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From LEK to LAB: The case of the blue crab *Portunus segnis* in the Pelagie Islands Marine Protected Area, central Mediterranean Sea

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ABSTRACT

The Non-Indigenous Species (NIS) and particularly the Invasive Alien Species (IAS) affect biodiversity at ecosystem, habitat and species level. NIS management is primarily based on species monitoring in order to prevent their spread and to limit their impact. Different NIS monitoring methods have been developed involving citizens, especially to detect easily recognizable species and, in particular, through Local Ecological Knowledge (LEK) data collection. In the present study, we report the LEK data collection conducted in the Pelagie Islands Marine Protected Area (MPA) interviewing fishers and dive masters, particularly focusing on the Lessepsian blue crab *Portunus segnis* (Forskål, 1775) and the subsequent sampling and validation of the species occurrence. *Fistularia commersonii*, *Siganus rivulatus* and *Percnon gibbesi* were the most reported species by the interviewed fishers and dive masters. *P. segnis* was first signaled by dive mastersand subsequently sampled for morphological and molecular validation. The LEK experience showed the importance of the collaboration between researchers and citizens in the NIS/IAS surveillance/monitoring activity; some considerations on the importance of NIS/IAS early detection and on how to achieve a good NIS/IAS surveillance system for MPAs are discussed.

1. Introduction

In the Mediterranean Sea, the records of Non-Indigenous Species (NIS) are continuously increasing and contribute to changes in biodiversity at local scale (Zenetos and Galanidi, 2020). Among these, the Invasive Alien Species (IAS) mainly affect biodiversity at ecosystem, habitat and species level, due to their life history features. These species usually have a strong capability to adapt to several habitats, tolerating different environmental conditions (Ricciardi and Rasmussen, 1998); in some cases, they show competitive behavior in the resource use. Lastly, some of them are highly fecund allowing rapid population expansion) (Geburzi and McCarthy, 2018; Hänfling et al., 2011).

The first detection of a NIS in the recipient area is usually followed by silent period (lag time) when the species seems absent, some species may not even reappear for decades. Afterwards, when conditions are favorable to the establishment of reproducing individuals, the species may reappear with a consistent population, and may proceed into an expansive phase, starting to spread elsewhere (Azzurro et al., 2016a; Crooks, 2011; Olenin et al., 2017). This is the crucial time to limit the effects that these species may have on biodiversity since the NIS population is still small and can be more easily managed. It is therefore fundamental to strengthen NIS monitoring activities to early detect any population increase; as already stated by many authors, NIS early detection is a key step in management rapid response of the biological invasions (Giakoumi et al., 2016; Katsanevakis et al., 2015). In the last decades, different monitoring methods (e.g. volunteer based monitoring, interviews to selected citizens, online questionnaires) have been developed involving the public to detect easily recognizable species. In the case of IAS, collaboration between local communities and researchers is an important tool to monitor the phenomenon of alien species spreading, and to promptly respond to new arrivals, activating information and awareness campaigns and management actions. Take for instance the alert campaigns on the toxic silver-cheeked toadfish *Lagocephalus sceleratus*, that allowed researchers to track its spread in the Italian and Maltese waters thanks to citizens' reports (Andaloro et al., 2016; Azzurro et al., 2016b). Currently, the number of citizen science initiatives for monitoring IAS has increased enormously thanks to the recent adoption of information and communications technology (ICT), such as internet and/or mobile application-based interfaces for citizen training and data generation (Johnson et al., 2020). The success of

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citizen science application in marine ecosystems has been demonstrated in the involvement of divers and fishers for reporting data on the presence of alien/invasive species (Boudreau and Yan, 2004; Mannino and Balistreri, 2018). However, IAS detection by citizens is limited only to species of large size (marine megafauna and megaflora) and bearing easily recognizable morphological characters as clearly suggested by Hewitt and Martin (1996 and 2001). In the marine environment, the selection of citizens able to help scientists in collecting data on IAS is restricted to specific categories, such as divers and fishers who are familiar with this environment. Instead, the support of other citizen categories is often limited in time and space so that it rarely adds value to the information.

