILES* IN CYPRUS AT DEPTHS BEYOND RECREATIONAL DIVING LIMITS

Abstract
Biological invasions are a worldwide environmental phenomenon contributing to a wave of massive ecological disruption. Removal of individuals is one action to maintain low levels of an invasive species in order to lessen the ecological impacts. Since the Indo-Pacific lionfish Pterois miles *(Osteichthyes: Scorpaenidae) was first observed at a few localities in Cyprus in 2012, this species has successfully expanded, establishing almost all around the island. Maintenance management by periodical removals, mostly conducted by volunteers (e.g. recreational divers), is suggested as a form of control. In this study, lionfish abundance below the recreational diving depth limit (30m) is presented. Technical divers, spearfishermen, fishermen, first-hand monitoring by technical scuba diving, and consultation of project reports and published results allowed to produce a general estimation of lionfish abundance at depths between 30m and 150m. Our data suggest, unsurprisingly, that lionfish populations thrive at great depths and adults and juveniles are found in various substrate types throughout the year. This on-going study generates more questions than it answers but strongly calls for attention to those deep-water populations of lionfish. These individuals may be the source of recruits that repopulate shallower areas particularly after removal activities, which are limited to less than 40m. It is only when experienced spearfishermen or trained technical divers participate in the removals that the depth limit can be extended. The potential role of deep-water habitats as refugia for lionfish needs to be considered in managing actions. Possibly due to the fact that these areas are technically difficult to reach, they are usually kept out of the discussion table and most resources and actions are limited to shallower areas.*

Key-words: Invasive species, species removal, maintenance management, population control, culling

Introduction
Few biological invasions in the marine realm have attracted such general attention as the on-going spread and establishment of the Indo Pacific lionfish *Pterois miles*/*volitans*. Within the last 33 years it has spread in areas of the Western Atlantic, Caribbean Sea and Gulf of Mexico (ca. 4 million square kilometres by one estimate; Hoag, 2014). This remarkable dispersal and wide distribution of lionfish prevent eradication. In consequence, many removal (culling) programs have been initiated in the invaded areas to reduce populations at least locally (e.g. Akins, 2012; Green *et al*., 2017a). However, removal activities are highly controversial no matter which organism is causing the invasion (see review in Simberloff, 2013) and they may benefit if socio-political aspects are considered before their implementation (Crowley *et al*., 2017). In Cyprus, stakeholders' perceptions on culling were diverse (Jimenez *et al*., 2017) and opposition

was one common stand. The fact that lionfish is capable to inhabit deep-water habitats raise concerns on the efficacy of shallow-water removals if those populations beyond reach of removal activities may act as source of new recruits (Andradi-Brown *et al.,* 2017; Gress *et al*., 2017). Normally, targeted removals have the participation of volunteer divers restricted by the depth limit imposed by recreational diving regulations (-30m), leaving deeper fish communities undisturbed. In Cyprus, the first targeted removal (tournament) had the participation of volunteer divers and freedivers that extracted over 300 lionfish (Jimenez *et al*., 2018). It is envisioned that teams of volunteers will continue performing periodic removals in shallow habitats during similar organized activities in Cyprus and elsewhere (e.g. ReLionMed-LIFE Project; http://relionmed.eu/). However, little attention is paid to deep-water habitats. The aim of this study was to: (i) record lionfish presence in deep-water habitats (>30m), (ii) determine the presence of juveniles or adults, (iii) compare their distribution around the island according to the substrate type, and (iv) raise awareness about the potential role of these deep-water populations in repopulating shallower areas.

Materials and Methods

Lionfish distribution and abundance at depths greater than 30m around Cyprus were determined by interviewing freedivers, technical divers and fishermen. This approach to obtain lionfish distribution knowledge from local people has proven to be effective in the Levantine region (see Azzurro & Bariche 2017). Most of respondents were already participating in other research projects related to natural predation of lionfish and deep-water marine caves (Jimenez in prep.). They were asked on the presence/capture of lionfish in deep-waters, when and where, and if possible, provide samples or photographs. In addition to the interviews, periodic monitoring of areas (40-55m depth) by means of technical diving allowed us to produce first-hand data on the abundance, behaviour and seasonality (data not presented here) of lionfish. Due to constrains in the bottom time, technical divers inspected the substrate and fish communities following the bottom relief and observation time was according to each particular place (e.g. depth); observations were all standardized by time (data not presented here). Important information was also extracted from published information (e.g. Jimenez *et al.*, 2016a; Kletou *et al.*, 2016; Orejas *et al.*, 2018) as well as unpublished reports of a research trawl survey conducted by the project Protecting Mediterranean East (PROTOMEDEA; http://www.protomedea.eu/en/). Commercial trawler companies and interviews to crew members provided also records of lionfish.

Results

Lionfish appear to have a wide spatial and bathymetrical distribution in Cyprus. Even though the areas from where data was produced around the island (Fig. 1) are very diverse in terms of substrate type, the main sources of information confirmed that lionfish are present in all localities and well below the recreational diving limit (Tab. 1). The first reports of lionfish in 2012 were of juveniles below -30m; in the following years the depth of the sightings continued to increase and since 2016/2017 have been substantial year-round. This pattern applies as well to the fishermen catches using nets (Tab. 1); from single or very few individuals caught in the nets (2014/2015), the catches started to increase in quantity and weight. Not surprisingly, the trawlers and the nets provided the deepest records of lionfish (Tab. 1), which are between -80 and -90m and between -90m and -150m, respectively. Trawled areas are distributed along a considerable section of the coast (Fig. 1) but the sampling effort is minimal or none during the summer months, mostly due to the cessation of commercial trawling during this time of the year. Only trawling for research is currently done during that period. The deepest depth record of lionfish from the nets is interesting (and worrisome) and is because the specimens of lionfish came from a coral habitat. The nets were cast in an area known locally as the "coral bank" at the eastern coast of Cyprus.

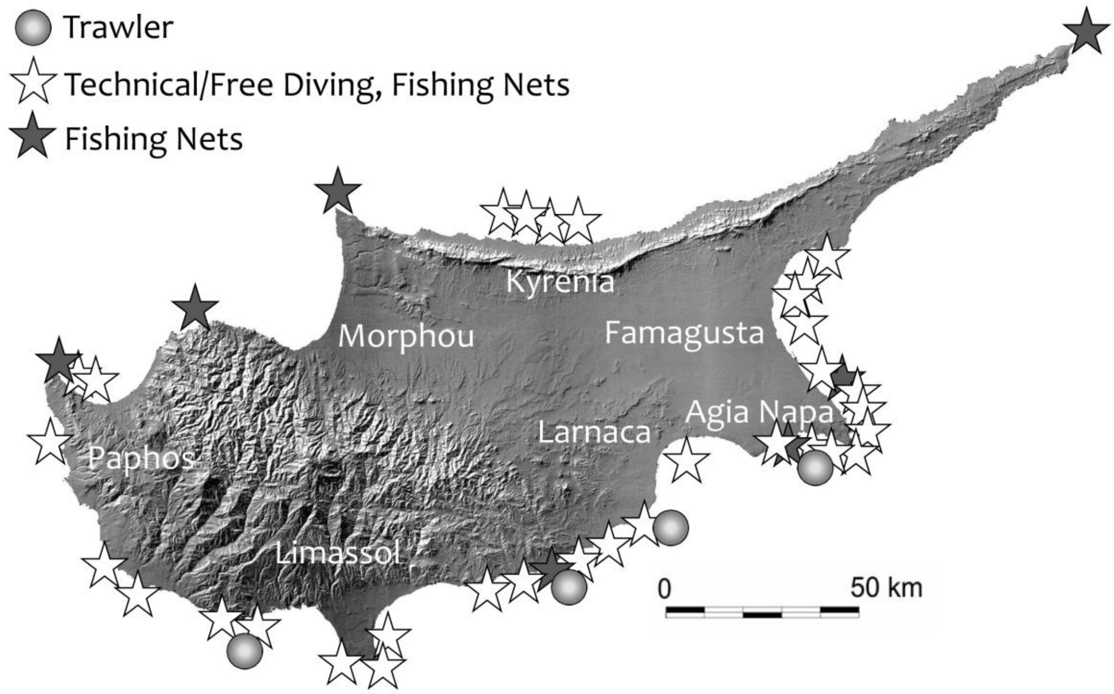

Fig. 1: Locations around Cyprus from where information was gathered on lionfish abundance at depths between -30m and -150m from Technical/Free Diving and fishermen catches with nets (white stars) or exclusively from catches (grey stars), and from commercial and research trawlers (circles). Due to the small scale of the map, symbols may represent more than one location.

Tab. 1: Lionfish sightings (number of individuals) in Cyprus below the recreational diving depth limit (-30m) by means of technical diving (TD), freediving (FD), fishing nets (FN), and research (TW1) and commercial (TW2) trawlers. Sources of the data: a-Jimenez *et al.* (2016a); b-this study; c -Kletou *et al.* (2016); d-PROTOMEDEA 2016 Trawl Survey (unpublished interim report); e-Orejas *et al.* (2018).

Depth (m)	2012	2013	2014	2015	2016	2017	2018	Sources
30-40	2	2	16	25	67	176	472	TD-a, FD-a, FN-a
40-50			10	29	74	127	479	TD-a,b, FD-b,c, FN-b
50-60				1	44	77	164	TD-b, FN-b
60-70					1	39	51	TD-b, FN-b, TW1-d, TW2-b
70-80					3	30	39	FN-b, TW2-b
80-90						4	37	FN-b, TW2-b
90-150						5	2	FN-b,e

Noteworthy is the fact that the specimens from the deepest records (nets and trawlers) were all juveniles (9.1cm TL the smallest) or young adults (15.6cm TL the largest) (Fig. 2a,b).

Large adults were absent from these collections and the commercial trawlers seem not to have collected any since they are requested to keep records/samples.

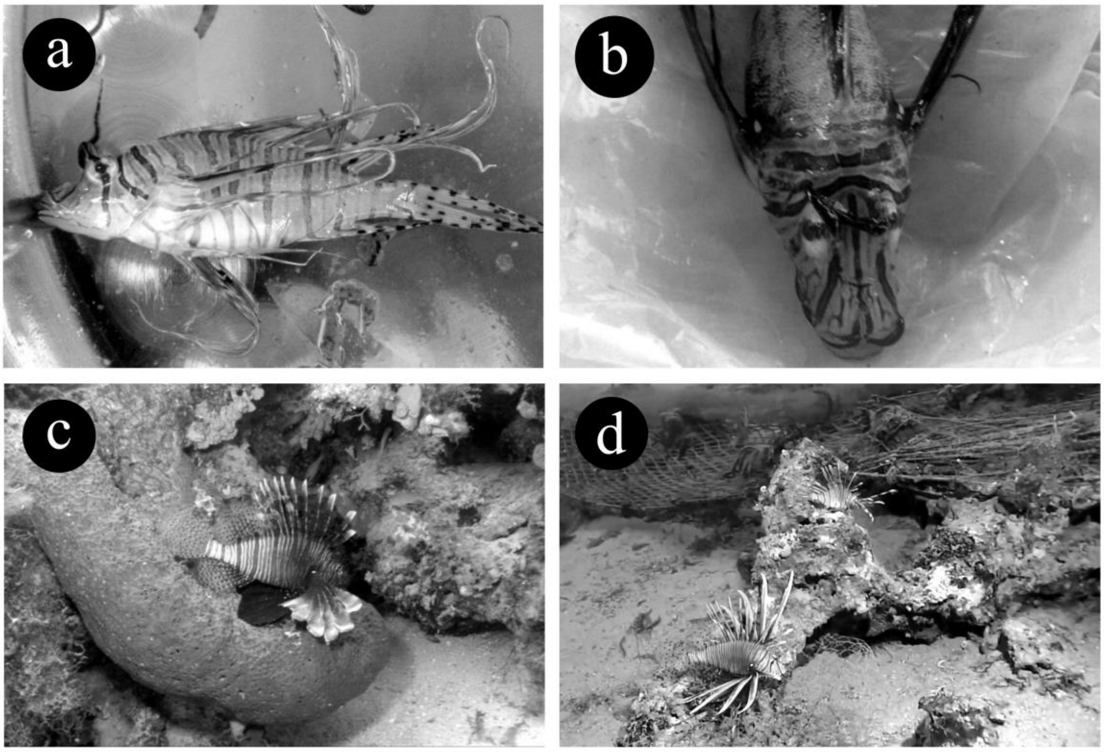

Fig. 2: Examples of lionfish from different depths around Cyprus. Juveniles/young adults from a sandy bottom at -68m approx.; PROTOMEDEA Trawl Survey 2016 (a). Juvenile from a sandy bottom at -89m approx.; commercial trawler (b). Large adults from coralligenous habitats at -56m (c) and -58m (d); technical diving monitoring for this study. Derelict fishing gear can also be seeing (d) spreading further into deeper water.

The periodic monitoring of selected areas by means of technical diving were concentrated on coralligenous habitats. Lionfish abundance varied considerably along the year particularly between -45m and -60m. Single individuals (Fig. 2c) or pairs (Fig. 2d) are commonly found active, moving around substrate. Adults (20-35cm TL) tend to be more abundant than juveniles at least during the time of the surveys (between 07:30 and 14:00 hrs). On very few opportunities, lionfish were found in groups larger than 2-3 individuals (max. 4).

Discussion and conclusions

Until this study, most of the research on lionfish in Cyprus has focused on shallow habitats (-30m depth max.), no information was available on populations inhabiting the deep-water coralligenous habitats or any other habitat at those depths. This situation is common since logistics and technical constrains in the shallows are less problematic and less expensive than what is needed to do research in deeper waters. The results of this study, somehow expected, suggest that deep-water habitats, beyond reach by conventional (e.g. recreational) diving techniques, harbour large populations of lionfish. For example, at the beginning of the spread and establishment of lionfish around Cyprus, there were very few sightings in shallow and deep-water habitats (Jimenez *et al*., 2016a; Kletou *et al*., 2016). It was only in 2015 that fishermen started catching lionfish

(1-2 individuals) regularly in deep-water (Jimenez *et al*., 2016a; this study). Nowadays it's not unheard-of to have catches of 10kg or more of lionfish usually below the -45 m depth mark (Jimenez, in prep.). Given the evidence of adult lionfish in deep-waters elsewhere (e.g. Gress *et al*., 2017), the apparent scarcity of large specimens in the catches deeper than 85m may be the result of the small sample/effort size (1 trawler and 2 fishermen); this perception may change with an increase in the sampling effort (temporal and spatial).

The ecological effects of lionfish on the deep-water habitats can only be inferred for the moment but the find that the species is already in the coral habitat built by the coral *Dendrophyllia ramea* (Orejas *et al.*, 2017) is of major concern. Bioinvasions are a threat to deep-water coral habitats in the Mediterranean (Galil *et al*. 2018), and this coral habitat in Cyprus is unique in the entire Mediterranean Sea (Orejas *et al*., 2018). Lionfish populations from deep-water habitats may function as sources of new recruits that will inhabit shallower areas due to seasonal and ontogenetic migrations between shallow and deep habitats (Andradi-Brown *et al*., 2017b; Harms-Tuohy *et al*., 2018). The implications of this "reloading" of the lionfish effects on the already stressed shallow habitats of Cyprus (e.g. Jimenez *et al*., 2016b) are not only ecological. Management activities for the purpose to lessen lionfish ecological impacts by decreasing population numbers, for example, will be weakened if not ineffective. The mere existence of these deep-water lionfish populations, out of reach, sight and of most managing plans, force to reconsider the overly enthusiastic expectations of the targeted removals (Green *et al*., 2017a; Gress *et al*., 2017; Côté & Smith, 2018). Culling of lionfish during organized activities (tournaments, festivals, derbies) is a viable activity with important educational outreach (Jimenez *et al*. 2018; Kleitou *et al*., submitted), although of limited benefits under particular conditions (see Tuohy *et al*., 2018). However, managers need to consider this action among the options for controlling lionfish populations. Possibly the best option is to continue performing targeted removals, frequently enough and in a wide geographical scale, at the same time that the possibilities and the means to target deep-water populations of lionfish are evaluated and tested.

