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NON-INDIGENOUS SPECIES IN THE MEDITERRANEAN MARINE PROTECTED AREAS: DIVERSITY, DISTRIBUTION AND IMPACTS

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Contents

1. Introduction

1.1. Relevance of the thesis

Non-indigenous species (NIS), introduced by humans outside of their natural range and their natural dispersal potential, may profoundly affect the ecosystem functioning and the integrity of native communities and habitats, as well as economy and human health in invaded regions (Grosholz 2002; Katsanevakis et al. 2014; Olenin et al. 2010).

The Mediterranean Sea is heavily affected by biological invasions (Galil 2009, Zenetos et al. 2012; Galil et al. 2014) and marine protected areas (MPAs) are not immune to NIS introductions and spread (Simberloff 2000; De Poorter et al. 2007; Monaco and Genovesi 2014; Guidetti et al. 2014). However, the effects of MPAs on NIS establishment and spreading are generally unrecognized. Moreover, due to limited research efforts and a lack of regular monitoring programs, there is a lack of information on numbers, identity, and distribution of marine NIS in some Mediterranean countries (e.g. in Croatia) (Galil et al. 2016). Such information represents a necessary scientific basis for risk assessment and an adequate response to the threat of NIS. Regular NIS monitoring programs within MPAs and surrounding areas could enable identification of NIS at an early stage of colonization, when it has limited spatial distribution and its eradication may still be possible, at least on a local scale (Otero et al. 2013; Mannino et al. 2016).

The quantitative measures of impacts of NIS often remain unknown (Olenin et al. 2010; Ojaveer et al. 2015; Cardeccia et al. 2016). The study of the trophic traits of NIS can therefore be a useful tool for evaluating the impacts of NIS. Characterisation of trophic interactions is critically important in conservation ecology, because changes in the diets of consumers can potentially reflect environmental changes (Hardy et al. 2010). Quantitative understanding of the predator-prey dynamics might offer a tool for measuring early responses to disturbance (Carreon-Martinez and Heath, 2010) and thus functional changes in the ecosystem.

In this study, the distribution of NIS and the effect of MPAs on NIS establishment and spread was assessed using rapid assessment, inside and outside some selected Mediterranean MPAs. Furthermore, trophic interactions between the indigenous benthic taxa and the non-indigenous species (NIS): the green alga *Caulerpa cylindracea*, the red alga *Asparagopsis taxiformis*, the crab *Percnon gibbesi* and the sea hare *Aplysia dactylomela*, were investigated using stable isotope ratios and subsequent isotopic population metrics, in order to determine the role of NIS in invaded ecosystems and the possible impacts it has on indigenous taxa. Moreover, the impacts of *C. cylindracea* on fish and microbenthic assemblages were evaluated inside the MPAs.

1.2. Aim and main tasks of the study

The aim of the study is to assess the diversity and distribution of NIS and their impacts in the Mediterranean Marine Protected Areas (MPAs).

The main tasks are:

1. To quantify and describe the distribution of marine NIS on Lampedusa Island and to assess the effects of protection on NIS and macrobenthic and fish assemblages.

2. To evaluate the trophic interactions between NIS and indigenous species on Lampedusa Island.

3. To investigate the identity, abundance and distribution of marine NIS on mooring lines in berthing areas along the eastern Adriatic coast, Croatia, and to evaluate the effects of MPAs on NIS establishment and spreading in berthing areas.

4. To assess the vulnerability of benthic habitats to the invasion of the green alga *Caulerpa cylindracea* and its role in macrobenthic and fish assemblages.

5. To estimate the impact of *Caulerpa cylindracea* on trophic interactions in the MPAs.

1.3. Novelty of the study

This study provides the data on abundance and distribution of some non-indigenous and cryptogenic macroalgae, sessile and mobile macroinvertebrate and fish species in the central and northern parts of the Mediterannean Sea. For the first time, the efficiency of Mediterranean MPAs Pelagie Islands (Italy, central part of the Mediterannean Sea), Lastovo Archipelago, and Mljet Island (Croatia, Adriatic Sea) was assessed in relation to NIS introductions and spreading.

The records of fouling NIS and cryptogenic species *S. plicata, C. brunnea* and *W. subtorquata* represent the first ones in Croatia, and those of *C. brunnea* and *W. subtorquata* are new to the whole Adriatic Sea.

For the first time, the trophic positions and trophic interactions of non-indigenous herbivores *Aplysia dactylomela* and *Percnon gibbesi* were assessed using stable isotope analysis (SIA), thus providing the evidence for the invasion meltdown hypothesis: the mixing models indicated that the non-indigenous green alga *C. cylindracea* was the most important resource in the diet of the non-indigenous crab *Percon gibbesi.*

Furthermore, the SIA was used to document the differences in the trophic niches of native invertebrates between the sites invaded by *C. cylindracea* and non-invaded sites for the first time in the Adriatic Sea.

1.4. Scientific and applied significance of the results

The results of this study contributed to the body of knowledge on the distribution and trophic role of non-indigenous invertebrates and algae in the Mediterranean Sea. The data obtained during this study is available for further use in the modeling of other biological and ecological processes, such as nutrition, predation, dispersal, etc.

The rapid assessment methods used in this study proved to be an effective method for recording sessile species and monitoring NIS presence, which could be undertaken for regular NIS surveys in the Mediterranean Sea, especially in MPAs, considering the fact that these methods have minimal impact on the communities. The results of rapid assessments may be utilised as a baseline for future assessments of the occurrence of NIS in the Mediterranean Sea. Also these data may be utilized for the NIS assessements for the Marine Strategy Framework Directive (MSFD) purposes.

1.5. Defensive statements

1. There is no evidence of the protection regime effect on the abundance and distribution of NIS *Caulerpa cylindracea*, *Asparagopsis taxiformis* and *Percnon gibbesi* on Lampedusa Island.
2. All fouling NIS and cryptogenic species on mooring lines were found outside of the Adriatic MPAs, which may be due to reduced levels of boat activity and anthropogenic pollution in the MPAs.
3. The records of fouling NIS and cryptogenic species *Styela plicata, Celleporaria brunnea,* and *Watersipora*

subtorquata represent the first ones for Croatia. Furthermore, *C. brunnea* and *W. subtorquata* are new to the whole Adriatic Sea.