Citizen science initiatives include informative campaigns on a specific topic as well as interviews to local divers and fishers following specific questionnaires aimed at collecting data on the presence and distribution of species, the so-called Local Ecological Knowledge (LEK) (Anadón et al., 2009; Brook and Mclachlan, 2008; Huntington, 2000). Such a method allows retrieval of more information on ecological phenomena by people who live in close contact with the environment and for this reason it has been considered as an alternative information source on species' occurrence and distribution (Anadòn et al., 2009). The questionnaires could also be distributed online through social media or through an intermediary (e.g. head of fishers' cooperative), although personal contacts between interviewers and interviewed citizens are the most fruitful in terms of data quality. The success of such an approach also depends on the expertise and ability of the interviewers/researchers in defining questions and in building up a trust-based relationship with interviewees. LEK studies are being increasingly used to understand ecosystem processes (Le Fur et al., 2011), to study animal abundances (Anadón et al., 2009) and to investigate historical trends of marine organisms (Rosa et al., 2014; Silvano and Begossi, 2012) mostly for fish species (Azzurro et al., 2011; Boughedir et al., 2015; Damalas et al., 2015; Azzurro and Bariche, 2017), also for benthic organisms (Bastari et al., 2017). Moreover, detection of NIS arrival and spread history are also becoming topics of LEK studies (Boughedir et al., 2015; Azzurro and Cerri, 2021; Cerri et al., 2020).

In some cases, MPAs staff might act as intermediary since they can train local communities on specific issues such as the presence of NIS; local communities can then be involved in the MPAs monitoring activities, thereby reinforcing the standard monitoring effort. In the protected areas the participatory management by all users is considered as best practices to better assess and understand the marine environment and subsequently improve decision making among stakeholders (Berkes et al., 2007).

In the framework of the project HARMONY (Interreg V-A Italia-Malta, 2014–2020), aimed at monitoring selected habitats and invasive alien species occurrence in Natura 2000 sites, a LEK survey was carried out in five Sicilian and two Maltese sites on IAS detection. In the present study, we focus on the arrival of the Lessepsian species *Portunus segnis* (Forskål, 1775) in the Pelagie Islands MPA and we report the subsequent research activity aimed at validating citizens' contribution. The blue swimming crab *P. segnis* is one of the earliest Lessepsian invader of the Mediterranean (Fox, 1924), being its native distribution the Indian Ocean, from Pakistan, Red Sea and Persian Gulf to east Africa; it lives in a wide range of inshore and continental shelf areas, including sandy, muddy or algae and seagrass habitats, from the intertidal zone to at least 50 m depth (Carpenter et al., 1997). Its impact on native biota is undetermined but being an omnivorous predator much larger than any of the sea's native portunid crabs, it can be assumed that it has the potential to outcompete native taxa (CABI, 2021).

2. Material and methods

2.1. Study area

The Pelagie Archipelago, constituted by two major Islands (Lampedusa and Linosa) and one islet (Lampione), is located in the Strait of Sicily (central Mediterranean Sea) between Malta and Tunisia, about 180 km from the Sicilian coast and 150 km from the African one. The Pelagie Islands MPA, established by ministerial decree of October 21, 2002, covers 4136 ha, including more than 70% of the coastal zone of the two major islands of Lampedusa and Linosa excluding the harbours, urban centers and neighbor urbanization areas, and totally the islet of Lampione. The islands are different in geological formation and in seabed profile creating a heterogeneous seascape, two of them (Lampedusa and Lampione) belonging to the African platform while Linosa Island is of volcanic origins (Grasso et al., 1985; Innangi et al., 2018). These aspects contribute to identifying the Archipelago of Pelagie as a biodiversity hotspot in the central Mediterranean (Pulcini et al., 2013; Tonielli et al., 2016; Cattano et al., 2021; Visconti et al., in press). Being in strategic geographic position, like a gateway, between the eastern and the western Mediterranean basins, it is a crucial point of important migrations like those of the large marine mammals, the sea turtles and other pelagic organisms.

The MPA is organized in three principal protection degree areas, "No Take Zone" (A), "Partial Protection Zone" (B) and "General Protection Zone" (C) (Fig. 1). The MPA regulates activities of diving centers and artisanal fishers through a fixed number of authorizations. In the Pelagie Islands there are seven diving centers (five at Lampedusa and two at Linosa) authorized by MPA for 34 dive sites around the islands. The small-scale fishery is composed of 46 fishing boats, among them 22 are regularly authorized by MPA to work in B and C zones of the MPA (updated to 2019)

2.2. LEK and sampling surveys

The marine NIS phenomenon has been preliminary explained by ISPRA (Italian Institute for Environmental Protection and Research) researchers and MPA staff to fishers and divers introducing them on some characteristics and peculiarities on NIS such as their invasiveness, the possible interactions with habitat and native species and the main differences with native similar species. Furthermore, the actions to be taken in case of sighting/capture of a NIS were also addressed.