Acknowledgements

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TACKLING THE LIONFISH INVASION IN THE MEDITERRANEAN. THE EU-LIFE RELIONMED PROJECT: PROGRESS AND RESULTS

Abstract
The lionfish invasion in the Western Atlantic has been characterised as one of the most ecologically harmful marine fish introductions to date; associated with habitat modifications, and severe impacts on native communities. In the Mediterranean, lionfish followed similar expansion trends and raised significant concerns among the scientific community due to its potential to cause devastating ecological and socioeconomic impacts. The coastal ecosystems of Cyprus, near the Suez Canal, are amongst the first Mediterranean waters to be affected by the lionfish invasion. Cyprus sentinel location offers therefore, an ideal site for the development of an early warning and rapid response system of marine bioinvasions. RELIONMED (Preventing a LIONfish invasion in the MEDiterranean through early response and targeted REmoval) is a four-year project, funded by the EU LIFE instrument, aiming to make Cyprus the first line of defence against the invasion of lionfish in the Mediterranean. The project has started successfully on September 2017 with a number of early (preparatory) project actions including stakeholder consultation and baseline assessment of social awareness, biological analyses of a small lionfish sample, and the development of a lionfish risk assessment following the guidelines of the Regulation 1143/2014 to include the species in the EU IAS priority list. Forthcoming project's actions strongly rely on citizen scientists' and stakeholders' participation and RELIONMED aims to develop the capacity and tools for control of lionfish, particularly in priority habitats. Preliminary results such as early maturity, high growth rates, generalist diet, and reproduction throughout the year indicate that lionfish can become a ferocious invader for the basin and RELIONMED calls for regional collaborations and coordinated management measures against lionfish invasion in the basin.

Key-words: Lionfish, Invasive species, *Pterois*, Mediterranean, Cyprus

Introduction

Non-Indigenous Species (NIS) are increasingly spreading across biogeographic barriers, shifting species' communities and assemblages (Geburzi & McCarthy, 2018). Despite a decreasing rate of NIS introductions via the Suez Canal (Zenetos, 2017), there are significant evidences that the ecosystems on continental shelves of the eastern Mediterranean are experiencing unprecedented shifts in species abundance and community composition (Arndt *et al.*, 2018; Givan *et al.*, 2018). The increasing alien species biomass is likely to obstruct any future fishery management measures and resilience to sea warming of the region, if its control is not included in an ecosystem-based management approach (Corrales *et al.*, 2018).
Evidence from throughout the eastern Mediterranean shows that a lionfish [*Pterois miles* (Bennett, 1828)] invasion is underway (Giovos *et al.*, 2018; Jimenez *et al.*, 2016; Kletou

et al., 2016). Following an unsuccessful invasion in 1991 (Golani & Sonin, 1992), the lionfish were recorded again in 2012 off Lebanon, and numbers have proliferated and spread, reaching the central Mediterranean Sea in just three years (Azzurro *et al.*, 2017; Bariche *et al.*, 2017). In the Western Atlantic, lionfish has been considered as one of the most harmful fish invasions to date, responsible for detrimental impacts on the biodiversity and ecological functions of the region (Albins & Hixon, 2013). The potential threats of lionfish in the Mediterranean were recognized in an EU horizon-scanning exercise conducted in 2014 which listed lionfish as second in a list of 95 new or emerging Invasive Alien Species (IAS) that should be prioritised for risk assessment for inclusion in the EU priority list according to the Regulation EC/2016/1141 (Roy *et al.*, 2015).

Cyprus is amongst the first Mediterranean countries to be affected by the invasion (Kletou *et al.*, 2016). Due to its sentinel location near the Suez Canal, it has a strategic role in understanding lionfish invasion dynamics, and exchanging information, data and best practices related to management programmes to the wider region. The RELIONMED project (Preventing a LIONfish invasion in the MEDiterranean through early response and targeted REmoval) is a four-year project funded by the EU LIFE instrument, which aims to make Cyprus the first line of defence against the invasion of lionfish in the Mediterranean. The project focuses on priority habitats and biodiversity hotspots such as MPAs and its main objectives are a) to develop the capacity to ensure that Cyprus can tackle the lionfish invasion, b) to assess the effectiveness of a range of lionfish invasion prevention measures such as the development and implementation of an early detection and removal system driven by motivated citizens and c) to develop tools that can be transferred and replicated in other countries of the region.

The RELIONMED project has started successfully on September 2017 with a number of early (preparatory) project actions including stakeholder consultation and baseline assessment of social awareness, biological analyses of a small lionfish sample, and the development of a lionfish risk assessment following the guidelines of the Regulation 1143/2014 to include the species in the EU IAS priority list. The findings up to date together with the forthcoming project actions are presented.

Materials and methods

<u>Early Preparatory Actions</u>

A series of preparatory actions have been conducted to set a strategic plan for the implementation of the project's core actions.

Specifically, consultations with key stakeholders were organised, while public and stakeholder interviews were carried out to understand lionfish awareness. A telephone survey of 300 Cypriot permanent residents was conducted while 108 stakeholders (i.e. defined as people who make use of the marine environment as a resource or who are involved in the decision-making) were interviewed during meetings carried out across different districts of Cyprus. Additional stakeholder-focused questionnaire surveys were carried out with 20 commercial fishers, 6 dive business owners, 20 recreational fishers, 10 restaurant owners, 100 beach visitors, and 5 aquarium/pet shop owners.

A literature review was conducted to describe and assess the current distribution of lionfish, its expansion rates and the invasion pathway in the Mediterranean. High risk areas around Cyprus and the wider regions were identified and displayed on GIS risk maps. Furthermore, lionfish samplings were carried out throughout the first year of the project (September, 2017 – August, 2018), and a total of 268 lionfish (instead of 50 anticipated by the project proposal) were collected. Morphometric analysis was conducted in all specimens, reproductive developmental stage and gonadosomatic indices were calculated for 164 lionfish, lionfish growth rates were evaluated using the otoliths of 53 individuals, stomach contents were macro- and microscopically examined from 65 specimens, and lionfish tissues from 56 individuals were genetically analysed. For the latter, the Cytochrome c oxidase subunit 1 (COI) gene and the Control Region (CR) were targeted while Bayesian inference (BI) and Maximum Likelihood (ML) methods were recruited for the phylogenetic analysis.

All of the above complemented the development of a risk assessment following the guidelines of the EU Regulation 1143/2014 and guided the strategic implementation plan of the project.

Future Core Actions

The core actions of the project are expected to start in 2019. RELIONMED has a demonstrative character with three major areas of development, namely the demonstration of surveillance and early detection system, the demonstration of a removal response system, and the lionfish market exploitation. The surveillance and early detection system will be developed in collaboration with MedMIS and will include a GIS tracking online interactive platform and a mobile phone application to alert stakeholders to the presence of lionfish. The Removal response system will be demonstrated with motivated fishers and divers who will be equipped with toolkits and will be trained on how to remove lionfish. Removal Action Teams composed of volunteer divers will utilize targeted removals in priority areas. In addition, lionfish removal derbies/championships for divers will be organised every three months for two consecutive years. The lionfish market exploitation will be demonstrated by promoting and serving lionfish as a delicacy at the restaurant business and by exploring the potential of using lionfish fins in the local jewellery and artwork market.

Ecological and socio-economic indicators will be monitored throughout the project. The results acquired from all actions will be evaluated to develop a lionfish management guide which will indicate the most cost-effective practices identified, and make recommendations of an integrative efficient management strategy for the detection and control of lionfish. Suitable dissemination and transferability activities are planned so to raise public awareness about lionfish and invasive species, and share the identified best-practices to the wider scientific and management community of Cyprus and neighbouring countries.

Results

Public and stakeholder awareness

The interviews revealed differences between public and marine stakeholders' knowledge and perceptions; with the latter being significantly more informed. There was a divergence in opinion regarding the consumption of lionfish and the purchase of products made from lionfish (e.g. jewellery). The public was more opposed to such statements than the marine stakeholders. However, there was almost unanimous support towards further research and development of a management strategy to limit the spread of lionfish. The opinions in regards to the feasibility of lionfish control were contradictory amongst stakeholders. Currently there is no market value for lionfish and lionfish caught from fishers were discarded or given to friends. Restaurant owners characterised the demand as low to non-existent, mainly due to lack of consumer awareness and little supply. Most of them believed that a chef could benefit from knowing how to prepare lionfish in the future. Most commercial fishers catch lionfish on a daily basis (many reported an average of around five lionfish per day). Recreational fishers reported that they catch lionfish mainly using a speargun, but some reported fishing nets, fish traps, and hook and line as capture techniques.

Lionfish invasion dynamics

A total of 384 lionfish observations from Cyprus were recovered during the literature review and the RELIONMED project development or early project actions. Most lionfish were recorded within the warmer areas of eastern Cyprus; in the unexposed sites of Cape Greco MPA and Nissia, Famagusta bay and Larnaca (Fig. 1). These are areas with a predominant South - South East circulation pattern and known to favour the establishment of NIS. A total of 74 lionfish were caught in an MPA at Cape Greco area on 27th November, 2017 after four dives (composed of four divers each) in an area of around five hectares (500 m x 100 m); demonstrating the remarkable expansion that lionfish populations have experienced in recent years.

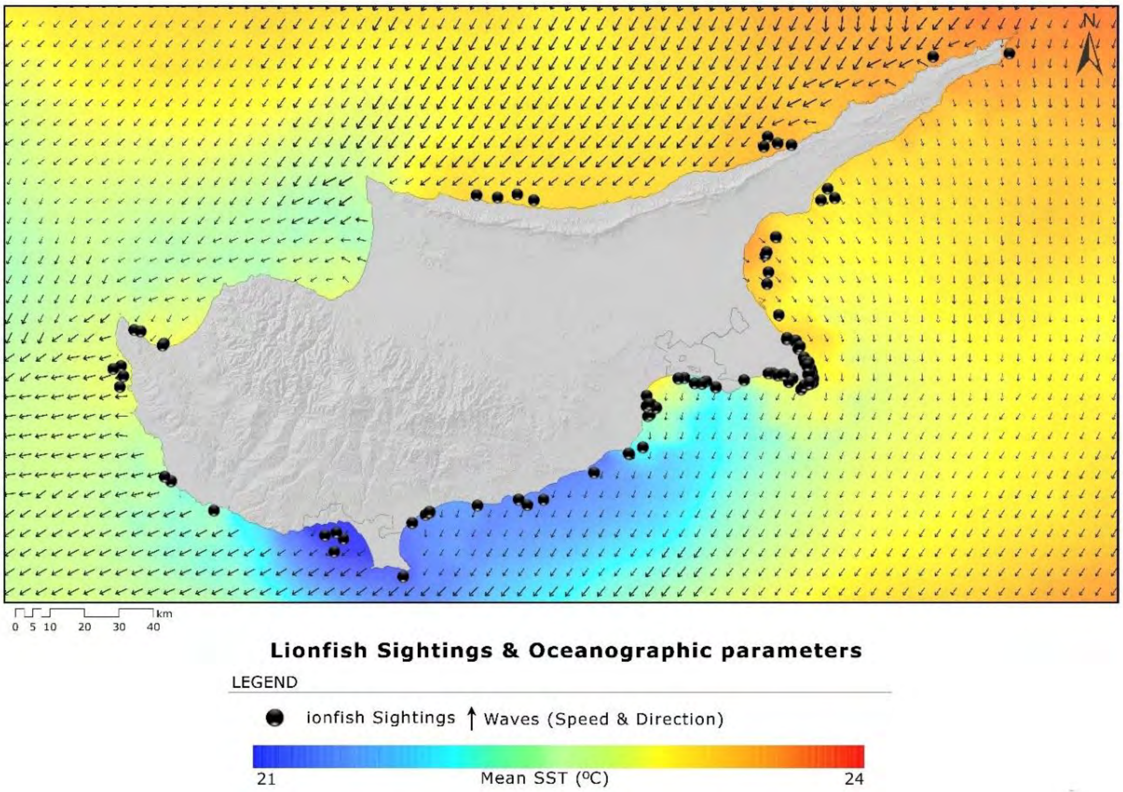

Fig. 1: Annual oceanographic conditions (SST and wave direction/speed) (Georgiou *et al.*, in prep) and lionfish sightings recovered from the grey literature

From the 268 lionfish caught (i.e. 126 females and 82 males), the biggest specimen was a male and attained a TL of 37.1 cm, a SL of 28.4 cm and weighted 755 g. The lionfish with the highest biomass was another 850 g male, having a TL of 36.8 cm and a SL of 27.6 cm. The highest recorded mouth gap area was 32.5 cm^2. Lower numbers of larger lionfish during the cold seasons (i.e. winter and spring) were detected suggesting a possible ontogenetic shift to deeper waters. Lionfish can be characterised as generalist predators, as their diet constituted of a range of native pisces and crustacean prey, whereby some are of economic (e.g. *Spicara smaris* and *Sparisoma cretense*) and ecological value (e.g. *Chromis chromis* and *Thalassoma pavo*). Otolith examination showed that lionfish grow fast and can reach 20 cm in one year of age (Fig. 2). Average age was 1.92±0.66 years for females and 2.15±0.80 for males with the maximum age detected being 4 years old in two individuals. Otolith and macroscopic gonad analyses suggest that lionfish may become mature in less than a year of age and that mature lionfish could be spawning year-round. High gonadosomatic indices indicate a peak in reproductive potential during summer (June – August), coinciding with seawater warming (Fig. 2). Genetic analyses demonstrated a low genetic diversity between the lionfish of the most distant and geographically distinct areas. Haplotypes resembled individuals from the Red Sea, although some showed higher genetic similarity to *P. miles* from the Indian Ocean.

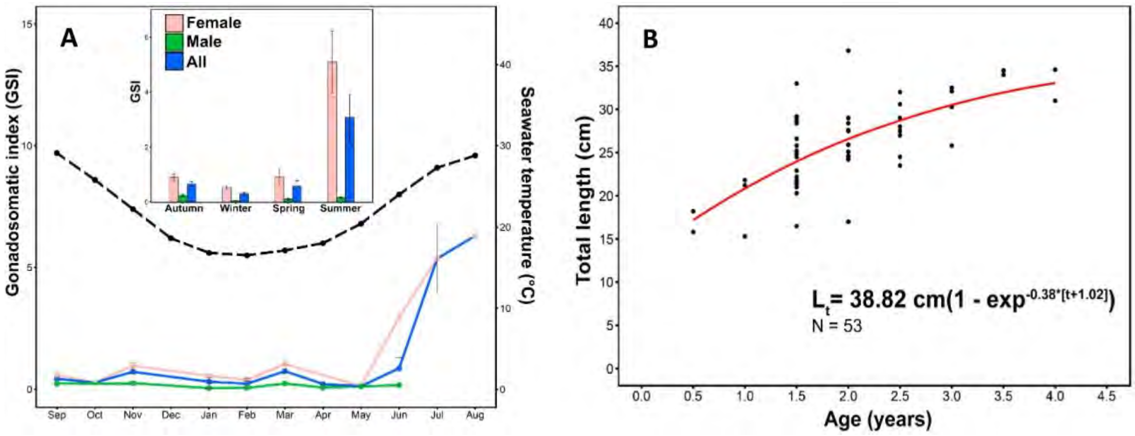

Fig. 2: (A) Mean gonadosomatic indices (GSI) ± SE for female, male and total fish (n=164) collected throughout the study period, with mean water temperatures (◦C) at 5 m depth (dashed line; acquired from data logger at eastern coast of Cyprus). (B) Growth curve fit to length (L_t) at age (t), data derived from the examined otoliths (n=53).

Discussion and conclusions

The preparatory actions of the project, particularly the biological analyses, suffered from low spatial and temporal sampling size. However, the high growth rates observed, the reproductive potential exhibited, and the generalist diet, together with the rapid expansion of lionfish in the region, indicate that the species is well-established and thriving, and can potentially become a ferocious invasive species for the region. Immediate action is required from national and regional management authorities.