4. The green alga *C. cylindracea* invaded all types of substrates in the Adriatic MPAs. Sandy substrate sites had the highest abundance of *C. cylindracea.* The sites dominated by rocky substrates had higher species richness at invaded than at non-invaded sites.
5. The isotopic niches of non-indigenous herbivores, the sea hare *A. dactylomela* and the crab *P. gibbesi,* showed no overlap with the niches of indigenous consumers. The invasive green alga *C. cylindracea* provided the largest contribution to the diet of *P. gibbesi.* Thus, this alga might facilitate the expansion of the herbivorous crab in the Mediterranean Sea.

1.6. Scientific approval

The results of this study were presented at several conferences, meetings and seminars:

- Summer School in Marine Ecology and Geobotany – MARECO, Erasmus Intensive Programme of 'Education and Culture DG - Life Long Learning Programme' (http://www.mareco-eu.info), FOR-MARE (www.for-mare.eu) and the University of Pavia.
- Second MARES Annual Meeting, Faro, Portugal, February 2013.
- Fourth MARES Annual Meeting, Ispra, Italy, February 2015.

- Seminar given at 'Oceanography at Sea: An Introduction to Practical Aspects of Oceanography, EUROFLEETS2', Technical University of Denmark, June 2015.
- YouMares conference, Bremen, Germany, September 2015.
- 12th Croatian biological congress, Sveti Martin na Muri, Croatia, September 2015.

The following peer-reviewed research papers have been produced during the PhD studies:

Marić M, Ferrario J, Marchini A, Occhipinti-Ambrogi A, Minchin D (2016) Rapid assessment of marine non-indigenous species on mooring lines of leisure craft: new records in Croatia (eastern Adriatic Sea). Marine Biodiversity.10.1007/s12526-016-0541-y

Marić M, De Troch M, Occhipinti-Ambrogi A, Olenin S (2016) Trophic interactions between indigenous and non-indigenous species in Lampedusa Island, Mediterranean Sea. Marine Environmental Research. 10.1016/j.marenvres.2016.08.005

Corsini-Foka M, Zenetos A, Crocetta F, Çinar ME, Koçak F, Golani D, Katsanevakis S, Tsiamis K, Marić M, et al. (2015) Inventory of Alien and Cryptogenic Species of the Dodecanese (Aegean Sea, Greece): Collaboration through Cost Action Training School. Management of Biological Invasions, 6 (4): 351-366.

1.7. Thesis structure

The dissertation is presented in the following chapters: Introduction, Literature review, Materials and methods, Results, Discussion, Conclusions, References and Annex. The volume of the dissertation is 173 pages. References include 227 sources. The body of the dissertation is written in English with Lithuanian summary. It contains 26 tables and 33 figures.

1.8. Abbreviations and definitions

MARES	Joint Doctoral Programme on Marine Ecosystem Health & Conservation funded through Erasmus Mundus
MPA	Marine protected area
MSFD	Marine Strategy Framework Directive
NIS	Non-indigenous species
ROV	Remote operated vehicle
SIA	Stable isotope analysis
TEF	Trophic enrichment factor
TP	Trophic position

Non-indigenous species (NIS; synonyms: alien, exotic, non-native, allochthonous) are species, subspecies or lower taxa introduced outside of their natural range (past or present) and outside of their natural dispersal potential. This includes any part, gamete or propagule of such species that might survive and subsequently reproduce. Their presence in the given region is due to intentional or unintentional introduction resulting from human activities. Natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS. However, secondary introductions of NIS from the area(s) of their first arrival could occur without human involvement due to spread by natural means (Olenin et al. 2010).

Invasive non-indigenous species/invasive alien species are a subset of established NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an adverse effect on biological diversity, ecosystem functioning, socio-economic values and/or human health in invaded regions (Olenin et al. 2010).

Cryptogenic species are species of unknown origin which can not be ascribed as being native or alien. They also may demonstrate invasive characteristics and should be included in invasive species impacts assessments (Olenin et al. 2010).

2. Literature review

2.1. Non-indigenous species in a marine environment: the basic concepts in marine bioinvasion studies

Non-indigenous species (NIS) continue to be recorded in European seas (EEA 2015). All established NIS are supposed to have an impact on the functioning of the ecosystem to some extent. Some of them may become invasive and have a more severe impact on biological diversity, ecosystem functioning, economy and human health in invaded regions (Bax et al. 2003; Grosholz 2002). Ecological impacts of invasive species range from single prey-predator interactions to population declines, changes in community composition, or massive impacts, such as changes in the entire ecosystem functioning (Olenin et al. 2007).

Biological invasions are one of the major threats to the integrity of native communities and they are considered as one of the drivers of global biodiversity changes (Mack et al. 2000). While some invasive NIS simply decrease the abundance of native members of a community, others can cause fundamental changes in the composition and community structure of invaded areas. Differential impacts on different species can be calculated using measures such as ranked order of species by abundance (Vitousek 1990). Olenin et al. (2007) proposed the NIS impact level assessment, termed "biopollution level index", which consider the abundance and distribution ranges of NIS with the levels of impact on communities, habitats and ecosystems. In each impact category, the scale involves five levels ranging from no impact (no measurable impact) to massive impact.

Invasive NIS can cause shifts in community composition and dominant species (Britton-Simmons 2004), displacement of native species (Neideman et al. 2003) and loss of type-specific communities and keystone species (Gophen et al. 1995).

NIS can change the physical habitat in ways that drastically alter ecosystem functioning (Rodriguez et al. 2005) and cause physical–chemical changes to the substrate (Vallet et al. 2001), sediment transport and water flow (Schwindt et al. 2001), nutrient regime and the transparency of water, as well as the replacement of the keystone habitat forming species (Grosholz 2002, Reise et al. 2006).

NIS can also modify ecosystem functioning through their abundance, distribution or their role as key species. When they belong to a different functional group, from that of native key species or ecosystem engineers, they can change the energy flow and food web structure (Boudouresque et al. 2005; Hejda et al. 2009).