LEK survey in the Pelagie Islands MPA has been carried out in May 2019, through semi-structured interviews using a questionnaire (modified from Garrabou et al., 2018) submitted to professional and recreational fishers as well as to dive masters. Respondents were shown a poster with 24 selected photos of alien and cryptogenic species; they were also shown other photos of morphologically similar indigenous and NIS with an electronic tablet, highlighting the main distinctive features, in order to verify whether the sighting was correct (Fig. 2). The selection of the species has been made according to defined criteria: degree of invasiveness, ease of recognition, interaction with the seabed, recruitment from local fishing systems, detection in coastal areas by visual census. For each species, it was asked for information about the first sighting and subsequent sightings (date/season, site, substrate type, abundance), the used fishing tool in the case of fishers and any available documentation (photo or video).

Since from the interviews, the occurrence of *P. segnis* was reported for the first time in this area, the subsequent post LEK activities focused on this species. The MPA staff invited fishers and diving centers to collaborate in the detection of *P. segnis* and asked them to collect specimens and any other documentation (photos/video) of this crab in order to validate its occurrence in the study area, also through morphological analysis in the laboratory.

2.3. Morphological and molecular analyses

Collected specimens of *Portunus* sp were measured for carapace length (CL) and width (CW). Species classification is based on the last genus revision by Lai et al. (2010).

Samples were stored at −20 °C or analysed immediately after

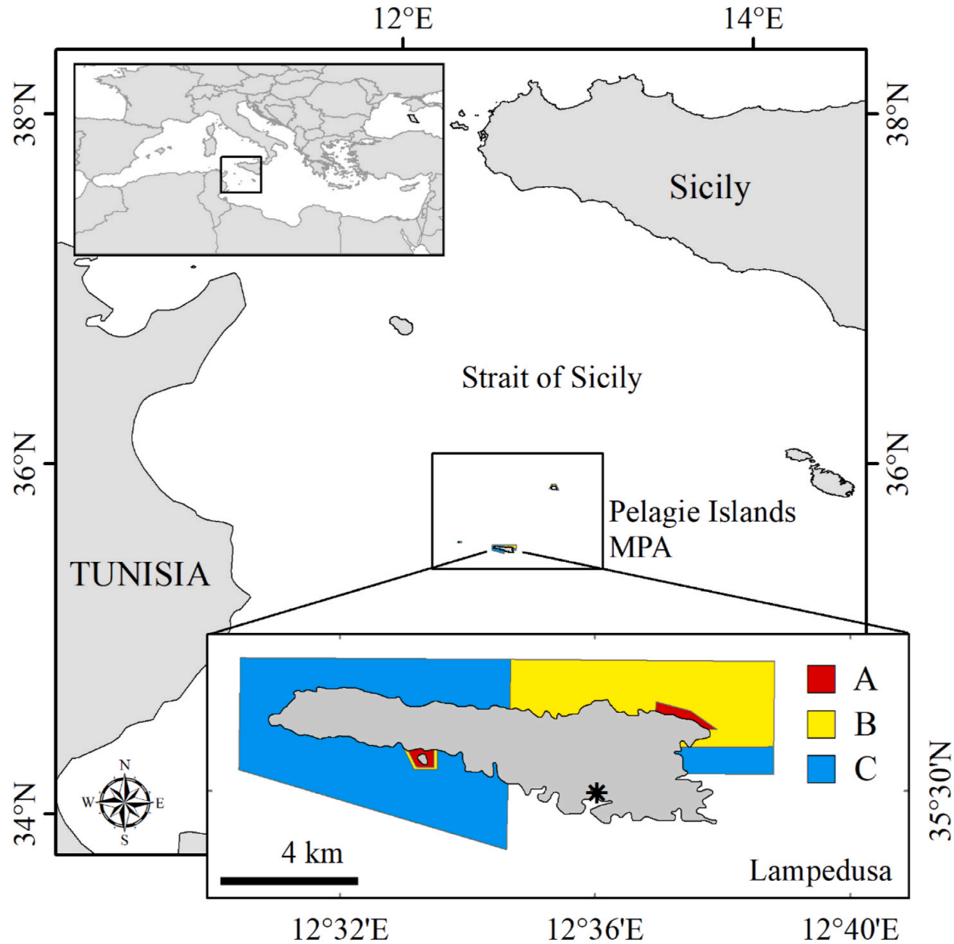

Fig. 1. Marine Protected Area Pelagie Islands; asterisk indicates the sampling site of *Portunus segnis.* Protection degree areas are also shown: A - No Take Zone (red), B - Partial Protection Zone (yellow) and C - General Protection Zone (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