RELIONMED is a demonstrative project and seeks for research collaborations on a multinational or regional level to study and better understand lionfish ecology and dynamics. Enormous biological material will be collected through the demonstration activities which can become valuable in answering important biological and ecological questions.

Through the forthcoming activities, RELIONMED aims to: establish rich and deep links with the local communities, scientific communities and policy makers; strengthen regional cooperation, responsibility and surveillance on corporate and social levels and; potentially turn lionfish from a threatening and dangerous invasive species to a stimuli for environmental consciousness, material for education, information and awareness. RELIONMED has the potential to demonstrate a notable example on how communities and regions can work together to protect and improve the ecosystem.

Acknowledgments
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MONITORING OF SPECIFIC BIODIVERSITY AND PRELIMINARY UPDATE INVENTORY OF ALIEN SPECIES FROM BIZERTE COASTAL AND LAGOON WATERS

Abstract

This work was carried out as part of a local regular monitoring of marine biodiversity in Bizerte region focus on the Invasive Alien Species (IAS) and especially on the invaders one. From 2016 to 2018 a field surveys was done to develop a standard and qualitative method to determine the present and historical knowledge of both IAS and local biota using the bibliography as an extra tools. Samples were collected from different sites: the coastal wave breaker in front of Bizerte channel, the harbour, the channel and the Bizerte lagoon as the main site of our surveys. Punctual surveys were made in the Cani islands. A total of 22 alien species has been recorded: Crustacean (32%), Chorda (18%) and Algae (18%). Mollusca and Cnidaria present almost (10 %). One corals (Oculinida) was reported as Oculina patagonica*.The top recent alien species which were recorded are* Caprella scaura*,* Balanus trigonus and Styela plicata*.*

Regarding the important risk to introduce IAS in Bizerte complex of harbour-channel-lagoon, more investigations must be done in this area with more performed protocol as Rapid Assessment Survey (RAS) and a Local Ecological Knowledge (LEK) protocol. This latter focused mainly on fish, crustacean and molluscs. We propose to implement a regular monitoring biodiversity and to get emergency call against invasive alien species, regarding their environmental and socio-economically impacts, particularly in higher anthropized and populated areas such as Bizerte.

Key-words: Alien species*,* Invasive species, NIS, Bizerte lagoon, Tunisia

Introduction

The Tunisian coasts situated between the western and eastern basins of the Mediterrean, can be considered as a hotspot for the introduction and arrival of Non Indigenous Species (NIS). In this context, the Subregional Committee for Central Mediterranean (SRC-CM) of the GFCM, as well as other Mediterranean instances, was recommended on April 2018 to focus monitoring programme and alert plan for IAS (especially for fishes and molluscs (crustacean and bivalve) in Central sub-region (Tunisia, South Italy, Sicily and Libya (Sirt Gulf). According Ounifi-Ben Amor *et al.*(2016), 136 alien species were recorded in Tunisian waters, 60 records in northern coasts, west Mediterranean and 76 in central and southern coasts, central Mediterranean. Nearly half of the first sightings in Tunisian waters took place in the Gulf of Gabes. Among them, 17 species with invasive behavior, 2 marine plants and 15 animal species especially molluscs and crustaceans (particularly *Metapenaeus monoceros*, *Libinia dubia* and *Portunus* spp.). Some IAS have already reported and established in Tunisian waters, especially in the southern regions (Gulf of Gabes) and East. Among these species to be monitored are *Halophila stipulacea*, *Caulerpa taxifolia*, *Portunus segnis* (and *Portunus* spp.), *Callinectes sapidus*, and *Libinia dubia* (Ounifi-Ben Amor *et al.,* 2016).The last confirmed record of a non indigenous species in the studied site was the amphipod *Caprella scaura* observed on November 2017, in the north-eastern part of Bizerte lagoon (Chebaane *et al.,* 2018).

Materials and methods

Sampling

The study focused mainly on the Bizerte lagoon, as well as the channel, the only communication with the sea. Surveys at the wave breaker in the area of the commercial outer harbour, as well as prospections in the Cani Islands have been punctual. These two field surveys took place respectively in September 2016 and May 2017.

Species identification and LEK investigations

The denomination of the recorded and sampling species and the attribution of their scientific name after validating identification is based on the taxonomic systems of the data base Word Register of Marine Species (http://marinespecies.org)(WoRMS, 2018).The Presence of all species was checked with site prospections mainly during summer period between August and September 2018. The Local Ecological Knowledge Protocol (LEK Protocol) developed by Azzurro *et al.,* 2011 and applied by several Mediterranean investigtions (e.g. Azzurro & Bariche, 2017; Azzurro *et al.,* 2018) was used to interview 17 fishermen from Menzel Abderrahmen locality to gather information on alien species and historical trends. These witnesses were also a tool for consolidating citizen sciences with local fishermen.

The questionnaire performed with the fishermen concerns mainly fauna species (by showing them photos of the targeted species) which they were not accustomed to see its in the last years and the changes in their abundances. More generally, questions have been asked about changes affecting biodiversity observed by fishermen in Bizerte lagoon on the last decennium.

Results

A total of twenty-twoalien species have been recorded in the study sites: Crustacean (32%), Chorda (18%) and Algae (18%). Mollusca and Cnidaria present almost (10 %). One corals (Oculinida) was reported as *Oculina patagonica*.The top recent alien species which were recorded are *Caprella scaura*, *Balanus trigonus*, *Melibe viridis*, *Styela* and *plicata.*Many species as *Percnon gibbesi*, *Eucrate crenata*, *Pinctada radiata*, *Bursatella leachii* and *Stephanolepis diaspros* were strongly presents in the region even well established.

We suspect greatly the presence of other invasive species, although they are not observed during this work as *Halophila stipulacea*, *Caulerpa taxifolia*, *Portunus segnis, Styela calva*, *Balanus spp.*and *Megabalanus spp*. due to the lack of observation effort.

The inventory of all observed species was presented as annex (Table.1, *see last section*) which includes the NIS as well as other cited species.

Some other species strongly established in Bizerte lagoon presents confusion concerning status and origin. Thus, *Phallusia mammillata* is considered as cryptogenic species (Goulletquer, 2016) whereas *Ciona intestinalis*, which considered as an exotic and invasive in French coasts, may represents in the lagoon with its two genetic type A and B, respectively with pacific and Atlantic origins (Goulletquer, 2016).

LEK Protocol results

The age of interviewed fishermen ranged between 40 and 70 years and over. The fishing experience was located between 20 and 55 years old and even more (Fig.1A). Only three age categories contributed significantly to the LEK investigations, when Fishing experience was spread over five categories of fishermen concerned by the LEK investigations (Fig.1B). The used fishing gear by the majority of fishermen is the trammel net as monofilamaent and multifilament nets. A total of 14 alien species were recognized by the fishermen. Additionally, they refer to the other species mainly crabs and ascidians which are observed rarely or for the first time on their nets (Fig.2). These species have not identified precisely (requires further investigations).

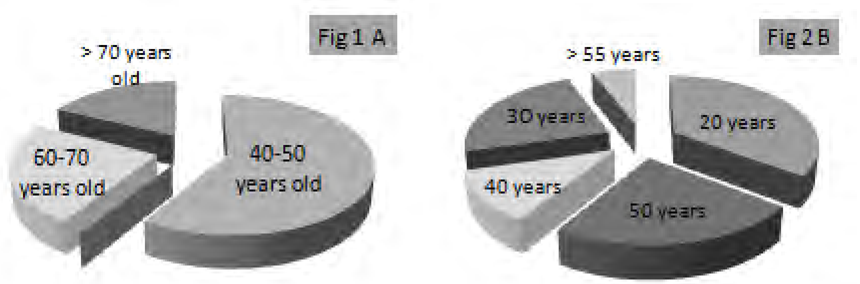

Fig. 1: (A) Age and (B) Fishing experience (n°of years) of fishermen involved by the LEK survey

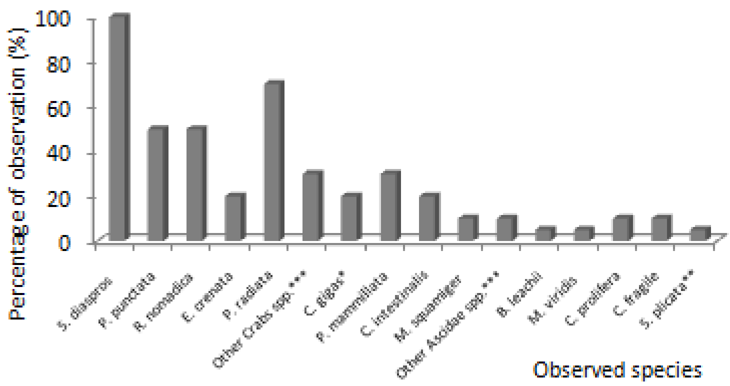

Fig. 2: Observed species according fishermen (LEK survey) of Menzel Abderrahmen locality (Bizerte lagoon)

Discussion

Local biodiversity and Invasive Alien Species (IAS)

New species of both, Atlantic and Indo-Pacific origin, have conquered Tunisian waters. The most exotic species currently fished and evoked by all fishermen was the *S. diaspros*. This alien species was recorded in Bizerte lagoon since 2004 (Bdioui *et al.,* 2004) and seems to be established and abundant, especially in the lagoon.

Crustaceans (crabs) were commonly captured taxa. However, the majority of these species are discarded and local fishermen mostly evoked exotic species, which have historically established in the lagoon, such as *Stephanolepis diaspros* and *Pincatada radiata*.

Species such as *S. diaspros* and *P. radiata*, but also *Melibe viridis* and *Bursatella leachii* resulted in increasing biomass being the highest values reported for *P. radiata* and *S. diaspros*. *Pachygrpsus maurus* (cryptogenic species) et *P. Transvsersus* are probably presented especially at the wave-breaker, channel, inner harbour and outer harbours. Their confusion with *P. marmoratus* (caught and used as bait) makes very difficult their perception and identification by non-scientists.

In the same context, there is strong suspicion on the occurrence of other exogenous crabs in the region particularly in Bizerte lagoon but due to limited monitoring efforts their occurrence in the lagoon cannot be confirmed.
The jellyfish *Rhopilema nomadica* and *Phyllorhiza punctata* have also been recognized and mentioned many times by the fishermen. According to their observations, the first sightings of these species occurred since 4-6 years, before the official records, which were published in the scientific literature (Daly Yahia *et al.,* 2013; Gueroun *et al.,* 2015).
NIS such as *Pinctada radiata*, *Crassostrea gigas*, *Fulvia fragilis*, *Bursatella leachii*, *Melibe viridis* and *Microcosmus squamiger*, as well as algae and phanerogams such as *Caulerpa cylindracea* are well established mainly in Bizerte lagoon. The invasive chlorophyta *Halophila stipulacea* was actively sought during this study but not reported yet.
New species was recently recorded as *Caprella scaura*, *Melibe viridis* and probably *Balanus trigonus* (in process of identification, short communication in redaction process) when other species was recently re-observed after their record on the past as *Alpheus inopinatus* (in southern Tunisia on 1956, Galil *et al*., 2002, 2018; WoRMS, 2018) and *Microcosmus squamiger* (in Bizerte waters on 1963, Goulletquer, 2016).
As far as other invertebrates (such as ascidians, gastropods, crustaceans, bryozoans, tunicates and polychaetes) are concerned, their difficult identification prevented the application of LEK to gather complementary information.
In the most of these cases LEK should be used with great cautions, to do most generate confusion leading to misidentification and taxonomical errors. For these species, our first inventory certainly needs to be complemented by further investigations and in some cases biomolecular and genetic techniques (barcoding and genomic sequencing, see also Barcode Of Life Data System, http://boldsystems.org/) will be needed to solve taxonomical doubts.
Finally, seaweed and phanerogams could be easily distinguished by fishermen but were not included in the LEK survey. Thus, it would be wise to integrate them into the future.
The complex of lagoon, channel, port and outer harbour of Bizerte (until wave breaker in front of channel and outer harbour) represents as strategic outpost were to detect NIS, including those with invasive potential (IAS). For this reason and on the basis of the present study, we propose to integrate this area in the Tunisian national monitoring program for biodiversity and non-native species of Tunisia validated in 2017 (made by SPA-RAC). Those sites were easy for direct observation of biodiversity and implementation of monitoring protocol and indicators.

Conclusion

This first inventory is just the beginning of a program of inventory and monitoring of marine biodiversity at the wave-breaker, the outer harbour, the inner harbour, the channel and the Bizerte lagoon. This work will continue to provide an exhaustive inventory especially for IAS listing as complete as possible. It will be primordial of this region as regard of its socio-economic (fishing and shellfish farming) and ecological impacts. In this context, some alien species, such as *Pinctada radiata* and *Stephanolepis diaspros*, are impacting local biodiversity and probably ecosystem functioning, with their increasing abundances. The evolution of these IAS in the ecosystem can also lead to socio-economic impacts. In this context, reflections on management options, particularly through adaptation strategies should be carefully evaluated.

Framework

This work was done under local prospection trips of the association Méditerranée Action-Nature (MAN, NGO) under the period from 2016 to 2018 on the sites of Cani Islands, Mezza Luna (wave-breaker), harbour and outer-harbour of Bizerte, Channel and Bizerte lagoon.

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Tab. 1: Unexhaustive list of remarkable/emblematic species and IAS in Bizerte lagoon and neirbouring coastal region (Cani Islands, wave-breaker,harbour and channel). (Protected Species (Ps) ; Emblematic Species (Es)/Patrimonial Species (PAs) ; Cani Islands (CI) ; Wave-Breaker (WB) ; Outer Harbour (OH) ; Harbour (H); Channel (C) ; Lagoon (L)