The abundance and impact of NIS will often co-vary with environmental variation. The relationship of invader abundance and, for example, the effect on species richness, may be non-linear. Therefore, a negative relationship of invader impacts and species richness, among sites with different disturbance regimes, may be caused by anthropogenic factors instead of being a genuine effect of the invader (Thiele et al. 2011). Human activities that are likely to affect species and ecosystem diversity are coastal development, dredging, dumping (solid waste), fishing (commercial fishing, trawling, overfishing and amateur fishing), ocean warming, ocean acidification and pollution (nutrients, organic matter, heavy metals, turbidity, aquaculture facilities) (Boudouresque et al. 2005; Orr et al. 2005).

Furthermore, ecological interactions between NIS are common. Simberloff and Von Holle (1999) suggested that non-indigenous species facilitate the establishment of subsequent invaders in various ways, increasing the likelihood of survival and/or of ecological impact, and possibly the magnitude of impact. The process by which a NIS facilitates the invasion of other species was termed 'invasional meltdown'. Since then, this hypothesis was often used to explain bioinvasions. Jeschke et al. (2012) revealed that out of 30 studies that explicitly tested the invasional meltdown hypothesis, 77% of those found evidence in support of the hypothesis. The search conducted using the Google Scholar service, with the input keywords "invasional meltdown", resulted in around 2900 references where this hypothesis was mentioned, thus confirming its importance in invasion biology.

2.2. Assessing abundance, distribution and impacts of non-indigenous species

Knowledge of the distribution and quantification of the impacts of invasive NIS is essential for the prioritisation of actions in order to prevent new invasions or for developing mitigation measures. Along with protecting marine biodiversity, Marine Protected Areas (MPAs) provide suitable locations for investigating the impacts of NIS without severe anthropogenic disturbances. These areas are often chosen because they include important marine resources and habitats. The impacts of marine NIS in these environments can be substantial and even highly detrimental to them. Therefore, it is important to address the problem of NIS in MPA planning and management (Trenouth and Campbell 2013; Campbell and Hewitt 2013). Moreover, NIS impact assessment is recommended at a local scale, in ″stepping stone areas″ or ″hot-spots″ for NIS introduction, and/or in areas of special interest, such as MPAs (Olenin et al. 2010).

2.2.1. Underwater visual census and video techniques

Underwater visual census techniques have minimal impact on the community (Harmelin-Vivien et al. 1985). For that reason, they are one of the main methods used for studies in MPAs (Azzurro et al. 2007a; Tunesi et al. 2006; Consoli et al. 2013). The community structure is assessed *in situ* by estimating the quantity of individual species or community descriptors (by counts of individuals or by percent cover). Transects and quadrats are the most common sampling units. Moreover, they allow simultaneous collecting of the data on the assemblages and environmental characteristics (Murray et al. 2006).

Underwater video techniques likewise have minimum impact on the environment. In addition, they are depth-independent and can help remove some major sources of observer bias in visual censuses by providing a simultaneous collection of a much wider suite of information in a permanent record that can be analysed later (Cappo et al. 2003). Therefore, they have proved to be a very useful tool for assessing marine populations and communities in MPAs (Assis et al. 2007; Moore 2014, 2015).

2.2.2. Non-indigenous species from berthing areas: rapid assessments

A large proportion (54%) of NIS recorded in the eastern part of the Adriatic Sea were fouling species (Pećarević et al. 2013). Leisure craft are capable of spreading non-indigenous species (NIS), both on an international and local level (Minchin et al. 2006), and in some regions are considered to be the most important vector of introduction of such species (Clarke-Murray et al. 2014).

The large number of marinas, which have been built over recent decades within some Mediterranean regions (Savini et al. 2006), and the behaviour patterns of leisure craft (Cornell 2002) enable NIS to become widely distributed beyond commercial shipping ports and areas where aquaculture is practiced. The layout of marinas, that are frequently endowed with extensive protective breakwaters offering substrate opportunities for benthic species, may also enhance the probability of NIS retention and development once introduced (Floerl and Inglis 2003; Airoldi et al. 2015).

Sampling for marine non-indigenous species (NIS) has been undertaken at marinas from various regions of the world, as a part of standard monitoring approaches. Very often, this has involved a team of specialists for species identification (Arenas et al. 2006; Pedersen et al. 2005). The development of a target list of NIS was proposed by Ashton et al. (2006), Minchin (2007) and Nall et al. (2015) in order to facilitate the search for potential invaders. This approach has the advantage of selecting particular NIS, ones most likely to be encountered, and involves sampling using a smaller research team. Sampling is normally made on immersed portions of the floating units supporting boardwalks and finger jetties to which small craft berth. The fouling often includes algae (Mathieson et al. 2008) as well as a wide range of invertebrates (Minchin and Nunn 2013).

2.2.3. Stable isotope analysis as a tool for detection of the impacts of non-indigenous species

Based on the experimental evidence that the stable isotope ratios of a consumer reflect those of its prey (DeNiro and Epstein 1978; 1981), SIA is a widely used method for understanding trophic interactions, estimating specific trophic positions, reconstructing the dietary composition and quantifying the ecological niche of a species within a community. Different than stomach content analysis, this method provides time-integrated information about the feeding relationships (Peterson and Fry 1987; Newsome et al. 2007; Layman et al. 2007). Moreover, it has been shown to be a powerful tool for the detection of the impacts of NIS on ecosystems and a possible niche overlap between NIS and indigenous species (Olsson et al. 2009; Bodey et al. 2011; Jackson et al. 2012; Hill et al. 2015; Fanelli et al. 2015).

2.3. Non-indigenous species in the Mediterranean Sea

The Mediterranean Sea is heavily affected by anthropogenic actions such as intense fishing activity, pollution and tourism (Micheli et al. 2013). It is one of the global hotspots of biological invasions (Galil 2009, Zenetos et al. 2012; Galil et al. 2014), mainly because of the opening of the Suez Canal in 1869 (Galil et al. 2015). Other important pathways of NIS introduction are shipping, aquaculture and aquarium trade (EEA 2015). The majority of NIS are thermophilic tropical species, from the Indo-Pacific Ocean, the Indian Ocean, the Red Sea and pan-tropical areas (Galil et al. 2009).