collection. Genomic DNA was purified using PureLink Genomic DNA Kits from muscle. Cytochrome Oxidase subunit I (COI) gene was amplified by PCR by using COIa and COIf primers (Kessing et al., 1989). 50 ng of purified DNA was used as the template for PCR amplification, in a total volume of 25 μl using Platinum Taq DNA Polymerase (Thermo Fisher Scientific) following producer instructions. PCR was performed following this protocol: hot start of 2 min at 95 °C, 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s, with a final 72 °C extension for 3 min. After PCR fragments visualization and purification, the PCR product was sequenced using an ABI Prism 373 automated sequencer. Sequences homology was checked on BLASTn and subsequently aligned using the MUSCLE plugin within the MEGA X (Kumar et al., 2018) with *Portunus* sequences from GenBank, in particular they were compared to published *Portunus* sequences from Lai et al. (2010). Genetic distances were calculated using the Tamura Nei model. Phylogenetic reconstructions were performed based on the Neighbor-Joining and Maximum likelihood method generated in MEGA X. To estimate support for the nodes, 1000 bootstrap replicates were performed and only the values supporting the nodes accounted for more than 50% of the bootstrap replicates were retained.

3. Results

3.1. LEK survey

A total of 20 citizens among fishers and dive masters were interviewed in the Pelagie Islands MPA in May 2019; age classes of experience of the interview ranged from 21-30 yrs to 61–70 yrs and the most represented was 41–50. The most reported species were *Fistularia commersoni Siganus rivulatus* and *Percnon gibbesi*, all invasive alien species settled in the islands for a long time (Fig. 3).

Regarding *P. segnis*, it was observed hiding inside crevices of Lampedusa Island harbour, by diving masters belonging to 21–30 yrs age class; unfortunately, no videos or photos were available.

Subsequently in the summer 2019 several divers and fishers reported the presence of different NIS along the coast of Lampedusa Island to MPA staff, among the reported NIS there was also *P. segnis.* On October 5, 2019, ISPRA researchers found a blue crab exoskeleton (Fig. 4A), whose carapace measured 14.4 cm CW x 6.7 cm CL, in the harbour of Lampedusa where they video-recorded a living *P. segnis* specimen during the night. The video constitutes the first documented report of *P. segnis* in the waters of the Pelagie Islands (snapshot in Fig. 4B). In the same period the MPA staff and ISPRA researchers were contacted by local fishers and citizens on further sightings of *P. segnis* in the harbour of Lampedusa Island, sometimes documenting them through photos and reporting the occurrence of several specimens from the dock of the harbour after sunset. On 13th October, MPA operators and local fishers caught an adult specimen of *P. segnis* (Fig. 4C) in the harbour at night, using a conic fish net. The specimen was used for the next laboratory analyses. Lastly at the end of 2019, ISPRA launched a press release to alert citizens on the presence of the new invader, inviting them to communicate any new record of *P. segnis*. ISPRA campaign led to a further documented record in July 2020 (Fig. 4D) when fishers alerted ISPRA researchers of a conspicuous presence of the blue crab in the

Fig. 2. Local Ecological Knowledge survey activity, carried out in May 2019 by ISPRA researchers, in the Pelagie Islands MPA with fishers (A, B) and dive masters (C, D).