Species	Status (IAS ; Ps ; Es/PAs)	Abundance r (rare) ; x ; X ; XX ; XXX ; XXX+	Establishment (or first record) CI ; WB ; OH ; H; C ; L	Species	Status (IAS ;Ps ; Es/PAs)	Abundance r (rare) ; x ; X ; XX ; XXX ; XXX+	Establishment (or first record) CI ; WB ; OH ; H; C ; L	Remarks
Fish				**Opisthobranchia**				
Stephanolepis diaspros	IAS	XX	L	*Aplysia fasciata*	Es	X	WB ; C ; L	**Need more investigations**
Bivalve				*Bursatella leachii*	IAS	XX	WB ; C ; L	- More commun during autumnal season
Pinna nobilis	Ps/ Es	X	C ;L	*Melibe viridis*	IAS	XX ?	C, L	- Observed since June 2016 (probably before)
								- More commun during autumnal season
Pinna rudis	Es	X	CI ; C ; L	**Ascidian**				
Solen solen		X	L	*Phallusia mammillata*	IAS	XX	WB ; OH ; H ; C ; L	- Exploited in Bizerte lagoon + Chennal as a bait
Solen marginatus		XX	L	*Styela plicata*	IAS	X ?	C ; L	Exploited in Bizerte lagoon + Chennal as a bait
Pinctada radiata	IAS	XXX+	CI ; WB ;H ; OH ; C ; L	*Styela calva*	IAS	?	?	- Not observed but probably present
Crassostrea gigas	IAS	XX	C ; L	*Ciona intestinalis*	IAS	XX ?	C ; L	- Introduced for shellfish farming
Ostrea edulis		XXX	BW ; C ; L	*Microcosmus squamiger*	IAS	XXX	C ; L	
Fulvia fragilis	IAS	XX	L	*Clavelina lepadiformis*	IAS	?	?	**Under validation**
Mytilus galloprovincialis	PAs	XXX+	WB ; C ; L	**Colonial ascidian**				
Tellina planata		XX	L	*Botryllus spp.*		XX	CI ; WB ; C, L	*B. leachii, B.violaceus and B. diegensis*
Ruditapes decussatus	PAs	XXX+	L	*Botryllus schlosseri* ?	IAS	?	L ?	**Under validation**
Venus verrucosa		XX	L	**Cirripedia**				
Flexopecten glaber		XXX	L	*Megabalanus coccopoma*?	IAS	?	?	**Need more investigations**
Modiolus barbatus		XXX	C ;L	*Megabalanus rosa* ?	IAS	?	?	**Need more investigations**
Ruditapes philippinarum	IAS	?	?	*Balanus trigonus*	IAS	X ?	L ?	- Introduced in the Gulf of Gabes but spread on whole Tunisian coasts (expansion limits ?)
Lithophaga lithophaga	PAs	X	C ;L	**Echinidea**				
Petricola lithophaga		X	C ;L	*Paracentautus lividus*	Es / PAs	XXX	CI ; WB ; OH ; H ; C ; L	- Observed in Bizerte lagoon since 2005 (personal observations)
Gastropoda				*Sphaerechinus granularis*		r	CI ; WB ; C ;L	
Hexaplex trunculus	PAs	XXX	CI ; WB; C ; L	**Echinozoa**				
Murex brandaris	Es	XX	C ; L	*Holothuria sanctori*		X	CI ; WB ; OH ; H ; C ; L	
Haliotis tuberculata	Es	r	CI ; WB	*Holothuria tubulosa*		XXX	CI ; WB ; OH ; H ; C ; L	
Patella spp.	Es	XXX	CI ; WB ; OH ; H ; C ; L	**Asterozoa**				
Cerithium sp.		XXX		*Asterias rubens*	Es	r	CI ; WB ; C	
Hamenia sp.		XX	C ; L					
				Anthozoa (Cnidaria)				
Crustacea				*Oculina patagonica*	IAS	X	CI ; WB ; C ;L	
Carcinus aestuarii	PAs	XXX		**Phanerogams**				- Surexploited in Bizerte lagoon as a bait
Ilia nucleus		XX		*Posidonia oceanica*	Ps, PAs	XX	CI ;WB ; C ; L	
Maja squinado	Es	X	L	*Caulerpa cylindracea*	IAS	XXX+	CI ; WB ; OH ; H ; C ; L	
Percnon gibbesi	IAS	?	CI ; WB	*Caulerpa prolifera*		XXX+	CI ; WB ; OH ; H ; C ; L	- Coastal marine waters (rocky environments) (observed in wave breacker on 2016 and in Cani Islands on 2017
Eucrate crenata	IAS	X ?		*Caulerpa ollivieri*		r	C ; L	- First record in Bizerte lagoon in 2009 and even before
Pachyrapsus marmauratus	PAs	XXX		*Cymodocea nodosa*	PAs	XX	WB ; C ; L	-Exploited in the channel as a bait
Pachyrapsus maurus		XX ?		*Cystoseira* spp.	PAs	X	C ; L	- cryptogenicspecies
Pachygrapsus transversus		X ?		*Halophila stipulacea* ?	IAS	?	?	- Species with probable presence but not observed
Portunus segnis	IAS	?						- Suspicions of its presence but no evidence yet
Pinnotheres pisum		?	L	*Caulerpa taxifolia* ?	IAS	?	?	- Probably introduced in Bizerte lagoon via shellfish farming
Caprella spp.	IAS	?		**Algae**				
Caprella scaura	IAS	?		**Chlorophyta**				- Invasive behavior, first record in 2017
Alpheus inopinatus	IAS	r ?		*Codium fragile*	IAS	X	CI ; WB ; OH ; H ; C ; L	- First record on 1956
Cnidaria				*Codium tomentosum ?*		X	C ?; L ?	
Aurelia aurita	Es	X	WB ;OH ; H ; C ; L	*Ulva* spp.		X	C ;L	- Very variable biomass according to seasons and eutrophication degree of the different ecosystems notably in the Bizerte lagoon
Phyllorhiza punctata	IAS	X	WB ; OH ; H ; C ; L	*Chaetomorpha* spp.		X	C ; L	**Idem**
Rhopilema nomadica	IAS	X	WB ; OH ; H ; C ; L	*Enteromorpha* spp.		X	C ; L	**Idem**
				Rodophyta				
				Asparagopsis armata	IAS	X	CI ; WB	**Need more investigations**
				Asparagopsis taxiformis	IAS	X	CI ; WB	**Need more investigations**
				Gracilaria verrucosa		X	C, L	- Very variable biomass according to seasons (mainly in Bizerte lagoon)
				Desmospongia				
				Crambe crambe		X	CI ; WB ; H ; OH ; C ; L	

***Remarkable species:** A rare or endangered species under the Habitats and Birds Directives, in particular those on the Red List of Threatened Species / **Emblematic species:** A wild species of cultural, religious or economic importance for humans in a certain region (according European Directives Natura 2000).**The authority names of all species have not displayed in the table as well in the text (for space constraint required by the editor instructions). ***This table is not intended to make a complete inventory of the whole biodiversity of Bizerte lagoon and neighboring marine areas. It is aimed to present the most remarkable, emblematic and patrimonial species and especially IAS (old and new observations).

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ADDRESSING INVASIVE ALIEN SPECIES THREATS AT KEY MARINE BIODIVERSITY AREAS IN TURKEY: GEF VI PROJECT

Abstract
Invasive alien species are considered as one of the most important causes for biodiversity loss and changes in the ecosystem services, while threatening biodiversity for the last four centuries. They have become one of the main threats to the world at both national and international levels as these species have spread and multiplied rapidly due to the lack of natural predators and competitors. In Turkey, several invasive alien species (including fish, plants, mammals and insects) are observed and they pose a great threat to our natural wealth. The opening of Suez Canal, effects of climate change, and lack of biodiversity in the recipient environment have facilitated the establishment of new species. Currently, while over 1000 IAS are found in Mediterranean, over 450 IAS are found along the Turkish Mediterranean coast and 21 species in the Turkish Black Sea. This number continues to grow. 66% of the 450 species are coming via the Suez Canal to the Mediterranean Sea. About 80% of IAS coming via ballast water to the Black Sea. With this project, we aim to ensure resilience of marine and coastal ecosystems through strengthened capacities and investment in prevention, detection, control and management of Invasive Alien Species in Turkish Seas

Key-words: Invasive alien species, key marine biodiversity areas, Turkish Seas

Introduction

Turkey is a country situated on the continents of Asia and Europe, bounded on the north by the Black Sea, on the west by the Aegean Sea and on the south by the Mediterranean. Total surface area is 780,576 km^2.

Turkey has three major bio-geographical regions namely Euro–Siberian, Mediterranean and Irano-Turanian and has very different types of ecosystems such as mountain, forests, steppes and wetlands, as well as coastal and marine ecosystems. The country has rich flora and fauna diversity, high endemism and wider genetic diversity. The total size of protected areas covers 7.24 % of country surface area.

Turkey has a coastline of approximately 8,592 km (excluding islands), bordering four different major seas – the Mediterranean, Aegean, Marmara and Black Seas. Different hydrographical and ecological features of these seas support Turkey's overall high level of marine biodiversity. Typical habitats found within the marine waters along the coast of Turkey include dense meadows of the endemic seagrass known as Neptune Sea Grass (*Posidonia oceanica*) that grow in shallow-water sandy bottom of the Aegean and Mediterranean coasts. Seagrass meadows (*P. oceanica* as well as *Cymodocea nodosa* and *Zostera* spp.) are important ecosystems in Turkish marine waters, as they stabilize the sediment and act as a sink for nutrients and carbon, weaken the hydrodynamic force of wave action and thus help protect the beaches, and serve as spawning area and a nursery for many species, among them fishes and large invertebrates of economic importance.

The Convention on Biological Diversity defines invasive species as alien species which threaten ecosystems, habitats or other species by causing economic or environmental damages via its establishment and invasion.
In total, nearly 8,000 plant and animal species have been identified in Turkey's marine waters. Some 472 species of marine fish have been identified, of which 50% are believed to be at risk of decline due to a combination of threats. While the Aegean and Mediterranean coasts of Turkey have higher biological diversity, the Black Sea has historically supported substantially more productive fisheries. The Black Sea has a lower salinity level (surface water: 18‰), and the number of species living in it is only 20% of the number that live in saline water (> 34‰) of the Aegean and Mediterranean Seas. The difference in diversity is due partly to the fact that the continental shelf of the Black Sea is very narrow and deep water (>150 m) is azoic due to the presence of hydrogen sulphide, which limits the abundance and species variability of benthos. The Aegean Sea and its islands contain abundant microhabitats – including those dominated by seagrasses and algae (*Posidonia oceanica* and *Cystoseira* spp., coralligenous) – which play an important role in the sustainability of the ecosystem.
This vulnerability is mainly due to the fact that Turkey is surrounded by three different marine environments, with high endemism but at the same time having high risk of entry of IAS. Currently, approximately 450 IAS have been reported along the coast of Turkey and 21 species in the Turkish Black Sea. Figure 1 shows data from 2011, since which the number of IAS has increased.

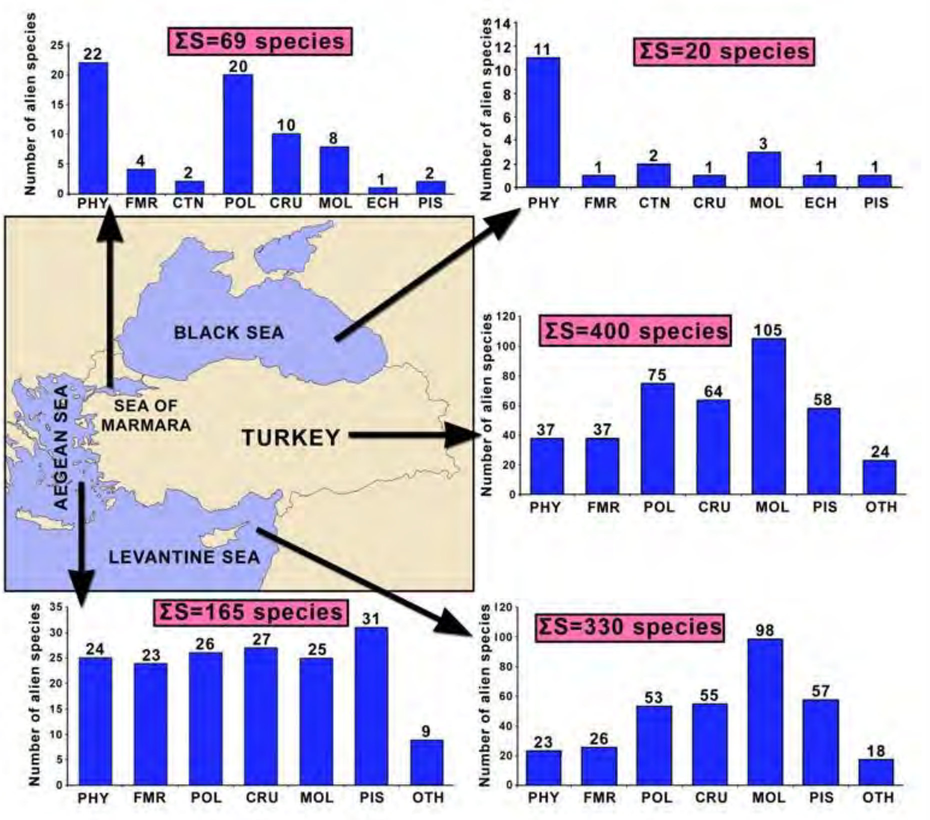

Fig. 1: Number of invasive species along Turkey's Coasts (Cinar et al., 2011)

There are two major pathways for IAS into Turkey's marine waters: 1.) The Suez Canal (opened in 1869), and "ship-mediated transport" (commonly through transport of ballast water, but also possible via external adhesion (hull fouling) or other ship-related means). In the 2011 national review of IAS in marine waters (Cinar et al, 2011), it was found that 66% of the total IAS in Turkey's coastal waters arrived via the Suez Canal, while 30% arrived via ship transport. The majority of species (306 species, 76% of total number of species) have become established in the area, while 59 species are classified as casual (15%), 23 species as questionable (6%) and 13 species as cryptogenic (3%).
One new alien species was introduced to the coasts of Turkey every 4 weeks between 1991 and 2010. The aliens species dominate in soft substratum with 198 species and in shallow waters by 319 species in between 0-10 m. Some species such as *Caulerpa cylindracea*, *Amphistegina lobifera*, *Amphisorus hemprichii*, *Rhopilema nomadica*, *Mnemiopsis leidyi*, *Hydroides spp.*, *Ficopomatus enigmaticus*, *Charybdis longicollis*, *Rapana venosa*, *Asterias rubens*, *Siganus spp.* and *Lagocephalus sceleratus* show a highly invasive character, and have great impacts both on the prevailing ecosystems and humans.
Target 9 of Aichi targets of Convention on Biological Diversity focuses on IAS that by 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment. Identification, eradication and management of Invasive Alien Species are cited on the National Strategy and Action Plan for Biodiversity in Turkey.
Key Biodiversity Areas Analysis: All of Turkey's coastal area is recognized as a globally significant marine biodiversity zone, 3,000 plant and animal species have been identified in Turkey's coastal (Turkey Environment Report, 2016)), but there are multiple individual Key Biodiversity Areas within this region as well. Turkey is one of the few countries for which a multi-taxon national Key Biodiversity Areas assessment has been conducted. The results of this assessment have been published in a scientific peer reviewed journal, in July 2016: "Identifying key biodiversity areas in Turkey: a multi-taxon approach. This assessment primarily focused on terrestrial KBAs, but does still have relevance for Turkey's coastal ecosystems as well. All four planned project pilot areas are considered part of Key Biodiversity Areas, as indicated in **Erreur ! Source du renvoi introuvable.** 2. The scientific analysis was conducted to identify KBAs that are "unprotected", "partially protected", or "fully protected". Two of the proposed pilot sites are identified as "partially protected" while two are identified as "unprotected". All sites except Igneada are part of the "Mediterranean Basin" hotspot (one of 36 globally recognized), and part of the Global 200 marine temperate shelves and seas ecoregion "Mediterranean" (one of 238 globally).
The project works at both the national level and at the site level, at four proposed pilot sites. The key characteristics of the pilot sites are summarized in **Erreur ! Référence non valide pour un signet.** 1.