The recorded species of NIS in the Mediterranean Sea vary according to different reviews. Zenetos et al. (2010) reported 955 NIS in the Mediterranean Sea. The majority was reported in the eastern Mediterranean (718), followed by the central Mediterranean (268) and the Adriatic Sea (171) (Zenetos et al 2010). Galil et al. (2014) reported 680 NIS in the Mediterranean Sea. The number of established NIS, after excluding extinct and single records, is 614 (Galil et al. 2016). The number of NIS differs among Mediterranean countries, and is substantially greater in the east than in the west Mediterranean. Of the 614 established NIS, 324, 295, 190 and 175 species were recorded off Israel, Turkey, Lebanon and Egypt respectively, as compared with 105 and 69 from the Mediterranean coasts of France and Spain (Galil et al. 2016).

More than 5% of the total number of species in the Mediterranean Sea are considered NIS (Zenetos et al. 2012). Some of the Mediterranean species are considered cryptogenic (Carlton 1996), since their origin is not clear. A more accurate analysis (e.g. a molecular comparison of populations in different parts of the world) or new data on transport vectors can clarify their status. Therefore, their number has been changing over time.

2.3.1. Non-indigenous species from Lampedusa Island

Placed in the centre of the Mediterranean, between the eastern and western basin, Lampedusa Island is a remote and relatively pristine island, free of severe anthropogenic impacts and direct NIS introduction vectors, but still exposed to strong propagule pressure of NIS as a result of their secondary spread from other parts of the Mediterranean Sea.

Some of the most successful marine invaders in the Mediterranean Sea have established a population on Lampedusa Island, including the widely distributed and/or locally highly abundant macroalgae *Caulerpa cylindracea* Sonder, 1845 and *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon, 1845, the sea hare *Aplysia dactylomela* Rang 1828 and the crab *Percnon gibbesi* (H. Milne Edwards, 1853).

The green alga *C. cylindracea* is widely distributed in the Mediterranean Sea (Klein and Verlaque 2008). It was introduced into the Mediterranean Sea from south-western Australia (Verlaque et al. 2003). *C. cylindracea* has a high invasive ability and strong competitive characteristics. It can completely cover the surface of all substrates and consequently outcompete native communities, decrease structural complexity and alter trophic interactions (Piazzi et al. 2001; Piazzi and Balata 2008; Katsanevakis et al. 2010; Deudero et al. 2011).

Although the destruction of natural habitats, increased sedimentation and nutrient load enhances the spreading of *C. cylindracea* (Piazzi et al. 2016), MPAs are not immune to the *C. cylindracea* invasion. This algae has been found at both polluted and pristine sites, and on all types of substrates between 0 and

70 m of depth, both in polluted and in unpolluted areas (Piazzi et al. 2005; Klein and Verlaque 2008) (Figure 1).

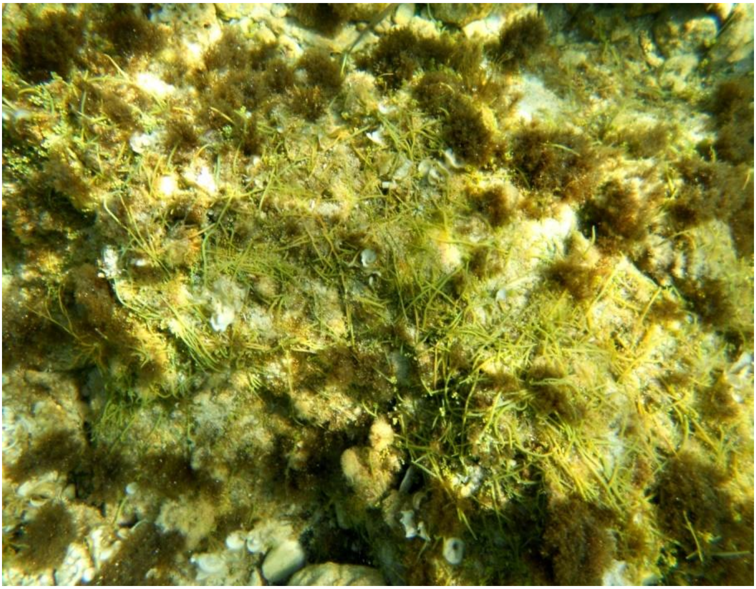


Figure 1. The green alga *Caulerpa cylindracea* on Lampedusa Island (Photo: Fabio Giardina).

Despite the secondary metabolites produced by *C. cylindracea*, such as caulerpenyne, several fish and invertebrate species incorporated this alga into their diet (Ruitton et al. 2006; Box et al. 2009; Tomas et al. 2011; Terlizzi et al. 2011). The species was first reported from Lampedusa Island in 1993 (Alongi et al. 1993).

Asparagopsis taxiformis is a red alga with a heteromorphic life cycle. The erect gametophytic phase alternates with the filamentous tetrasporophyte known as the 'Falkenbergia' stage.

The species has a wide distribution in tropical and subtropical areas. *A. taxiformis* embodies six cryptic mitochondrial lineages. One of them was introduced into the Mediterranean Sea from the Indo-Pacific and exhibits a highly invasive pattern (Andreakis et al. 2009; 2015). It was first recorded from Sicily in 2000 (Barone et al. 2003) and spread throughout the Mediterranean basin and to the Atlantic south coast of Portugal (Andreakis et al. 2009; Katsanevakis et al. 2014). Ecological impacts of *A. taxiformis* are poorly known, but the species can probably outcompete the indigenous species for space and light (Otero et al. 2013) (Figure 2).

Figure 2. The red alga *Asparagopsis taxiformis* on Lampedusa Island (Photo: MARECO Summer School).