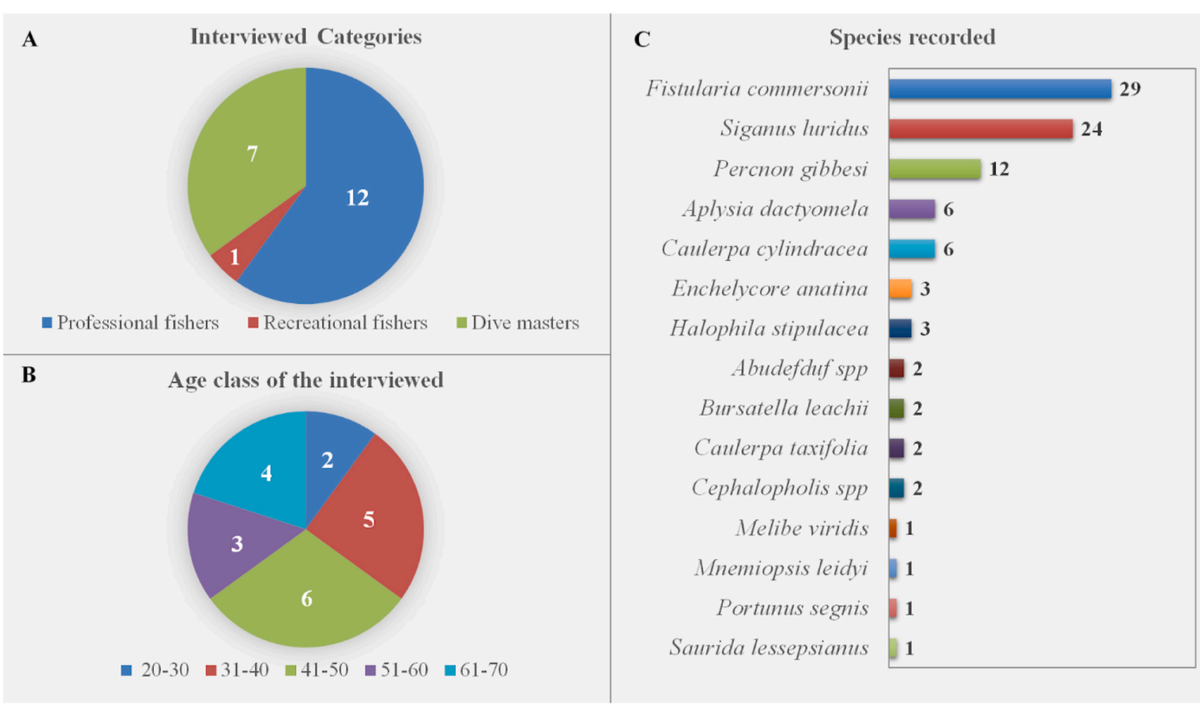

Fig. 3. LEK survey results. Number of interviewed citizens per category (A); number of interviewed citizens per age class (B); total number of reports/sightings per species (C).

harbour of Lampedusa; further records of several specimens were reported in October and November 2020 confirming the blue crab population increase in the area.

3.2. Morphological and molecular analyses

The specimen collected on October 13, 2019 (Fig. 4C), measuring 15.8 cm CW and 8.4 cm CL, showed the following characteristics: large

Fig. 4. Documentation of *Portunus segnis* records from Lampedusa Island: A: blue crab exoskeleton found in the harbour (October 5, 2019); B: snapshot from the video documentation (October 5, 2019) (ISPRA, 2019); C: *P.segnis* adult caught by local fishers (October 13, 2019) D: local fisher photo documentation (July 2020)

carapace with minute median frontal teeth and fine granulation; narrow elongated chelipeds bearing three spines in the anterior margin of merus, elongated ambulatory legs with merus of pereiopods longer than wide, elongate oval natatorial paddle. Coloration: carapace dark olive green with many pale white spots on surface particularly posteriorly and anterolaterally; the longest carapace spines end in a whitish tip; chelipeds and natatorial pereiopods with the same color pattern except for whitish and pale blue natatorial paddles; the other pereiopods light blue with whitish spots brownish bordered on merus.

The COI gene was successfully amplified for the two collected samples; the sequences did not show any nucleotide substitutions; BLASTn search reported 99.3% sequences homology with *P. segnis* from Israelian coast sequenced and deposited by Lai et al. (2010) (Accession Number EF661958); subsequently they were aligned with other *Portunus* spp. sequences from Lai et al., (2010) (Accession Number from EF 661877 to EF661976). The alignment resulted in 573 base pairs (bp); of the total 573 bp, 472 resulted conserved, 101 were variable with 73 parsimony informative and 28 singleton substitutions. The nucleotide frequencies were 0.277 (A), 0.378 (T), 0.187 (C), and 0.159 (G), revealing a thymine bias. The overall transition/transversion bias (R = 1.24) showed that the greater part of nucleotide variation was due to transitions, as is common in the protein coding gene. Estimates of Tamura Nei (1993) evolutionary divergence among *Portunus* species as identified by Lai et al. (2010) ranged from 0.02 (*P. reticulatus* vs *P. armatus*) to 0.08 (*P. armatus* vs *P. segnis* and *P. segnis* vs *P. reticulatus*) (Table 1). Meanwhile, within-group Tamura Nei distance ranged from 0.007 (*P. segnis*) to 0.013 (*P. pelagicus*). Phylogenetic analyses conducted with different approaches resulted in phylogenetic trees with low values of bootstrap support at nodes of species identification, supporting the weak species delimitation already identified by Lai et al. (2010) (Fig. 5).