Table. 1: Summary data for marine IAS project pilot areas

Site Name	**Area**	**National Status**	**IUCN PA Category**
Igneada Floodplain Forests National Park Coastal Seascape	34,200 ha of marine habitat, including 22 km of coastal habitat	Coastal area adjacent to National Park	PA has category II status; proposed marine ecosystem area does not have PA status
Marmara Islands Marine Ecosystems	46,600 ha of marine habitat, including 186.5 km of coastal habitat	No designated PA status; scientific analysis identified site as an unprotected KBA	
Ayvalik Islands National Park	19,624 ha including 13,969 ha of marine habitat, including approximately 112 km of coastal habitat	National PA	IUCN category V
Hatay Samandağ Sea Turtle Nesting Beach	32 ha of marine habitat, including 16 km of coastal habitat	Limited local protections related to sea turtle nesting beach status; no designated PA status; scientific analysis identified site as an unprotected KBA	

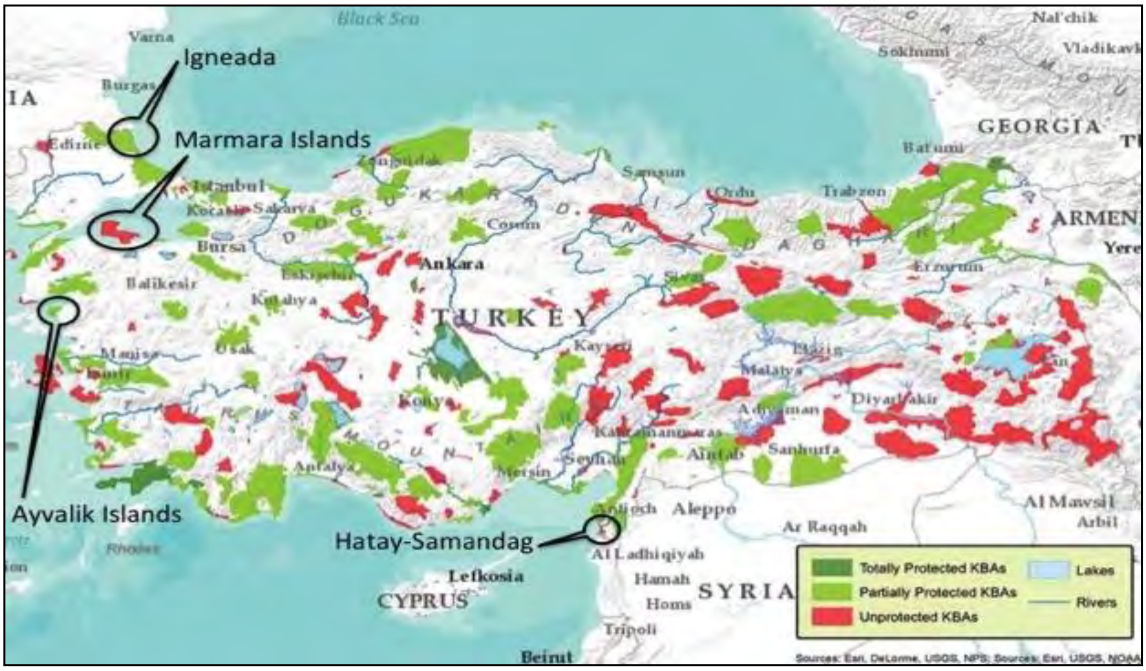

Figure. 2: 1 KBA confirmation for proposed marine IAS pilot sites

Results

The long-term project goal is to minimize negative impacts of IAS to support the conservation of the globally significant native biodiversity of Turkey's coastal and marine ecosystems. The project objective is "To ensure resilience of marine and coastal ecosystems through strengthened capacities and investment in prevention, detection, control and management of Invasive Alien Species." The project also seeks to promote gender equality and women's empowerment, to the extent relevant and feasible within the scope of the project. In order to achieve the project objective, and address the barriers, the project's intervention has been organized into three components:

- *Component 1.* Effective national policy framework on Invasive Alien Species
- *Component 2.* Capacity building, knowledge and information sharing systems to address the IAS threats
- *Component 3.* Investment in sustainable management, prevention, eradication, and control of IAS and restoration of IAS-degraded habitat at key marine and coastal areas

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DECLINE OF *CAULERPA TAXIFOLIA* IN THE ADRIATIC SEA

Abstract
Caulerpa taxifolia *(Vahl) C. Agardh (Chlorophyta, Bryopsidales), a green alga of Australian origin, was probably introduced in the Mediterranean in 1984. In the Adriatic Sea, three areas colonized by the alga were detected, all of them in Croatian waters: in Stari Grad Bay (Hvar Island) in 1994; Malinska (Krk Island) in 1994; and Barbat Channel (Rab Island) in 1996. The majority of the algal patches in Malinska and Barbat Channel were eradicated while the remaining fragments and colonies vanished during the cold winter of 2002/03. In Stari Grad Bay, the alga was detected in more than 15 stations. In 2000, the affected area of the primary station was 50 hectares. Sea bottom at a depth from 0 to 15 m was transformed into a continuous* C. taxifolia *settlement. From 1997 till 2003, eradication was carried out on secondary stations with virtually complete success. At the primary station, bordering areas were eradicated to prevent its further expansion. The applied methods included covering with a black PVC foil, manual collection, and extraction by suction pumps. In the winter of 2007/08,* C. taxifolia *radically declined: the covered area in the autumn of 2008 was 95% smaller than the year before. Remaining colonies were found only in a sparse* P. oceanica *meadow, between 8 and 12 m deep. During the following years, the covered area became even smaller and reached 30* m^2 *by the end of 2017. Through the preliminary survey at the end of 2018 the alga was not recorded. There is no apparent connection between the observed decline of* C. taxifolia *in Stari Grad Bay and abiotic parameters. Sea water temperature in the winter of 2007/08 and during the subsequent years was within the normal range. Seeing that a similar decline occurred throughout the Mediterranean, we hypothesized that an unidentified plant pathogen might be responsible for such a synchronous and wide scale vanishing. In addition, the alga was most likely affected by the native ascoglossan* Lobiger serradifalci*, which can cause significant damage to the* C. taxifolia *during autumn and winter seasons.*

Key-words: *Caulerpa taxifolia*, temperature, pathogens, decline, boom and bust

Introduction
A green alga of Australian origin, *Caulerpa taxifolia* (Vahl) C. Agardh, was first observed in the Mediterranean Sea in 1984, in Monaco. It was presumably accidentally released from the public aquarium (Meinesz & Hesse, 1991). Subsequently, it spread through several Mediterranean countries, transported through anchors over long distances, while fishing gears contributed to its local dispersion (Meinesz *et al*., 2001). In the nineties, *C. taxifolia* was considered as one of the major threats for the infralittoral communities of the Mediterranean Sea due to its rapid spread and significant impact (Meinesz *et al*., 1993; 1995).
In the Adriatic Sea, *Caulerpa taxifolia* was detected in three areas, all of them located in Croatia: Stari Grad Bay, Malinska, and Barbat Channel (Žuljević *et al*., 2002; Žuljević & Antolić, 2005) (Fig. 1). Present paper provides a review of the published information regarding the expansion and eradication of *C. taxifolia* in Croatia, with additional, previously unpublished data on sudden decline of the *C. taxifolia* settlements.

Materials and methods

Overview of the published data

We analysed and condensed all of the published data on *C. taxifolia* in Croatia, regarding its appearance, annual dynamics, spread, impact, eradication and interaction with native ascoglossans.

Mapping and temperature data collection

Every station with *C. taxifolia* in Croatia was surveyed at least once per year (during autumn), when the alga reaches maximum of development. Locations were surveyed by scuba divers and mapped according to the standard mapping procedure (de Vaugelas *et al*., 1999). Sea water temperature in Stari Grad Bay and Malinska was recorded by data-loggers which were positioned inside of the settlements of *C. taxifolia*.

Eradication

Eradication was attempted at each station. The applied methodology was selected based on the size of the *C. taxifolia* settlements and substrate. Methodology included the removal by air powered suction pumps, algal covering with black PVC foil, and hand collection of fragments and small thalli, sometimes with the help of a hammer and a chisel.

Observation of the interaction with ascoglossan sea slugs

During each field survey the presence of ascoglossan sea slugs (*Oxynoe olivacea* and *Lobiger serradifalci*) was determined based on the species-specific marks which they produce on the algal thalli during feeding. Ascoglossans are difficult to find, therefore, their abundance was estimated based on the spatial distribution of bite marks across the algal settlements.

Results

Stations of *Caulerpa taxifolia* in Barbat Channel

First observations were made by a local spear fisherman in September 1996. Alga was most likely introduced into the area from Malinska, in 1995, by the anchors of nautical boats. At the time of its discovery, about 20 m^2 of the surface, out of 350 m^2 of the affected zone, were covered by *C. taxifolia*. The alga developed in the photophilic biocoenosis, on a hard substrate within a *Cymodocea nodosa* seagrass bed, at depths between 2.5 and 8 m (Žuljević & Antolić, 2001a; Žuljević & Antolić, 2002). Manual eradication was successfully performed in October 1996. A new station of approximately 100 m^2 was discovered in the spring of 2001. It was located at an approximate distance of 500 m from the previously eradicated one. Soon after its discovery, the colony was eradicated by covering of the algae with plastic foils. Before covering, an emulsion of lime was added around the algal thalli. We did not observe the alga during a detailed survey of the area in the following two years, therefore we concluded that the alga was no longer present in the area.

Stations of *Caulerpa taxifolia* in Malinska

The alga was first observed in Malinska port in 1994. By the end of 2001, four algae stations were discovered in area of the Malinska town, affecting muddy-sandy substrate, at depths between 3 and 12 m. Following eradication by a suction water pump, carried out during 1996 and 1997, alga was completely removed from the station inside the harbor, where it had previously covered about 1300 m^2, out of 16000 m^2 of the affected sea bottom. The alga was only partially removed from the three remaining stations outside of the harbor (Žuljević & Antolić, 2002).

A drastic reduction in remaining stations occurred in the winter of 2002/03 caused most likely by a prolonged period of low seawater temperature. It was below 11°C for a period of 62 almost consecutive days, with an average value of 10.3°C and a minimum of 9.4°C (Fig. 2.) (Iveša *et al*., 2006; Žuljević, 2005). At the end of 2005, the alga was detected in a form of a 1 m^2 small colony and was subsequently eradicated by manual extraction (Žuljević, 2005). No additional records of *C. taxifolia* in Malinska have been reported since.

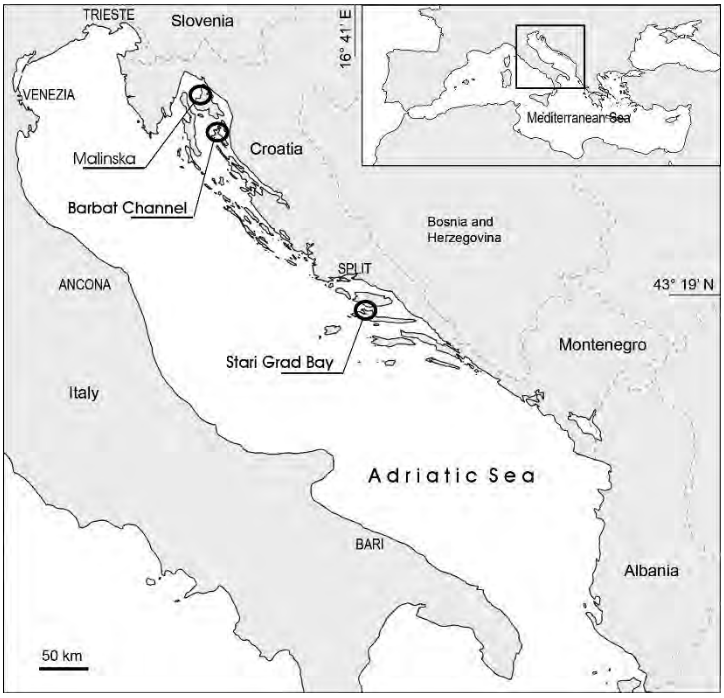

Fig. 1: Detected areas with *Caulerpa taxifolia* in the Adriatic Sea.

<u>Stations of *Caulerpa taxifolia* in Stari Grad Bay</u>

Caulerpa taxifolia was first observed in Stari Grad bay during the summer of 1994. Presumably, it had been introduced into this area in 1991 (Žuljević *et al*., 1998). By the end of 2001, it was found at >10 stations throughout the Bay. The alga was spread across 45 ha (affected area) at the central station, while it covered <100 m^2 in total at secondary stations where it was most likely introduced by fishing nets. It was found at depths between 0.5 and 20 m, growing on a hard substrate with photophilic and sciaphilic biocenoses, on sandy and muddy substrates and inside meadows of seagrasses *Posidonia oceanica, Cymodocea nodosa* and *Zostera noltei*. Sea bottom at the central station between 0 and 15 m was transformed into a continuous *C. taxifolia* settlement by the end of 2001 (Žuljević, 2005).

Eradication started in 1997 and was periodically done till 2005 (Žuljević & Antolić, 2001b). The main goals were to stop further expansion of the central station by eradicating the bordering colonies, and to completely eradicate the algae on all distant stations. Complete eradication was carried out on eight distant stations while the remaining two stations merged with the central station as it increased in size. Eradication was carried out by suction pumps, covering of the algae with black PVC foil, and by manual extraction.

In the winter of 2007/08, *C. taxifolia* vanished from more than 90% of the affected area, which was estimated to stretch across 70 ha at the end of 2007. The only remaining colonies of *C. taxifolia* were found in a sparse meadow of *P. oceanica*, at depths between 8 and 12 m. It disappeared from the areas at different depths with different substrate types (muddy, sand and rocky) which had previously been completely covered across the areas of thousands of square meters. After a drastic decline in Stari Grad Bay, the size of the *C. taxifolia* settlements has not enlarged. Only a slight increase in density and coverage was observed in the middle part of the central station, in the autumn of 2012. Progressive decline in size of the colonies resulted in 30 m^2 of the covered area at the end of 2017. At the end of 2018, preliminary survey did not encounter any thalli of the *C. taxifolia* in Stari Grad Bay.

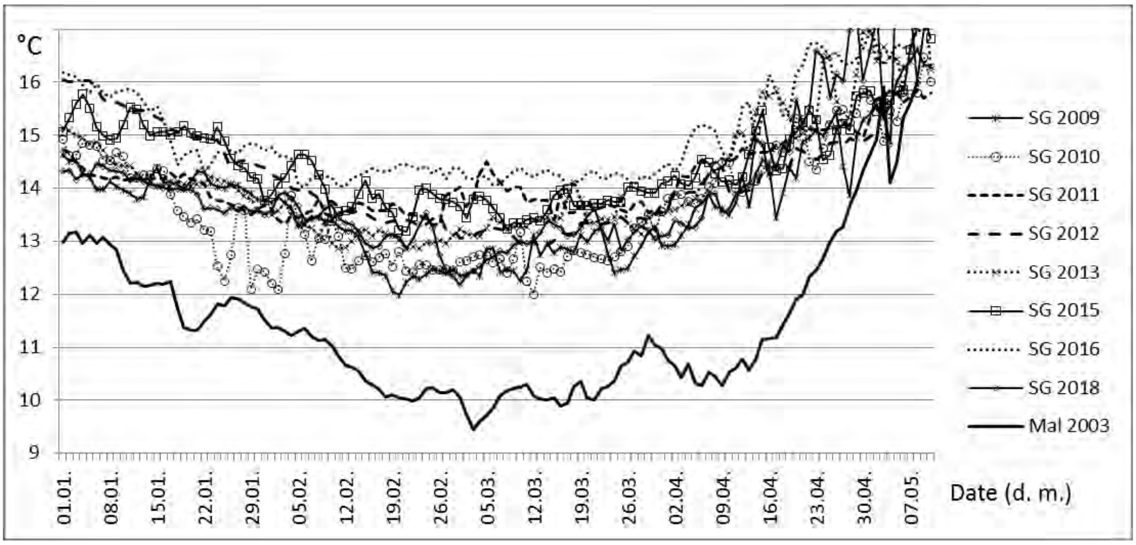

Fig. 2: Daily average of seawater temperature measured inside *Caulerpa taxifolia* settlement in Stari Grad Bay (SG) at 10 m depth and Malinska (Mal) at 7 m depth during the coldest period of the several years.

There were no measurements of seawater temperature by data-loggers in the winter of 2007/08, but from a meteorological standpoint, this was a standard winter with no significant temperature oscillations (DHMZ).

Water temperature data-loggers that were placed at a depth of 10 m inside of the *C. taxifolia* colonies, have not recorded any unusual sea bottom temperature patterns since the autumn of 2008 (Fig. 2).

Ascoglossan sea slug *Lobiger serradifalci* was commonly observed in every field observation carried out during autumn season. Its distribution was always patchy and its abundance was estimated as sporadic or abundant. *Oxynoe olivacea* was detected only sporadically.

Discussion

Eradication of *Caulerpa taxifolia* in Croatia demonstrated that a total eradication of a small colony can be done successfully. Following eradication, an extended period of cold seawater in Malinska probably caused a severe reduction of the algal colonies (Fig. 1). Recorded temperature in Malinska corresponds to the experimentally obtained lethal values - this species can survives three months at 10°C, while at 9°C, fragments die in less than 2 months (Komatsu *et al*., 1997).

In Stari Grad Bay, seawater winter temperatures rarely fall below 13°C. Between mid-February and mid-March, it can sporadically, reach values between 12.5 and 13.0°C, (Fig. 2). Seawater temperature at meteorological stations located nearby Stari Grad Bay (Towns of Hvar, Komiža and Split) (DHMZ), did not record any unusually situations in the autumn/winter/spring period of 1997/98, when a drastic decline of *C. taxifolia* occurred. Moreover, based on measurements by data-loggers during the following years, there were no records of unusually cold periods, either in the region or inside the *C. taxifolia* colonies (Fig. 2). Even though *C. taxifolia* has been progressively vanished.