The sea hare *Aplysia dactylomela*, native to the Atlantic Ocean (Valdés et al. 2013), was first recorded in the Mediterranean Sea at Lampedusa Island in 2002 (Trainito 2003). Since then it has spread across the central and eastern Mediterranean (Pasternak and Galil, 2010). It remains unresolved whether or not the introduction of *A. dactylomela* in the Mediterranean Sea has been a human-mediated or natural event (Valdés et al. 2013; Stasolla et al. 2014; Mannino et al. 2016).

One of the most invasive decapod species in the Mediterranean Sea, *P. gibbesi,* is native to the eastern Pacific (California to Chile), the western Atlantic (Florida to Brazil) and the eastern Atlantic (Madeira to the Gulf of Guinea) (Nizinski 2003). The species appeared in the Mediterranean Sea in 1999 at Linosa Island, Sicily and Ibiza (Relini et al. 2000; Mori and Vacchi 2002; Müller 2001) and spread rapidly throughout the basin. Its vector of introduction in the Mediterranean Sea is still under debate (Abelló et al. 2003; Mannino et al. 2016). The stomach content analysis, the feeding adaptations of the gastric mill and the morphological characteristics of the chela indicated that *P. gibbesi* is strictly herbivorous (Ferrer and Frau 2005; Puccio et al. 2006), although some studies reported opportunistic feeding behaviour of *P. gibbesi* (Deudero et al. 2005; Cannicci et al. 2004).

Despite their rapid spread, little work has been done on the impacts of *A. dactylomela* and *P. gibbesi*. Studies suggest that these species may have a negative ecological impact on algal abundance and diversity. It is unclear whether or not they compete with other herbivores, such as sea urchins (Katsanevakis et al. 2011; Sciberras and Schembri 2008; Otero et al. 2013). Lapointe et al. (2004) studied the environmental impact of *A. dactylomela* in the Bahamas and showed that herbivory was an important factor in controlling macroalgal biomass, but only specific prey items. *Percnon gibbesi* was considered to be a potential competitor for territory with the indigenous crab *Pachygrapsus marmoratus*. However, laboratory studies showed that *P. marmoratus* is unlikely to be excluded from its natural habitat by *P. gibbesi* (Sciberras and Schembri 2008).

The herbivorous rabbit fish *Siganus luridus* is considered among the most successful 'lessepsian' migrants (Golani et al. 2002). It is distributed along the Red Sea and eastern Africa to Mauritius and Reunion Island to the Arabian Gulf and the eastern Mediterranean. The species is abundant and commercially exploited in many areas of the eastern Mediterranean Sea (Azzurro and Andaloro 2004; Bariche 2002). In 2003, *S. luridus* was commonly observed in Linosa and also noticed in Lampedusa, at a depth range between 1 and 10 m (Azzurro and Andaloro 2004). It can be found mainly on rocky bottoms but also on seagrass beds. They are often noticed within schools of *Sparisoma cretense*. In the eastern Mediterranean, *S. luridus* grazes on common macrophytes (e.g,. *Halopteris* spp., *Padina* spp., *Sphacelaria* spp., *Polysiphonia* spp., *Ulva* spp., *Sargassum* spp.) and occasionally ingests *C. cylindracea* (Bariche 2006). The invasion of *S. luridus,* and the other invasive rabbit fish in the Mediterranean Sea, *Siganus rivulatus,* is causing a decline in habitat complexity, biodiversity and biomass of macroalgae. In the eastern Mediterranean, they are turning well-developed macroalgal assemblages into bare rocks (Katsanevakis et al. 2014; Galil et al. 2015).

2.3.2. Non-indigenous species from the eastern part of the Adriatic Sea

Unlike the Eastern and central Mediterranean basins, where the main pathway of introduction is the Suez Canal (Galil et al. 2016), in the Adriatic Sea, like in other coasts of Europe, most NIS have been introduced by shipping and aquaculture (David & Jakomin 2003; Pećarević et al. 2013; Galil et al. 2014; Marchini et al. 2015a). Moreover, the northward expansion of NIS, already established in the Ionian Sea via the East Adriatic Current, is also recognized as a further important pathway of expansion into the Adriatic Sea (Katsanevakis et al. 2011). According to the latest review by Pećarević et al. (2013), the number of introduced species in the eastern part of the Adriatic Sea was 113, of which 61 are NIS, and others resulting from natural range expansion.

The northern part includes a hotspot of NIS introductions, *i.e.* the lagoon of Venice with its 71 NIS (Marchini et al. 2015a). According to Pećarević et al. (2013), most of marine NIS in Croatia (Eastern Adriatic Sea) are known from the northern shallow part (29), followed by the southern Adriatic (15), and have been observed the least (11) in the middle part.

Caulerpa cylindracea has been reported from the Adriatic Sea in Croatia in September 2000 (Žuljević et al. 2003). The impacts of *C. cylindracea* in the Adriatic Sea have been described in only a few studies (Antolić et al. 2008; Kružić et al. 2008; Žuljević et al. 2011). To this date, the impact of *C. cylindracea* has never been studied in Croatian MPAs. Moreover, there is a lack of data on the impacts of *C. cylindracea* on trophic interactions or on the structure of benthic communities other than *P. oceanica* in the eastern part of the Adriatic Sea.

3. Materials and methods

Due to complexity of the biological invasion problem, different methods are required to reveal peculiarities of the NIS distribution, dispersal mechanism and impacts on invaded ecosystems (Lehtiniemi et al. 2015). The array of the methods and approaches used in this study was aimed to obtain as complete as possible the picture of NIS spread and effects on the selected Mediterranean Sea ecosystems (Figure 3).