4. Discussion

This study reports the first documented record of the blue swimming crab *P. segnis* in Lampedusa Island; this area represents a strategic site for NIS invasion in Mediterranean Sea and it can be considered an outpost of observation within the central Mediterranean Sea receiving NIS from the Atlantic Ocean through the Gibraltar Strait and from the eastern Mediterranean and Red Sea as well as from Tunisian and Maltese waters (Azzurro et al., 2014).

P. segnis is one of the earliest Lessepsian invaders of the Mediterranean Sea. It was first recorded as *Neptunus pelagicus*, off Egypt (Port Said) in 1898 (Fox, 1924), and subsequently followed the northward and westward expansion in the Mediterranean. It soon became a fishing resource along Turkish and Egyptian coasts due to its edibility and large size. Regarding the Strait of Sicily, it reached Malta in 1972 but it was misidentified as *Callinectes sapidus* (Schembri and Lanfranco, 1984). More recently in 2014, it was reported in the Tunisian waters with few specimens in shallow sandy areas mostly covered by seagrass and algal beds (Rifi et al., 2014; Raboui and El Zrelli, 2015). Since 2015, *P. segnis* population has increased enormously in abundance in the coastal areas of the Gulf of Gabès (Crocetta et al., 2015) where the species is currently sold in the local markets. In Sicily, it was present on the southeastern coast since 1966, misidentified as *Callinectes sapidus* (Torchio, 1967), and commercially exploited from the 1960's to the 1980's, then seriously declining (Galil et al., 2002). Therefore, *P. segnis* was already present in the Strait of Sicily but not yet in the Pelagie Islands. Since in this archipelago it has been documented only from a harbour, it is plausible to assume that the species arrived through maritime traffic, as already reported for other Mediterranean areas (Deidun and Sciberras, 2016; Hajjej et al., 2016), probably from Tunisia or Malta where it is currently very abundant. However, the hypothesis of immigration cannot be discarded as the natural dispersion of the species can be favored by the transport of the long-lived planktonic larvae by currents. Whatever the pattern of introduction is, the first occurrence of *P. segnis* in the Pelagie Archipelago highlights the need for a strict cross-border coordination between countries bordering the Strait of Sicily in order to monitor the NIS arrival.

Citizen involvement plays an important role, as demonstrated here, but the validation by researchers is mandatory, especially in cases where

Table 1
Pairwise genetic distance (Tamura Nei model) among the *Portunus* species as identified by Lai et al. (2010), using Tamura Nei substitution model.

	P. armatus	*P. pelagicus*	*P. reticulatus*	*P. segnis*
P. armatus				
P. pelagicus	0.0739			
P. reticulatus	0.0157	0.0712		
P. segnis	0.0764	0.0391	0.0793	