Contrary to numerous scientific papers on the initial spread of *C. taxifolia* in the Mediterranean, there is quite a few studies describing its decline, seemingly occurring in all of the Mediterranean stations (see Montefalcone *et al*., 2015; Meinesz *et al*., 2010). It was suggested that cold winter temperatures may play a role in the observed regressions. Other factors, such as depletion of the substrate, genetic degeneration, weakening of the endosymbiont bacteria, and high rate of gametogenesis, have also been considered as possible factors influencing algal decline in the Mediterranean, however, none of those have been studied in depth (Meinesz *et al*., 2010). The first sign of regression in France was recorded in 2004, but severe regression was reported in 2008 and 2009 (Meinesz *et al*., 2010), with particularly noticeable regression in the sheltered shallow areas and ports. The described period of regression in France coincided with the period of a drastic decline in Stari Grad Bay. The reported decline of *C. taxifolia* in Malinska should not be considered as a part of the widespread Mediterranean decline as it occurred earlier (between 2002 and 2003) and was a result of the eradication and low seawater temperatures during winter (Fig. 2).

How can we explain the decline of *C. taxifolia* over the entire Mediterranean region? Global climate change might be considered as a possible factor, however, in Stari Grad Bay, constant seawater measurements did not reveal any colder periods characterized by temperatures close to the lethal levels for *C. taxifolia* (Fig. 2) (Komatsu *et al*., 1997). Our hypothesis is that a pathogen might cause such as drastic and wide scale decline. Algae are also plagued with bacterial and eukaryotic pathogens (Gachon *et al*., 2010), however, our knowledge on such a subject, especially regarding pathogens on *Caulerpa* species, is limited.

Following a drastic decline in Stari Grad Bay, a negative impact on remaining colonies could have been also caused by a native ascoglossan sea slug, *Lobiger serradifalci*. It is known that its feeding on *C. taxifolia* during summer results in algal fragmentation and the subsequent vegetative propagation and spreading of the alga due to its fast regeneration processes (Žuljević *et al*., 2001; Žuljević & Antolić, 2002). When seawater temperature during autumn reaches values below 18°C, regeneration process becomes slower, while *Lobiger serradifalci* continues to produce fragments. Survival of such fragments during winter period is probably low and might result in reduction of algal pool for the expansion during the following vegetation period, or even a total fading of the algal colony (Žuljević, 2005). *Caulerpa taxifolia* was a textbook example of introduction, spreading and impact of a marine invasive species. With its rapid decline it now represents one of the most remarkable examples of boom-and-bust phenomenon, when a widely established non-indigenous species undergoes a rapid collapse, usually with no reasonable explanation (Boudouresque & Verlaque, 2012; Simberloff & Gibbons, 2004).

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POSTERS

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PARASITE FAUNA OF THE HERBIVOROUS FISH *SIGANUS LURIUS* (RUPPELL, 1829), *SIGANUS RIVULATUS* (FORSSKAL, 1775) AND *SARPA SALPA* (LINNAEUS, 1758) IN THE WESTERN COAST OF LIBYA

Abstract
A parasitological study was carried out along the western coast of Libya on three herbivorous fish species: S. salpa*,* Siganus luridus *and* S. rivulatus*. Overall a toptal of 70 , 87 and 87 speciemens were collected and analysed, respectively. Sixteen different species of parasites (total N of individuals = 15709) were extracted and identified from the studied fishes. The highest frequency (7077 individuals) was recorded for* T. plectocirra*. Some of parasites could not be classified to the species level; four of these parasites species are originally from Red sea/Indo-pacific region. The study of the parasites of alien fishes in the Mediterranean contributes to fill the gap of knowledge on the biological and ecological traits of these native and alien fishes.*

Key-words: Host, Alien species, South Mediterranean

Introduction

Marine invasive species are regarded as one of the main causes of biodiversity loss in the Mediterranean (Galil, 2007). A major risk associated with introductions of fish is the transmission of their parasitic fauna to native fish host that may be highly susceptible to infection (Kirk, 2003). There is limited knowledge on stdy on this issue (e.g. Stefani et al., 2012) therefore the goal is Whether parasites accompanied with alien fish species come from the origin area (Red Sea), or the native parasites have found new host in the Libyan water

Materials and Methods

The study was carried out along the western coast of Libya on 70 specimens of S. salpa, 87 of Siganus luridus and 87 of S. rivulatus. Fishes were collected directly from fishermen, during the period from 2014 to 2016. The parasites examination was carried out in the laboratory according to (Euzet and trilles, 1960).

Results

Sixteen different parasites were extracted and identified in herbivores fishes studied, some of parasites could not be classified to the species level; four of these parasites species are originally from Red sea/Indo-pacific region (Tab. 1).

Tab. 1. Parasites have been found in the herbivores fishes (*Red Sea/Indo-Pacific; ✓ present and × absent).

Parasite	*S. salpa*	*S. luridus*	*S. rivulatus*
*Glyphidohaptor plectocirra**	×	✓	✓
Tetrancistrum polymorphum *	×	✓	✓
*Apounurs sigani**	×	✓	✓
*Hatschekia siganicola**	×	✓	✓
Nerocila bivittata	×	✓	×
Nothobomolochus neomediterraneus	×	×	✓
Hirudinea	✓	✓	✓
Nematoda	×	✓	×
Gnathia sp	✓	✓	✓
Atrispinum salpae	✓	×	×
Lamellodiscus confuses	✓	×	×
Centroderma spinosissima	✓	×	×
Robphildollfusium fractum	✓	×	×
Mesometra orbicularis	✓	×	×
Mesometra brachcoelia	✓	×	×
Clavellotis pagri	✓	×	×

Discussion

Alien parasites are a peculiar kind of biological invader, because their populations are structured by the dynamics and movements of their hosts (McCoy and Major 2003). Parasites are potentially able to control host populations and reduce host density by affecting their growth, reproduction, and survival (Torchin and Mitchell, 2004). The study of the parasites of alien fishes in the Mediterranean contributes to fill the gap of knowledge on the biological and ecological traits of these native and alien fishes. Future studies should help to increase the knowledge on the impact of alien species. Moreover it is necessary to collect more data on the parasites of alien fishes in order to examine the Mediterranean juvenile fishes, and to investigate whether the possible intermediate hosts of the natural Indo- Pacific parasites found in the Mediterranean or not.

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A PRELIMINARY ASSESSMENT OF IMPORTS OF EXOTIC AQUATIC SPECIES TO THE MALTESE ISLANDS (CENTRAL MEDITERRANEAN)

Abstract
The increasing reliance of human societies on globalised maritime trade has spearheaded the spread of exotic aquatic species worldwide, with the Mediterranean Sea being one of the regional seas mostly affected by the phenomenon of Non-Indigenous Species (NIS) introductions, by virtue of its geographical location. This study attempts to characterise the flow of exotic aquatic biota being imported from non-EU countries to the Maltese Islands by assessing the information contained within a sample of importation licenses.

Key-words: maritime trade, exotic aquatic species, NIS, importation licenses

Introduction

Invasive species are ranked as the second most common cause of species extinctions (Bax *et al.,* 2003; Bellard *et al.,* 2016). A number of introduction pathways mediate the transmission of these species from the region of origin to the region of invasion, with global trade being one of the most prominent of these pathways. In fact, the magnitude of merchandise imports is a significant determinant of the number of species, as well as the rate of new species introductions of a wide range of alien taxa (Hulme, 2009). Despite the globalisation of trade and our increasing reliance on maritime routes for global trade, holistic investigations of the databases of imported exotic species are rare, with studies often focusing on individual importation drivers only (e.g. aquarium/pet industry). EU Regulation 1143/2014 lists the Invasive Alien Species (IAS) of Union Concern which should be the target or management measures and in which no commercial trade is allowed. Currently, this Regulation lists only terrestrial and freshwater species, and not marine ones. This study represents a preliminary attempt at characterising the exotic aquatic biota imported to the Maltese Islands by using data extracted from twelve months of importation licenses.

Materials and Methods

The authors were given access by the Environment and Resources Authority (ERA), as the responsible national authority handling these aspects, to a twelve-month excerpt from the importation license database, stretching from September 2016 to September 2017, for all aquatic species imported to the Maltese Islands from non-EU countries. The total of 498 licenses issued by the ERA over the period under scrutiny were provided in raw format such that all salient information had to be extracted and transcribed online manually.

Results

The exotic aquatic species featured within the importation licenses studied were exported to Malta from thirty-six different countries located in six different continents, of which the continents with the highest representation in terms of country of origin of the same imports were Asia (fourteen countries), Africa (eleven countries) and South America (five countries) The three countries from which most imports to the Maltese Islands originated were Oman, Senegal, Mauritania, Singapore, Morocco, Sri Lanka and Indonesia, whilst Africa exhibited the highest number of exotic aquatic species exported to Malta (412), followed by Asia (203) and North America (15). Although the economic value of imports of exotic aquatic species to Malta for Fisheries purposes was the highest, the Aquarium/Pet Industry was responsible for the highest number of imported individuals, i.e. 570,000, although most probably this number represents the maximum number of exotic individuals for which importers were licensed, rather than the actual number of exotic aquatic individuals imported to Malta. The vast majority of imported exotic species (494) are of marine affinity, whilst 94 imported species are reported to have a marine/brackish water affinity and 40 species a freshwater one. The vast majority of imported species were fish, followed by crustaceans, echinoderms (sea urchins and sea cucumbers), molluscs (largely cephalopods) and even jellyfish.

Discussion and Conclusions

In terms of openings for future work, one would recommend strengthening further the current risk assessment processes in relation to the trade in flora and fauna, possibly with more comprehensive risk analyses using the protocols adopted within global databases for NIS, including DAISIE and EASIN. On the basis of the data extracted from the importation licenses, a number of preliminary considerations to this effect can be made. For instance, although (1) the vast majority of aquatic biota imported to the Maltese Islands from non-EU countries for non-aquarium purposes (e.g. fisheries, aquaculture, research and the food industry, which might represent a higher probability of escape into the wild) consisted of dead individuals, a small number of these imports consisted of live specimens, and these belonged to the following species: *Homarus* spp. *Palinurus regius, Palinurus mauritanicus, Sparus aurata*, *Oreochromis niloticus* and *Thunnus thynnus*. Legal obligations and mitigation measures are in place to avoid potential introduction of NIS into the marine environment from land-based operations. Notwithstanding this, a number of species imported to the Maltese Islands have actually already been recorded from the wild from the same region as range-expanding species, including *Acanthurus monroviae* and *Cephalopholis taeniops* (imported to Malta from Senegal and Tunisia), whilst the *Lutjannus* sp. cited on the importation licenses and introduced from the same two countries might refer to *Lutjannus fulviflamma* already recorded from Maltese waters.

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PRELIMINARY RESULTS FROM MSFD-PRESCRIBED NATIONAL MONITORING WITHIN MALTESE WATERS FOR DESCRIPTOR 2 (NIS)

Abstract
The Marine Strategy Framework Directive (MSFD) applies an ecosystem-based model in aspiring to lead EU Member States to the achievement of a Good Environment Status (GES) for eleven different Descriptors of the marine environment. Descriptor 2 of the MSFD focuses on Non-Indigenous Species (NIS), and this study reports the data collected from the Maltese Islands over the 2017-2018 period for this Descriptor.

Key-words: MSFD, NIS, monitoring, Maltese Islands

Introduction

The European Union's Marine Strategy Framework Directive (MSFD) is a wide-ranging framework directive (2008/56/EC) with the overall objective of achieving or maintaining Good Environmental Status (GES) in Europe's seas by 2020 (MSFD, 2008). Eleven high level qualitative Descriptors of GES have been defined in Annex I of the MSFD, including Descriptor 2, for which GES has been defined as 'Non-Indigenous Species introduced by human activities are at levels that do not adversely alter the ecosystem.' In order to achieve the GES within each of the eleven MSFD Descriptors, EU Member States are obliged to implement a monitoring strategy within their waters pursuant to collecting data addressing a number of indicators identified for each Descriptor.

Materials and Methods

The monitoring programme for MSFD Descriptor 2 (Non-indigenous species or NIS) has been implemented in 2017-2018 as part of the EMFF 8.3.1 project, funded under Union Priority 6 of Malta's Operational Programme for the European Maritime and Fisheries Fund 2014-2020. A two-tiered approach was adopted for the monitoring of NIS within Maltese waters – one within Marine Protected Areas (MPAs) and within hotspots or likely entry points for NIS. Within the seven sites located within the confines of designated MPAs, two scientific divers swam along two linear, shore-normal and geo-referenced transects as per the protocol delineated within Otero *et al.,* (2013), with the aim of recording all observed vagile and sessile NIS, with the former being counted as individuals whilst the latter being quantified in terms of coverage through the Braun-Blanquet cover index. Within hotspots, represented by ports and harbours (Grand Harbour and Marsaxlokk Bay), two scientific divers swam for a fixed, pre-established length of time (30 minutes at each site) along jetties, wharves, pontoons to mooring dolphins, as per protocols specified in Minchin (2007) and UNEP (2014). The selected sites were sampled twice – in summer 2017 and in late spring/early summer 2018.

Results

A total of eleven and of seven NIS were recorded from Marsaxlokk Bay and from the Grand Habour respectively, for a total of thirteen species from both sites, with *Amathia verticillata* and *Pinctada radiata imbricata* being the most abundant at the first site, whilst *Branchiomma bairdii* being the most abundant at the second site, with all three NIS reaching Braun-Blanquet cover index values of 4. With five species, crustaceans were the most represented in terms of NIS within the sampled hotspots, followed by polychaetes, bivalves, bryozoans and ascidians, with two species each. A total of eleven NIS were recorded within the monitored MPA sites, with seven of these representing algal species, and phanerogams, bivalves, decapod crustaceans and ascidians being represented by a single species each. The sessile NIS most commonly encountered within the sampled MPAs was *Lophocladia lallemandii*, with *Percnon gibbesi* being the most common vagile species. During the monitoring surveys in question, two new records of sublittoral species for the Maltese Islands were made – *Symplegma* cfr. *brakenhielmi*, a Lessepsian ascidian, and *Oculina patagonica*, a cryptogenic coral, both recorded from the Marsaxlokk Bay hotspot.

Discussion and Conclusions

Although the number of NIS recorded within the monitoring sites located within Maltese waters was relatively low, algal NIS generally exhibited high coverage values and their impacts on the status of benthic habitats needs to be evaluated. The fact that no fish NIS were recorded during the survey under review is probably due to the snapshot nature of the same survey. The high coverage and diversity exhibited by Maltese NIS within the surveyed hotspots is consistent with the status of these locations as maritime traffic and transport hubs, even for vessels hailing from tropical and sub-tropical seas. Monitoring carried out in hotspots as part of the EMFF 8.3.1 project is considered to have contributed significantly to the emergence of new knowledge on this pressure within Malta's marine waters.

Data property

The data used for the purpose of this publication emanates from the EU funded project EMFF 8.3.1 under the European Maritime and Fisheries Fund 2014-2020. The copyright of such data is the property of the project in line with the provisions of CT 3031/2016 and will remain vested in the project in question.

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ARE THERE TWO *PORTUNUS* SPECIES PRESENT IN THE GULF OF GABES? RESERVATIONS ON RESULTS PRODUCED USING DNA BARCODING IDENTIFICATION

Abstract
Blue crab is a commonly used name for the genus Portunus. It has been recorded in the gulf of Gabes since 2014 where it became very common, and the species Portunus segnis *was reported in this area. During the genetic analysis of the blue crab populations in the region, a DNA sequencing of the mitochondrion gene cytochrome c oxidase subunit I (COI) was conducted on 10 specimens collected from four regions in the Gabes Gulf (Djerba, Zarat, Gabes and Kerkennah). Due to the similarity in the morphological traits between* Portunus segnis *and* Portunus pelagicus*, doubts at the morphological level were verified by molecular methods. After alignment, two of the haplotypes found in the region of Djerba and Zarat were revealed to be more similar to* P. pelagicus *sequences available on GenBank with an identification percentage of 94% and 99%, respectively.*

Key-words: *Portunus pelagicus*, mtDNA sequencing, DNA identification, blue crab, Gulf of Gabes

Introduction

Blue crab is the common name for decapod crustacean belonging to the family of *Portunidae*. Its appearance and expansion in the Gulf of Gabes since 2014 is of increasing scientific interest. Around the world, blue crab represents a very popular fishing resource. However, in Tunisia, the management of its fisheries is still poorly controlled. Up to now, no population genetic studies have been conducted in Tunisian coasts. In this context, this work aims to the verification of species of the genus *Portunus* in the Gulf of Gabes.