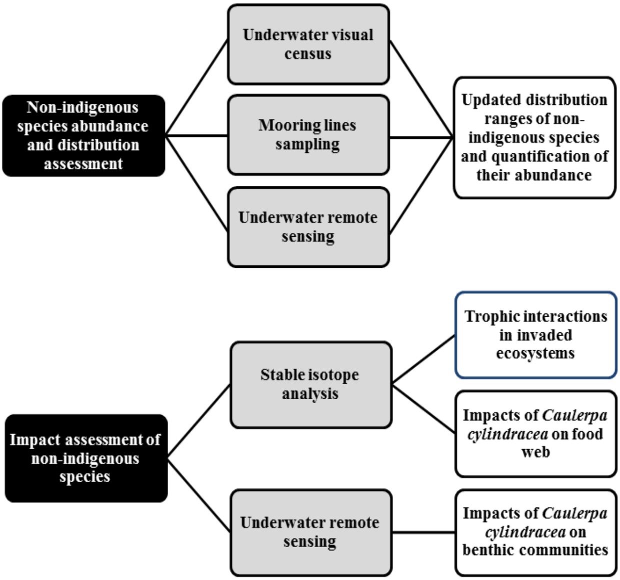


Figure 3. Scheme of the assessment of abundance, distribution and impacts of non-indigenous species: aims (black), methods (grey) and expected outputs of this study (white).

3.1. Case study 1: Lampedusa Island (inside and outside of Marine Protected Area Pelagie Islands)

The study was conducted in June 2013 on Lampedusa Island, the largest of the Pelagie Islands, with an area of 20.2 square kilometers and a coastline of 36 km (Figure 4). The Italian MPA Pelagie Islands is located in the Mediterranean Sea, between Malta and Tunisia. It was established in 2002 and includes all three Pelagie Islands (Lampedusa, Linosa and Lampione). The littoral zone is characterised by rocky substrate and sand.

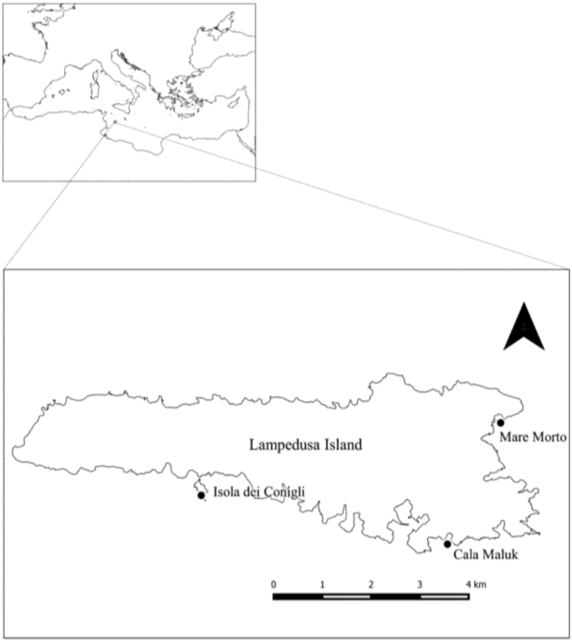


Figure 4. Map of Lampedusa Island showing the three sampling sites: Isola dei Conigli, Cala Maluk and Mare Morto.

The MPA is implemented according to three protection levels (Figure 5): 1. Integral reserve (only authorized personnel are allowed access for monitoring, research, and maintenance); 2. General reserve: low-impact tourism is allowed (boating, swimming, snorkeling, fishing, and scuba diving are allowed with restrictions on numbers, size, and types of boats and on fishing techniques); 3. Partial reserve: restricted recreational navigation and some sporting and commercial fishing activities are allowed (Villa et al. 2002).

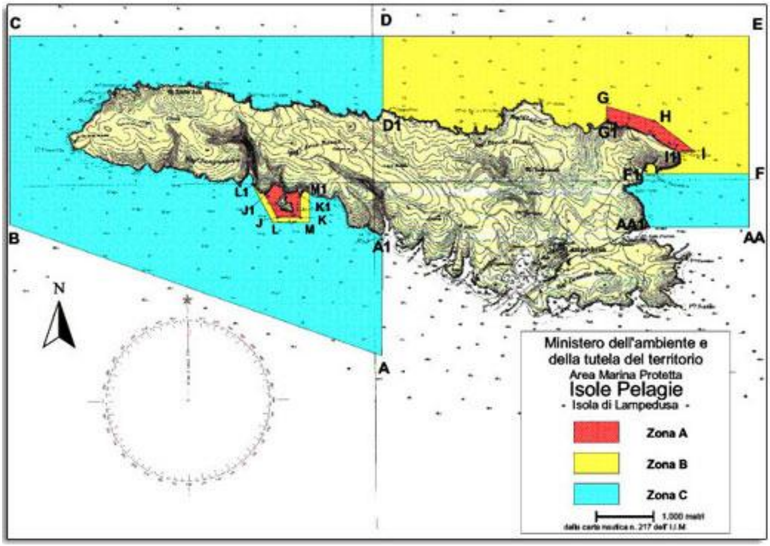


Figure 5. Map of Lampedusa Island showing zones with different level of protection in MPA Pelagie Islands: integral reserve (A), general reserve (B) and partial reserve (C).

Visual assessment of benthic community structure and collection of samples for SIA were conducted at three sites at Lampedusa Island: Isola dei Conigli (35.511037° N, 12.559057° E) in the integral reserve, Mare Morto (35.514816° N, 12.626914° E) in the general reserve, and one area outside the MPA, Cala Maluk (35.494539° N, 12.612508° E).

3.1.1. Underwater visual census

Community composition, NIS distribution and abundance were assessed considering a pre-agreed list of macrophytes, macroinvertebrates and fish taxa which are easily recognized underwater (Annexes 1, 2 and 3). The list was compiled according to the available literature and personal communication with experts and local divers.

3.1.1.1. Underwater visual census of macrophytes assemblages

The percent coverage of 20 macrophytes (Annex 1) was estimated using the 50 x 50 cm quadrat. At each site, 60 quadrats were randomly placed at depths between 0 and 20 m.

3.1.1.2. Underwater visual census of macroinvertebrates assemblages

Abundance of 25 taxa (Annex 2) was assessed by a visual census, which involved swimming along a 30 m transect in constant speed and recording all target species seen within a 4 meter belt (total surface area=120 m^2). Transects were placed at a depth of approximately 2 m. Number of transects per each study site was 8.

3.1.1.3 Underwater visual census of fish assemblages

Abundance of 29 taxa (Annex 3) was assessed by a visual census, which involved swimming along a 30 m transect in constant speed and recording all target species seen within a 4 meter belt (total surface area=120 m^2). The abundance was estimated by two divers. Transects were placed at depths between 1 and 20 m. Number of transects per each study site was 20.