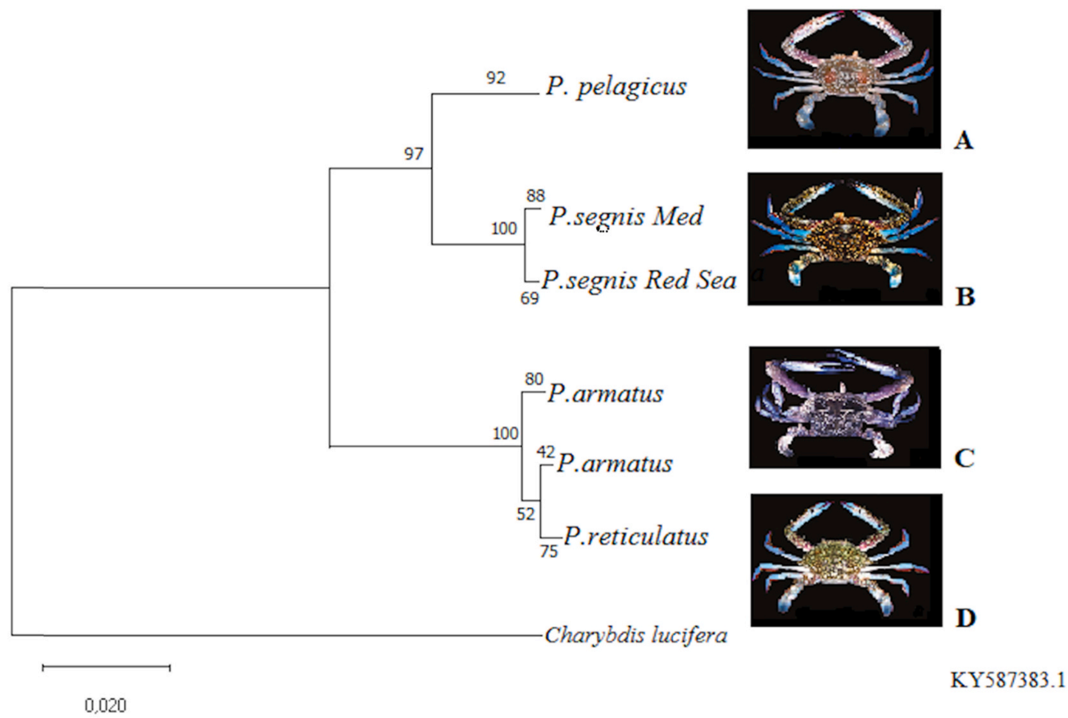

Fig. 5. Neighbor-Joining tree constructed using the evolutionary distances computed (Tamura-Nei method) among *Portunus* spp. Identification photos from Lai et al. (2010): A, *P. pelagicus* specimen ZRC 2007.0235; B, *P. segnis* photograph: H.H. Tan; C, *P. reticulatus* photograph: Z. Jaafar; D: *P. armatus* photograph: P.J.F. Davie.

the species found could be easily misidentified with other non-indigenous species having similar features. This is the case of *P. segnis* that over the years has been mistaken for *C. sapidus* even by specialists. In the present LEK survey, although interviewees were shown the photos of the two similar species highlighting the morphological differences between them, they were distracted by the most glaring character, i.e. the blue color of ambulatory and natatory legs, identifying the two species as one, the blue crab. In such cases, the documentation through photos/videos, or better yet the collection of specimens, are essential in order to validate the citizen reports and correctly identify the species. More than ever in the case of *P. segnis* it was also essential the use of molecular markers (e.g. COI) to identify the species, given the many taxonomic uncertainties of the genus *Portunus*, as pointed out and partially resolved by Lai et al. (2010). However, given the low degree of divergence among *Portunus* species, that may have resulted from recent lineage divergence followed by a secondary contact or hybridization as hypothesized by Bagheri et al. (2020), the use of different molecular markers for further insights is essential to understand the correct species delimitation.

The finding of *P. segnis* in the Pelagie Archipelago through LEK is a further example of the importance of citizen involvement for NIS/IAS early detection. Surveillance through monitoring and early detection allows to intercept species before their establishment and spread and therefore to undertake appropriate management strategies (Giakoumi et al., 2016; Katsanevakis et al., 2015). Identifying the most effective management measures to be applied in the presence of NIS is essential, especially when it comes to invasive species. While in the terrestrial environment, NIS/IAS management strategies in several cases proved to be effective leading to many successful eradications (Robertson et al., 2017), eradication of marine IAS has only been achieved in rare cases, when the species were in restricted areas and were detected early, and management responded rapidly (Giakoumi et al., 2019). The managing goal of established marine IAS has generally been to keep their populations below the densities that cause significant environmental damage to native ecosystems, to ensure their resilience (Green et al., 2014). Long-term management should be the last option, since the steps to be taken to eradicate/contain already established IAS may be impossible or too expensive (Simberloff et al., 2013). Several interventions, both in the terrestrial and aquatic environment, have succeeded in containing the populations of IAS below an acceptable threshold value, but in the long run they have proved to be unsustainable and management interest decreases once the populations control has been reached. Another problem of long-term maintenance management is the continuing reinvasion possibility from an unmanaged site to a managed site, depending on the invasion spatial extent and the connectivity between the sites (Simberloff, 2021).

According to the guiding principles on IAS adopted by the Convention on Biological Damalas et al. (2015), prevention is the priority response; when prevention fails, early diagnosis, rapid response and possibly eradication should follow. In addition, surveillance of NIS is considered extremely important by European Union as one of the three measures envisaged to combat IAS: Member States have to set up a monitoring and surveillance system to detect the presence of IAS of Union concern as early as possible and take rapid eradication measures to prevent them from establishing. Within the monitoring and surveillance system the contribution of citizens has become increasingly important mainly because it is a not expensive way of collecting data, able to fill gaps of the institutional and research monitoring programs.