Materials and methods

Ten specimens of the blue crab were collected using gillnets from four localities in the gulf of Gabes between spring and autumn of 2017 offshore of Djerba, Zarat, Gabes and Kerkennah. DNA was extracted based on the salting out method and the polymerase chain reaction was carried out to amplify a 700bp fragment of the COI gene using a pair of primers (LCO1490, HCO2198) following Folmer et *al.* (1994). The purified PCR products were subject to cycle sequencing and the sequences obtained were processed with online tools of identification provided by NCBI and GenBank (Morgulis et *al.*, 2008).

Results

According to the sequences analyzed, two of the specimens of blue crab belong to the species *Portunus pelagicus* (Tab.1, Fig.1).

Tab. 1: Details of species and specimens. GenBank accession numbers given, along with geographic locality.

Specimen	Location	Best matched species	Genbank accession number	% similarity
Zf1	Zarat	*P.segnis*	MF670482.1	93%
Zm1	Zarat	*P.segnis*	MF670456.1	93%
Zf2	Zarat	*Portunus sp.*	LC081233.1	96%
Zf3	Zarat	*P.pelagicus*	KF793331.2	99%
Zf4	Zarat	*P.segnis*	MF670432.1	92%
Dm1	Djerba	*P.pelagicus*	KJ168060.1	94%
Dm2	Djerba	*Portunus sp.*	LC081233.1	97%
Kf1	Kerkennah	*P.segnis*	MF670432.1	95%
Gf1	Gabes	*P.segnis*	MF670434.1	98%
Gf2	Gabes	*Portunus sp.*	LC081233.1	95%

Fig. 1: The two species of *Portunidae* in the gulf of Gabes

Discussion and conclusion

The few works found on the occurrence of the blue crab in the gulf of Gabes have signaled *Portunus segnis* as the only species introduced in this area (Rabaoui et *al.*, 2015). Therefore, it is the first study to confirm the presence of two species of *Portunidae*: *Portunus segnis* and *Portunus pelagicus,* using molecular tools.

However, since some studies question the accuracy of sequences of indigenous species in genetic databases , this work could be more complete if coupled with taxonomic study.

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HALOPHILA STIPULACEA VERSUS *CYMODOCEA NODOSA*: A COMPARISON OF ASSOCIATED AMPHIPODS ASSEMBLAGES

Abstract
The study aims to compare the amphipod assemblages associated with the non-native seagrass Halophila stipulacea *and native one* Cymodocea nodosa *in the Marina of Monastir during two periods, June 2017 and March 2018. A total of 15 amphipods species were recorded and a difference of the diversity and composition of Amphipoda assemblages were observed mostly during March 2018. The most abundant species in* C. nodosa *were* Dexamine spiniventris *and* Quadrimaera inaequipes*, whereas* Lembos websteri*,* Leptocherus guttatus *and* Leucothoe incisa *were dominant in* H. stipulacea*. Density, species richness* and *Shannon diversity were significantly higher in* H. stipulacea *and mixed habitat than in* C. nodosa*.*

Key-words: seagrass, amphipod, non-native, diversity, Tunisia

Introduction
Halophila stipulacea (Forsskål) Ascherson is seagrass native to the western Indian Ocean. This lessepsian migrant was reported since 2003 in Sfax harbor (southern Tunisian coast). In 2011, the species was found in Marina Monastir (center of Tunisia) within *Cymodocea nodosa* meadows (Sghaier *et al.,* 2011). Study of meadows descriptors of the two species was investigated in 2014 shown a regression of *C. nodosa* facing to *H. stipulacea* (Sghaier *et al*., 2014). The aims of the present work were to study and compare amphipod assemblages and diversity associated with these two seagrasses in the Marina Monastir.

Materials and Methods
Sampling was conducted in June 2017 and March 2018 at 0.5 to 2m depth. Samples of amphipods associated with *C. nodosa*, *H. stipulacea* and a mixed meadows of *H. stipulacea* and *C. cylindracea* were collected using a box quadrat of 20 × 20cm. Three replicates were done at each habitat. In the laboratory, amphipods were sorted, identified at the species level and counted. The mean species richness (S), the mean density (D) expressed by the number of amphipod individual by square meter, the diversity index (H', $\log_{10}$) and Pielou's evenness (J) were calculated for each habitat. A one-way ANOVA, followed by a post hoc test, was performed to ascertain whether the species richness and density vary between the three habitats. A probability of 0.05 or lower was considered significant. Statistical processing was conducted using the statistical package IBM SPSS Version 20.0.

Results and discussion
A total of 15 amphipods species were recorded during the present study (Table 1). Regardless the habitat composition, the species richness and mean density were significantly higher in March 2018 compared to June 2017. Highest number of species (9

species) and mean density (213.33±149.78 ind.m^{-2}) were recorded in the mixed habitat ($p<0.05$), whereas the lowest values (4 species and 28.33±5.77 ind.m^{-2}) were found in *C. nodosa* meadows.

Tab. 1: Mean species richness (S), mean density (D), diversity index (*H'*, $\log_{10}$) and Pielou's evenness *(J)* of Amphipoda associated with different seagrass during the two sampling periods (June 2017 and March 2018).

Species	*Halophila stipulacea*		*Cymodocea nodosa*		*Mixed habitat*	
	June	*March*	*June*	*March*	*June*	*March*
Lembos websteri	+	+		+	+	+
Dexamine spinosa	+	+		+	+	+
Dexamine spiniventris	+		+		+	
Amphithoe ramondi	+	+	+		+	+
Gammarella fucicola	+		+	+	+	+
Elasmopus pocillimanus		+			+	
Quadrimaera inaequipes			+			
Gammarus insensibilis					+	
Leucothoe incisa		+			+	
Hyale schmidtii				+		
Elasmopus brasiliensis				+		+
Leptocherus guttatus		+				+
Caprella liparotensis						+
Microdeutopus obtusatus						+
Monochorophium acherusucum		+		+		+
S (Mean±Standard Deviation)	5.00±0.00	5.33±0.58	3.67±0.58	5.67±0.58	7.00±1.00	9.00±0.00
D (Mean±Standard Deviation)	43.33±2.89	115.00±43.59	28.33±5.77	211.67±110.15	183.33±57.74	213.33±149.78
H'	2.17	1.76	1.76	1.65	2.26	2.13
J	0.43	0.33	0.48	0.29	0.32	0.27

The results suggested that amphipods can actively choose their habitats (Poore & Hill, 2006). Several studies showed that macrophytes architect complexity are an important component in habitat selection of amphipods. The presence/absence of epiphytes are also an essential factor (Zakhama-Sraieb *et al*., 2011). In Marina Monastir, mixed habitat displayed a habitat with higher architecture complexity comparing with *C. nodosa* or *H. stipulacea*. Very little is known about the influence of the seagrass *H. stipulacea* on altering marine communities, so complete faunistic studies dealing with other groups such as polychaetes or molluscs are necessary to properly address ecological and management programmes dealing with this non-native species.

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TUNISIAN LAGOONS: HOTSPOTS AND NURSERY GROUNDS FOR NON INDIGENOUS FAUNA

Abstract
Surveys conducted for updating marine biodiversity in Tunisian lagoons showed that 18% of non indigenous Tunisian fauna was firstly recorded in these confined areas: 18 species in Tunis Lagoon, 2 in Bizerte Lagoon, 3 in Boughrara and 2 in Bahiret El Bibane. Most of these species are now established in their new environment and we argue to consider Tunisian lagoons as hotspots and nursery areas for biological invaders before their further spread.

Key-words: Peri-Mediterranean Lagoons, bioinvasion, biodiversity, habitat, hotspots.

Introduction

The conservation of biodiversity and natural habitats in coastal areas is a matter of primary concern today, due to increasing human pressures and impacts (Edgar *et al.*, 2010). The largest Tunisian lagoons are Boughrara and Bahiret El Biban located in southern Tunisia. Then we have the Lagoon of Bizerte, Ghar El Meleh and Tunis, wich are smaller and located in the northern sectors of the country. Human activities (urbanization, industry, pollution, aquaculture, tourism, and overfishing) in these ecosystems recently lead to a drastic decrease of macro fauna (Ayari & Afli, 2003). The recent first records of alien species in Tunisian lagoons indicated that these confined areas are favorable transit sites. The present study aims to explain the settlement and the viability of invasive species in lagoons.

Materials and methods

Records of alien fauna from lagoons were based on regular surveys carried out seasonally during the last decade, as part of a study of biodiversity in these environments, mainly in Tunis Southern Lagoon using experimental and professional gears, scuba diving and also local ecological knowledge were used to detect and list non indigenous species, together with literature search. The nomenclature adopted in this article follows the World Register of Marine Species (WoRMS).

Results

Of the 136 alien fauna reported in Tunisian marine waters, 25 were firstly recorded in Tunisian lagoons (Ounifi Ben Amor *et al.,* 2016). Among these non indigenous species 18 were observed in the Tunis lagoon while 2 species were collected in the Bizerte lagoon. In the southern lagoons located in the Gulf of Gabès, 3 and 2 species were respectively caught at Boughrara and El Bibane. This alien fauna belongs to several taxa (Tab.1).

Tab.1: The non indigenous fauna of Tunisian lagoons: BL: Bizerte Lagoon; TL: Tunis Lagoon; BOL: Boughrara Lagoon; EBL: El Bibane Lagoon.

	Species (1st sighting Localities)
Cnidaria	*Haliscera bigelowi* Kramp, 1947 (BL); *Phyllorhiza punctata* Lendenfeld, 1884 (BL)
Polychaeta	*Hydroïdes dianthus* (Verrill, 1873) (TL); *Hydroïdes elegans* (Haswell, 1883) (TL)
Mollusca	*Venerupis philippinarum* A. Adams & Reeve, 1850 (TL); *Chromodoris quadricolor* Rüppell & Leuckart, 1830 (EBL); *Diodora ruppellii* G. B. Sowerby I, 1835 (TL); *Favorinus ghanensis* Edmunds, 1968 (TL); *Tayuva lilacina* (Gould, 1852) (TL).
Crustacea	*Amphibalanus eburneus* (Gould, 1841) (TL); *Caprella scaura* Templeton, 1836 (BOL); *Cymadusa filosa* (Savigny, 1816) (TL); *Gammaropsis togoensis* (Schellenberg, 1925) (BOL); *Anilocra pilchardi* Bariche & Trilles, 2006 (BOL); *Paracerceis sculpta* (Holmes, 1904) (TL); *Paradella dianae* (Menzies, 1962) (TL); *Sphaeroma walkeri* Stebbing, 1905 (TL); *Sphaeroma venustissimum* Monod, 1931 (TL); *Eucrate crenata* de Haan, 1835 (TL); *Hemigrapsus sanguineus* (de Haan, 1835) (TL); *Pilumnopeus vauquelini* (Audouin, 1826) (TL); *Rhithropanopeus harrisii* (Gould, 1841) (TL); *Erugosquilla massavensis* (Kossmann, 1880) (TL).
Bryozoa	*Tricellaria inopinata* d'Hondt & Occhipinti Ambrogi, 1985 (TL).
Actinopterygii	*Upeneus pori* Ben-Tuvia & Golani, 1989 (EBL).

Discussion and conclusions

The high number of recorded NIS in the Tunisian lagoons can be explained by their proximity to commercial ports, which are considered as main vectors for marine bioinvasions. We must also consider that these ecosystems are eutrophic to hypereutrophic and might provide favorable trophic conditions for NIS growth and reproduction and a relatively low biotic resistance (see Azzurro *et al.,* 2014) due to impoverished native communities. For these reasons, and based on empirical evidences, we argue that Tunisian lagoons are hotspots and nursery areas for the establishment and population grow of invasive species. Similar evidences were presented by Rilov & Galil (2009), which recognized the Suez Canal and its lagoons as important hotspots of invasion in the eastern Mediterranean. An intensification of shipping activities (Galil, 2009) and the global warming (Francour *et al*., 1994) expected to further enhance the introduction and establishment of NIS in these ecosystems.

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FIRST RECORD OF THE AMERICAN BLUE CRAB *CALLINECTES SAPIDUS* FROM THE MARCHICA LAGOON, MEDITERRANEAN COAST OF MOROCCO

Abstract
Callinectes sapidus *Rathbun, 1896 (Blue Crab) is a typical species of the Northern Atlantic Coasts, well caught and farmed in USA and Mexico. The occurrence of this species in Europe was first reported along the French coasts in 1900 and afterwards in the Mediterranean Sea. Specimens of the American blue crab* Callinectes sapidus*, were collected on August 17th 2017, November 9th 2017, May 5th - 6th 2018 from the Marchica coastal lagoon. Our finding constitutes the first documented record of the species from Mediterranean waters of Morocco. Two specimens were examined for their morphometric characters. These were a female and male with 6.2 cm and 9cm for the carapace Length, 11.1 cm and 15 cm for the carapace width and 115.66 g and 484.30 g for body length, respectively.*

Key-words: *Callinectes sapidus*; Invasive; Marchica, Morocco.

Introduction

The records of non- native species in terrestrial environments are more common and abundant than in marine environments (Bruno *et al*., 2005). Among marine environments, coastal and estuarine regions are the most invaded, with decapod crustaceans being commonly reported as non-native invertebrates, such as the American blue crab, *Callinectes sapidus* Rathbun, 1896, which is native of the estuaries and coastal waters of the western Atlantic (Nehring, 2011, 2012). The blue crab was introduced in Europe at the start of the twentieth century, the earliest confirmed record in the Mediterranean is from 1948 when two specimens were found in the Northern Adriatic (Giordani Soika, 1951), although its presence in the Aegean Sea was suspected as early as 1935 (Nehring, 2011). To date the species is recorded almost ubiquitously in the Mediterranean and Black Seas (Nehring, 2011; Castejón & Guerao, 2013); yet, established populations have been reported, besides the eastern Mediterranean Sea (Kevrekidis *et al.,* 2013), in particular the Adriatic Sea (Cilenti *et al.,* 2015). Though selected as one of the 100 “worst invasive” species in the Mediterranean (Streftaris & Zenetos 2006). Documentation of the presence of *C. sapidus* in the Marchica lagoon, Morocco, is presented here.

Material and methods

The Marchica Lagoon, (35.09′25″N; 002.50′43″W), (115 km2, 25 km long and 7.5 km wide) is the unique coastal lagoon on the Mediterranean coast of Morocco, with a maximum depth of approximately 8 meters. Crabs were caught, using trammel; Two specimens were examined for their morphometric characters. Furthermore, local fishermen have confirmed the presence of ovigerous females and juveniles in the Marchica area. The identification of the crabs was realized according to Williams (1974).

Results

Specimens of the American blue crab *Callinectes sapidus*, were collected on August 17th 2017, November 9th 2017, May 5th - 6th 2018 from the Marchica coastal lagoon. Two specimens were examined for their morphometric characters. These were a female and male with 6.2 cm and 9 cm for the carapace Length, 11.1 cm and 15 cm for the carapace width and115.66 g and 484.30 g for body length, respectively.

Discussion

The present marine biota of the Mediterranean is composed of species belonging to several biogeographic categories, like exotic species. The list of exotic animals and plants that invaded the Mediterranean is getting longer every day (Ribera & Boudouresque, 1995). The blue crab has been reported as a highly aggressive species and it has been selected among the 100 "worst invasive" species in the Mediterranean with impact on both biodiversity and socioeconomics (Streftaris & Zenetos 2006). Both immature and mature ovigerous (carrying eggs) females were found, as the (CW) size for mature females is between 120-170 mm, as indicated in studies of the Chesapeake Bay (Cadman & Weinstein 1985). On the other hand, all the three male crabs (measured and not measured) were mature, according to the detailed accounts of external morphology of the blue crab presented by Pyle and Cronin (1950). Further accurate researches are needed to study the ecological effect of the presence of this species on the ecosystem (competition), to confirm its fitting to evaluate the exploitation of the resource (fishery or aquaculture). Genetic studies could be also carrying out to mark the actual populations and monitoring any additional supplying of other new Atlantic specimens (probably transported in ballast).