3.1.2. Sampling for stable isotope analysis

The most common macroinvertebrate and macrophyte taxa were collected manually by snorkelling and SCUBA diving, at depths between 0 and 20 m. Fish were sampled by angling. Whenever possible, at least three individuals of each species were collected at each sampling site.

3.2. Case study 2: eastern Adriatic Sea, Croatia

The survey was carried out from June 22 until July 3 2014 in the middle part of the eastern Adriatic Sea. The Adriatic Sea is divided into three distinct biogeographic regions: a northern shallow eutrophic part, with the lowest values of surface temperature during winter (<9°C) and up to 23°C in summer, an intermediate middle part, and a deep oligotrophic southern part, where sea surface temperatures are above 13°C during winter and attain 25°C during summer (Zavatarelli et al. 1998; Lipizer et al. 2014; Viličić et al. 1989).

Lastovsko otočje (Lastovo Archipelago) Nature Park includes 44 islands, islets and reefs. The main island, Lastovo Island (40 km²), with its surrounding waters and islands, was declared a Protected Area (PA) in 2006, with a total surface area of 195.83 km², out of which 143.12 km² is marine (Figure 6).

The National Park Mljet was established in 1960. It is situated on the western part of the island Mljet, with a total surface area of 5.4 km² (Figure 6).

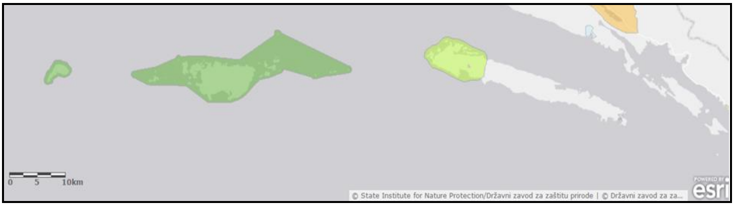


Figure 6. Map showing the MPA Lastovo Archipelago (dark green) and MPA Mljet (bright green).

3.2.1. Rapid assessment in berthing areas

The field survey was conducted at six marinas or berthing sites in the middle part of the Eastern Adriatic Sea, three of them being located in the mainland and three offshore, on coastal islands. The mainland sites were: marina "Kornati", Biograd na Moru (43.94083° N, 15.44222° E), marina "Zadar", Zadar (44.11964° N, 15.22928° E) and Rogoznica Quay (43.53293° N, 15.96616° E). The offshore sites were located within MPAs. Two sites were on Lastovo Island, within MPA Lastovo Archipelago: Pasadur (42.76581° N, 16.82224° E) and Ubli Quay (42.76555° N, 16.82249° E) and one site was in MPA Mljet: Pomena Quay (42.78777° N, 17.34333° E) (Figure 7). Offshore sites have lower number of berths and are characterized by having lower anthropogenic pressure in comparison with mainland marinas (Table 1).

Table 1. Geographical coordinates and the number of lines examined at the six sampling sites.

Berthing area	N	E	No. of lines examined	Comment
"Marina Kornati", Biograd na Moru	43.94083	15.44222	40	380 berths
"Marina Zadar", Zadar	44.11964	15.22928	30	300 berths
Rogoznica Quay	43.53293	15.96616	11	450 berths
Pasadur Quay	42.76581	16.82224	14	MPA, 30 berths
Ubli Quay	42.76555	16.82249	8	MPA, ferry terminal, temporary berths
Pomena Quay	42.78777	17.34333	9	MPA a few berths for visitors of the MPA

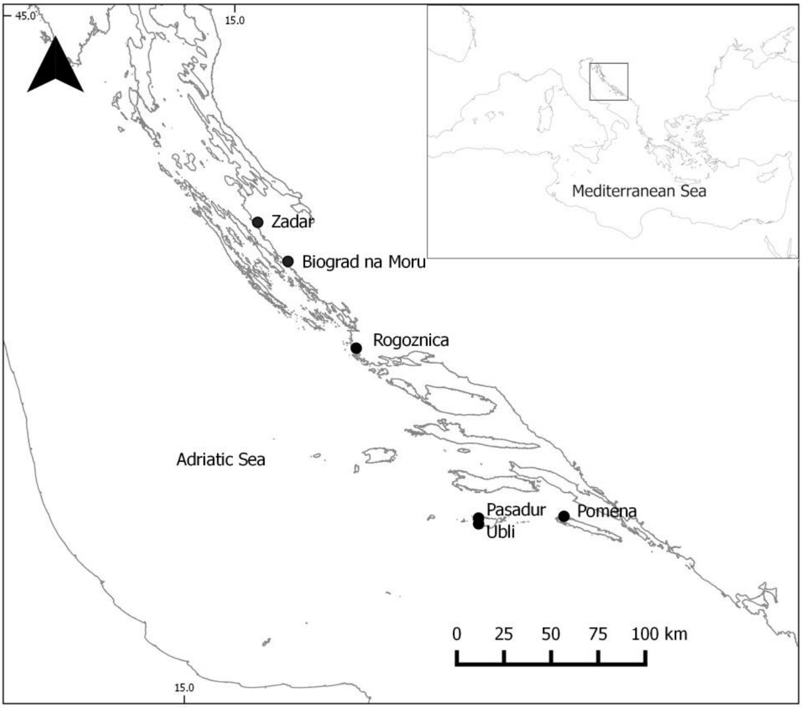


Figure 7. Map of the Croatian coast showing the location of six sampling sites.

Not all marinas have floating berthing units and in the Mediterranean Sea, which has a reduced tidal range, there are small leisure and fishing craft which berth with the stern of the vessel held close to the quay. Mooring lines, known as 'lazy lines', are fixed to an anchored block with paired lengths of chain and connected to a synthetic rope to the quay (Figure 8).

During berthing these ropes are lifted to either side of a craft and the raised chain, near the anchored block, is attached to the cleat, or samson post, at the bow. The paired 'lazy' lines are then slackened and returned to the water. As a result these are immersed over long periods during which fouling organisms may attach themselves to the rope. Two separate lines normally attach a craft to the quay. This method of mooring is used in the Adriatic and Aegean seas.