In this context, MPAs play a fundamental role in the NIS surveillance as a bridge between two extremes, research and environmental protection on the one hand and, on the other, the world of sea resource users - first of all divers and fishers (Fig. 6). For this reason, they can represent a hub for sharing information on environmental changes or anomalous events. To make this exchange effective, a high level of confidence between MPA staff and sea resource users should be established and maintained over time, ensuring a fruitful involvement.

At the same time, training on NIS/IAS issues to MPA staff as well as to sea resource users should be ensured, for the benefit of surveillance data quality as confirmed by several studies (Crall et al., 2011; Jordan et al., 2012; Goczał et al., 2017). Investing in the training of MPAs staff on the processes of NIS/IAS data collection and transfer to experts should be strengthened, producing specific surveillance guidelines, learning tools,

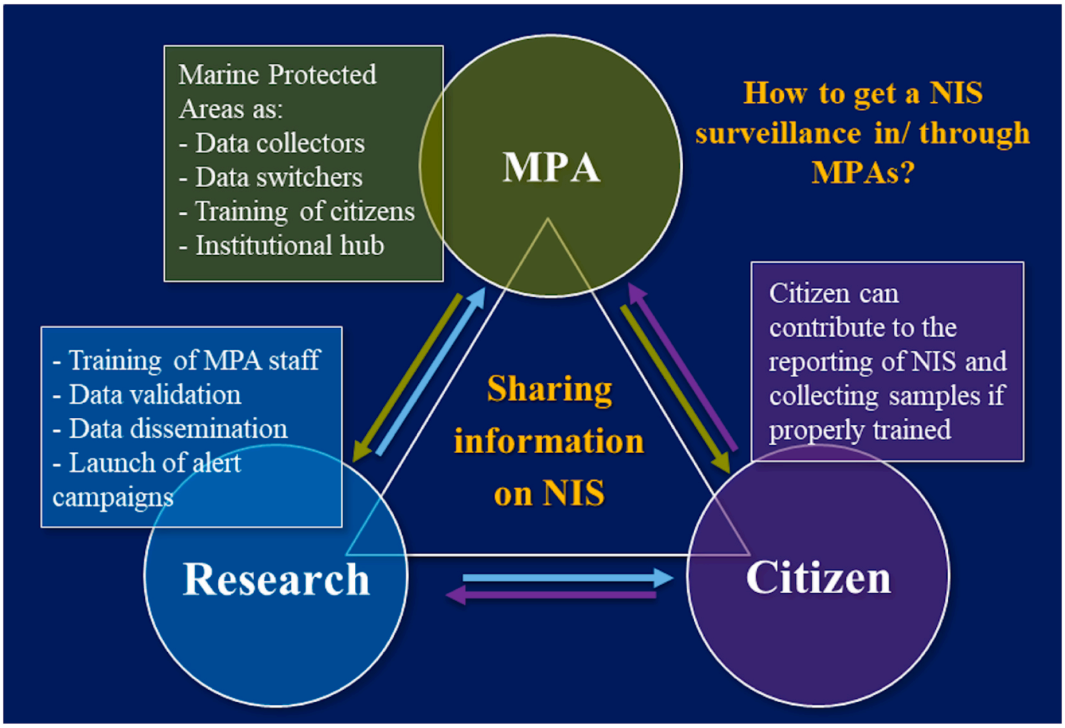

Fig. 6. NIS surveillance system scheme in/through Marine Protected Areas (MPAs).

dedicated exhibitions, workshops, etc. The MPA staff, in turn, should transfer the received training and skills to fishers and divers, so they can act as sea sentinels. In addition, containment measures of NIS/IAS involving local fishermen should be activated in the MPAs and neighboring areas.

5. Conclusions

The involvement of sea users is the way forward for effective activation of the NIS surveillance system as shown in the present LEK experience. In particular, the stakeholders' involvement in NIS data collection allowed us to establish a connection between researchers and other stakeholders through the active participation of the MPA staff. This strategy has led and continues to bring useful information regarding the presence of NIS/IAS in the Pelagie Islands MPA. Furthermore, citizen participation, if set up with the training support described above, can include volunteer-based monitoring activities as part of a surveillance system. These activities have proven to be effective for researchers in collecting data, filling the gaps in some cases (Fore et al., 2001; Lodge et al., 2006), as well as for citizens who benefit from hands-on learning experiences, feeling part of a community.

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6. Availability of data and material

The data and the material are available upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocecoaman.2022.106043.

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