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ADDITIONAL DATA ON THE INVASIVE BLUESPOTTED CORNETFISH *FISTULARIA COMMERSONII* RÜPPELL, 1883 IN TUNISIAN MARINE WATERS (CENTRAL MEDITERRANEAN SEA)

Abstract
Some biological traits of Fistularia commersonii *Rüppell, 1883 were studied at Cape Bon peninsula (Tunisia), based on 113 specimens. The sex ratio was significantly skewed towards females (1: 4.4) and length-weight relationship showed an isometric growth. Preliminary results on morphometric measurements, diet and reproduction of this species in Tunisian waters are also given.*

Key-words: Bioinvasion, trophic level, Lessepsian migration.

Introduction
The rapid geographical spread of *F. commersonii* and the increase of its abundance indicate the success of this Lessepsian migrant over the eastern and central sectors of the Mediterranean Sea. The species was firstly recorded off the coast of Israel (Golani, 2000), since then, the fish has spread to almost all Mediterranean coasts, including Tunisia in 2002 (Ben souissi *et al*., 2004).

Materials and methods
Overall, 113 specimens were collected, between October 2010 and February 2017 along the Cape Bon peninsula (Tunisia) by trawling (Tab. 1). In the laboratory, morphometric, meristic characters and weight were recorded and stomach contents were analyzed after preserving them in 70% alcohol. Prey items were identified to the lowest possible taxa.

Results
The overall sample was represented by 21 males (TL = 87.37 ± 2.49 cm) and 92 females (TL = 94.79 ± 0.95 cm). The overall sex ratio (1: 7.1) is significantly skewed towards females (χ^2, n=81, $p<0.05$). The morphometric characteristics are given in Table 2. Length-weight relationship resulted in a significant correlation (ANOVA, $p<0.001$) and the growth is isometric for both sexes ($p > 0.05$). Stomach analyses confirmed the piscivorous habit of *F. commersonii* (%*F* =100). Indeed fish preys were the predominant by both number (88.6%) and weight (98.41%) (Tab. 3). Isopoda and gastropoda were accidental preys. Examined fishes were in post spawning stage except three conspicuous females.

Discussion
So far, available information on both, distribution and the biology of *F. commersonii* in Tunisian waters was scarce. The present study provides significant information to assess the status of this invasion in this country. We also provided preliminary data on its growth

and reproduction, which sum to similar information provided in other Mediterranean countries (e.g. Mouine –Oueslati *et al*., 2017, Bariche *et al*., 2013).

Tab. 1: Number, sex and location of sampling sites of *Fistularia commersionii* from the cape bon Peninsula, Tunisia.

Specimens		Date of capture	Locality	Geographical coordinates		
Females	Males			Latitude	Longitude	Depth (m)
21	11	October 2010	Kelibia	36º48'59"	11º15'36"	108
26	3	February-March 2017	Kelibia	36º48'59"	11º15'36"	108
34	6		Korba	36º32'27"	11º13'20"	70
11	1		Menzel Temime	36º46'05"	11º10'17"	70

Tab. 2: Main morphometric measurements of *Fistularia commersionii* in the Tunisian marine waters. M: mean (in cm), s.e: standard error.

Measurement	m ± s.e (cm)	Maximum	Minimum
Total length (TL)	98.4±0.7	118	85.8
Fork length (FL)	83.4±0.7	106	70.8
Standard length (SL)	81.5±0.7	103.8	71.5
Snout length	29.4±0.3	39.6	24
Dorsal fin length	2.9±0.0	3.8	2.4
Pectoral fin length	1.7±0.0	3.6	1.3
Pelvic fin length	0.5±0.0	0.9	0.4
Anal fin length	2.8±0.0	3.9	2.2
Eye diameter	2.2±0.0	3.2	1.8
Total weight (TW), g	422.2±17.3	1135	250

Tab. 3: Number and weight percentage composition of prey items found in the stomachs of *Fistularia commersonii* in Tunisian marine waters %N: percentage by number, %W: percentage by weight, %F: frequency.

Prey group	Prey	%N	%W	%F	%IRI
Crustacea	**Isopoda**	9,65	0,14	10.29	1.34
Mollusca	**Gastropoda**	1.75	0.03	1.47	0.03
Fish	*Sardina pilchardus.*	7.89	10.36	11.76	2.85
	Spicara maena	11.40	13.53	13.24	4.38
	Chromis chromis	0.88	0.78	1.47	0.03
	Coris julis	3.51	5.50	5.88	0.70
	Boops boops	3.51	28.39	5.88	2.49
	Caranx rhonchus	0.88	4.37	1.47	0.10
	Unidentified fishes	60.53	35.48	69.12	88.07

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EXPANSION OF THE EXOTIC BROWN ALGAE *RUGULOPTERYX OKAMURAE* (E.Y. DAWSON) I.K. HWANG, W.J. LEE & H.S. KIM IN THE STRAIT OF GIBRALTAR

Abstract
The non-indigenous brown algae Rugulopteryx okamurae *(Dictyotales, Ochrophyta) has expanded since year 2015 on lit hard-bottoms of the Strait of Gibraltar. It has produced serious impacts over the indigenous benthic communities, frequent hooks on fishing nets and lines and the accumulation of thousands of tons of seaweed in nearby beaches and wrack zones. In this study, the geographical distribution of this algae in the Strait of Gibraltar and nearby areas of the Atlantic and Mediterranean is described. Its distribution is still restricted to the limits of the Strait, but it successfully colonizes a wide bathymetrical range, reaching more than 90 percent coverage in some areas. These results are especially worrisome, as they may indicate that non-indigenous* R. okamurae *may produce serious ecological impacts on native communities of the Strait of Gibraltar.*

Key-words: Strait of Gibraltar, NIS, Invasive Seaweed, Distribution, Impact

Introduction

Rugulopteryx okamurae is a Phaeophycea from temperate areas of the Pacific Ocean (Huang 1994). It was first cited in the Mediterranean by Verlaque *et al*. (2009), presumably introduced in the French coast through the importation of the Japanese oyster *Crassostrea gigas*. In the Strait of Gibraltar, it was cited for the first time by Altamirano *et al*. (2017) in the Spanish coasts and by Ocaña et al. 2016 and El-Aamri *et al*. (2018) in the coasts of Morocco. One year after being detected, *R. okamurae* covered most of the illuminated subtidal shallow areas, causing evident ecological impacts in the intertidal and subtidal and also requiring the municipal cleaning machines to remove from the beaches of the City of Ceuta more than 5,000 tons of *R. okamurae* detached biomass (Ocaña et al. 2016, García-Gómez et al. 2018). Seemingly, fishermen have reported continuous hooks on fishing nets and a reduction of captures on local media.

Materials and methods

During a field survey in 2016 on the Spanish coast of the Strait of Gibraltar (see sampled sites in García-Gómez *et al*. 2018), the occurrence of *Rugulopteryx okamurae* was checked, either attached to the substratum or as wrack deposits (floating in the water column, as rugs of loose specimens on the sea bottom or as supra-littoral wrack deposits).

Results

The geographical distribution of *R. okamurae* on the coasts of the Strait of Gibraltar and nearby areas is shown in Fig. 1.

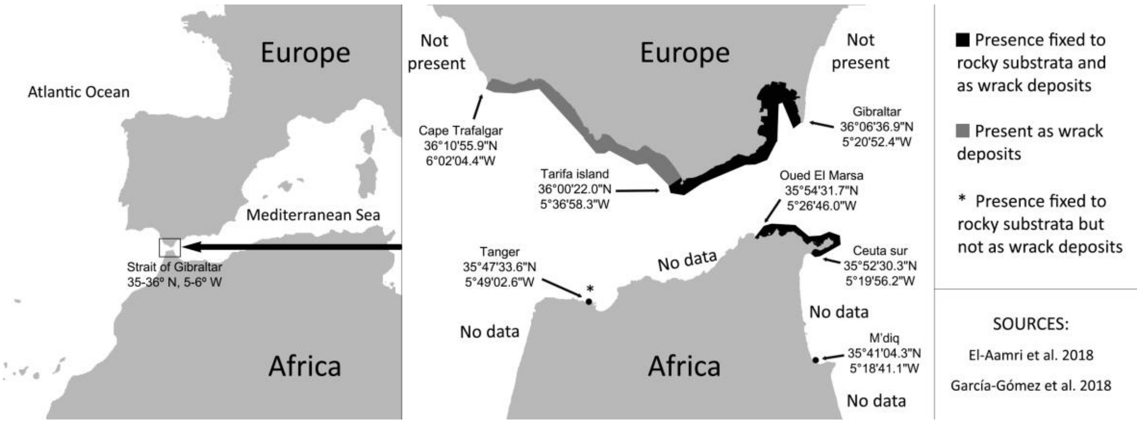

Fig. 1: *Rugulopteryx okamurae* distribution in the Strait of Gibraltar, years 2016-2017 (see sources).

Discussion and conclusions

The expansion of *Rugulopteryx okamurae* within the Strait of Gibraltar and the environmental impacts detected in this area are of great concern, given that this species may expand to both the Mediterranean and Atlantic. In the Pacific, *R. okamurae* inhabits shallow areas, from near surface to 15 metres depth (Hwang *et al*. 2009). It is also present throughout the year, although coverages are higher during warmer months. This situation is similar to that observed in the Strait, although here *R. okamurae* ranges from intertidal pools to over 50 metres deep, reaching up to 90% coverage in some horizontal, illuminated surfaces at 10-20 m depth and 70% at 5-30 m depth (unpub. data). The wide bathymetrical range colonized by this alga in high coverages is very worrisome and suggests that the ecological impacts may be high in the mid and long term (El-Aamri *et al*. 2018, García-Gómez *et al.* in press).

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REVIEW OF NON-INDIGENOUS AND CRYPTOGENIC MARINE AMPHIPODS IN TUNISIA

Abstract
In the present work, a review of the list and the geographical distribution of non-indigenous and cryptogenic marine amphipods along the Tunisian coast was performed. A total of seven species has been examined with one Gammaridea and six Senticaudata. For each species, the origin of the species, the mode of introduction, the locality(-ies), the year (or) period and the source of the first observation in Tunisia are given. The distribution and the status of species in Tunisia were evaluated and, where appropriate, discussed.

Key-words: Crustaceans, non-indigenous, invasive, cryptogenic, Mediterranean Sea

Introduction
Crustacean amphipod fauna of the Tunisian coast remained relatively unexplored until 2003 when investigations were conducted to establish a complete list of this group (Zakhama-Sraieb *et al*., 2009). Therefore, only the cryptogenic/alien species *Cymadusa filosa* was recorded from Tunisian coasts until 2003. Later, Zakhama-Sraieb & Charfi-Cheikhrouhou (2010) reported two new non-indigenous amphipods. During the last decade, the number of records of marine non-indigenous amphipods has been increased in Tunisian coasts. The aim of the present study is to give an updated list of the non-indigenous and cryptogenic marine amphipods of Tunisia.

Materials and methods
Investigations were conducted along the Tunisian coast from 2003 to 2018 in shallow water between 0 to -10m. Amphipods samples were collected by SCUBA diving or free diving at several marine biotopes. All samples were preserved in 70% alcohol. Amphipods were separated using a stereo dissecting microscope, sorted, and then identified. The specimens examined for this study are deposited in the Faculty of Sciences of Tunis. On compiling this list, all available published records of non-indigenous and cryptogenic Amphipoda along the Tunisian coasts were screened.

Results and discussion
As a result of investigations conducted along the Tunisian coasts and bibliography review, a total of seven non-indigenous and cryptogenic amphipod species belonging to two sub-orders, six families and seven genera were identified (Table 1). According to Marchini and Cardeccia (2017), *Caprella scaura* is considered as a valid alien in the Mediterranean Sea. The “alien” status of other amphipod species remains controversial according to the same authors. Further investigations on the taxonomy through molecular approach of the cryptogenic species should be undertaken to elucidate their status.

Tab. 1: List of non-indigenous and cryptogenic Amphipoda from the Tunisian coast

Species	Native range / Mode of introduction	Distribution along the Mediterranean coast	Distribution along Tunisian coast / Year of first detection / Reference
SUB-ORDER GAMMARIDEA			
FAMILY STENOTHOIDAE			
Stenothoe gallensis complex Walker, 1904	Cosmopolitan / Unkown	Egypt, Spain, France Italy and Greece	South: Zarzis (33°83′ N, 11°80′ W) / 2006 / Zakhama-Sraieb & Charfi-Cheikhrouha (2010)
SUB-ORDER SENTICAUDATA			
FAMILY AMPTHOIDAE			
Cymadusa filosa (Savigny, 1816)	Cryptogenic / Unkown	Algeria, Egypt, Libya and Malta	All the Tunisian coast / 1910 / Chevreux (1910)
FAMILY CAPRELLIDAE			
Caprella scaura Templeton, 1836	Indian Ocean / Fouling	Italy, Greece, Spain, Turkey, Israel, Malta and France	Boughrara lagoon (33º55' N, 10º71' W), El Bibane lagoon (33º27' N, 11º26' W) / 2009 / Ben Souissi *et al*. (2010) ; Tunis lagoon (36º47' N, 10º17' W) / 2014 / Ouni *et al*. (2016) ; Bizerte lagoon (37°13' N, 9°56' E) / 2018 / Chebaane *et al*. (2018)
FAMILY ISCHYROCERIDAE			
Jassa slatteryi Conlan, 1990	Cryptogenic / Fouling	Croatia, Spain, Malta and Italy	East: Monastir bay (35°47' N, 10°50'W) / 2018
FAMILY MAERIDAE			
Elasmopus pectenicrus (Spence Bate, 1862)	Cryptogenic Circumtropical / Passive dispersal	Egypt, Israel, Italy, Spain, Turkey and Algeria	South: Zarzis (33°83′ N, 11°80′ W) / 2007 / Zakhama-Sraieb & Charfi-Cheikhrouha (2010)
Hamimaera hamigera (Haswell, 1880)	Indo-Pacific / Passive dispersal	Egypt, Israel, Libya, Turkey and Cyprus	South: Boughrara lagoon / 2009 / Ben Souissi *et al*. (2010)
FAMILY PHOTIDAE			
Gammaropsis togoensis (Schellenberg, 1925)	Cryptogenic Indo-Pacific, Indian Ocean / Fouling	Turkey and Israel	South: Boughrara lagoon / 2009 / Ben Souissi *et al*. (2010)

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Recommendation of the 1st Mediterranean Symposium on the Non-Indigenous Species

1. Non-indigenous species (NIS) are spreading all over the Mediterranean with increasing impacts on native biodiversity, ecosystem functions and related services. Some NIS may also reach deep habitats.

2. Invasive species are also present in coastal lagoons and in MPAs. Eastern Mediterranean MPAs host very large abundances of these species.

3. Available knowledge on NIS is still poor and fragmented, especially in relation to their ecological and socio-economic consequences.

4. Mediterranean countries should enhance their mutual cooperation and coordinate monitoring activities in order to support appropriate responses at the sub-regional level.

5. The MAMIAS database was suggested as a possible repository of essential information to establish national, sub-regional and regional reference information related to NIS.

6. Local Ecological Knowledge, especially fishers' knowledge, was proved to be a cost-effective approach to establish large scale monitoring based on standard protocols. Results complement the knowledge generated by traditional surveys, while strengthening public awareness and participation.

7. Active monitoring networks should be established at national and regional levels, in line with the Barcelona Convention Integrated Monitoring and Assessment Programme (IMAP) principles and common indicators.

8. Risk assessment should be further promoted as an instrument to support policy makers in their decisions regarding the need for managing NIS.

9. Experiences and best practices on the management of invasive non-native species, should be transferred to all Mediterranean countries and MPAs.

10. Adaptive management should be implemented across coastal areas and MPAs to mitigate both ecological and socio-economical losses, especially in relation to the fishery and touristic sectors.

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