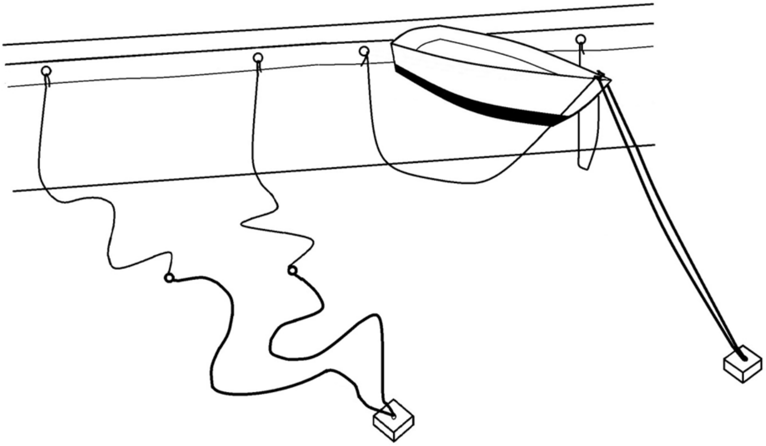


Figure 8. The berthing of small vessels in many Mediterranean regions involves the use of a specialised mooring system. The mooring lines (termed 'lazy' lines) before and after a berthing are shown here.

In each marina, a number of 'lazy' lines was sampled that was adequate to represent the occurring fouling community. Since the number of 'lazy' lines was variable across berthing areas, the following criterion was used: at sites with fewer than 30 lines present, all lines that were not recently cleaned were sampled. When the number of lines exceeded 30, a sub-sample corresponding to approximately 10% of all available lines was investigated, using a random criterion for lines selection. The number of sampled lines is shown in Table 1.

'Lazy' lines were lifted and drawn towards the shore. The abundance of NIS that could be identified in the field was visually estimated for each line and their frequency (the number of lines where NIS were discovered) was recorded.

Samples of small-sized species (<1cm) and species that were difficult to identify in the field (e.g. some polychaetes, bryozoans and crustaceans) were collected for further laboratory analyses. Samples were preserved in 90% ethanol and identified under stereoscopic microscopes. For the latter, only occurrence data is provided.

3.2.1.1 Abundance assessment

Abundance was evaluated according to the classification given in Olenin et al. (2007). Abundance was considered to be *'low'* where species were present in low numbers: three individuals or less for unitary organisms and three colonies or less for modular organisms. A *'moderate'* level was assigned when a taxon was frequent, but occupied less than half of the surface area available for attachment and *'high'* level when the species occupied more than half of the available surface for attachment, being clearly dominant.

3.2.2. Underwater surveys in Marine Protected Areas Lastovo Archipelago and Mljet

Underwater remote sensing survey, visual assessment of fish assemblages and collection of samples for SIA were conducted in MPA Lastovo Archipelago and MPA Mljet, Croatia (Figure 9; Table 2).

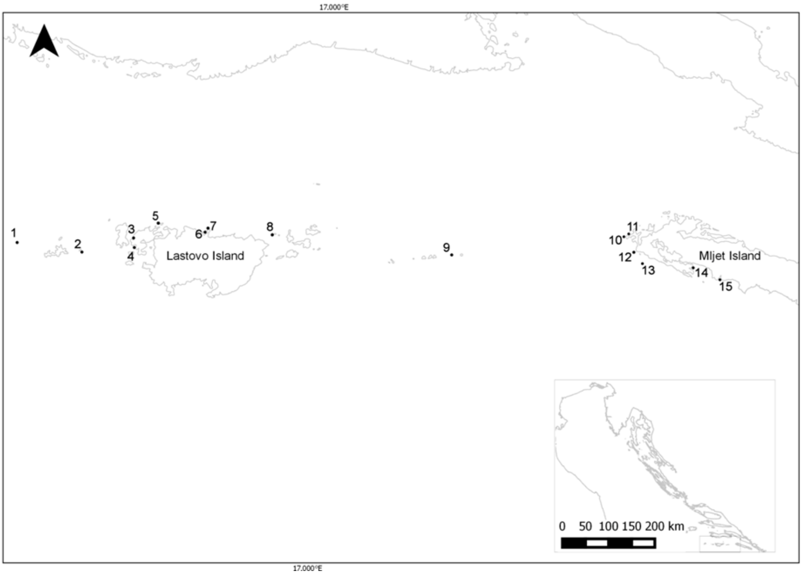


Figure 9. Map of the MPA Lastovo Archipelago and MPA Mljet showing the locations of 15 study sites. The underwater remote sensing survey was conducted at sites: 1, 2, 3, 6, 7, 9, 10, 11, 12, 13, 14 and 15. Samples for stable isotope analysis were taken and visual assessment of fish assemblages was conducted at sites: 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 14 and 15.

Table 2. Geographical coordinates of 15 study sites in MPA Lastovo Archipelago and MPA Mljet.

Site No.	Sites	N	E	Samples taken for SIA/visual assessment of fish assemblages	Underwater remote sensing survey
1	Bijelac	42.758117	16.679083	+	+
2	Crnac	42.754567	16.746433	+	+
3	Prežba (NW)	42.767433	16.802317	-	+
4	Prežba (SW)	42.755895	16.805861	+	-
5	Maslovnjak	42.778717	16.827717	+	-
6	Zaklopatica Bay	42.773683	16.875067	+	+
7	Zaklopatica Islet	42.775688	16.876726	+	-
8	Lukovac Mali	42.775350	16.946767	+	+
9	Mrkjenta	42.764317	17.134400	+	+
10	Goli Rat	42.786883	17.317183	+	+
11	Pomeštak	42.797065	17.339890	+	+
12	Korizmeni Rat	42.774983	17.328050	-	+
13	Mljet (SW)	42.759567	17.333703	-	+
14	Gonoturska	42.762817	17.388717	+	+
15	Grabova	42.755546	17.417781	+	+

3.2.2.1. Underwater remote sensing survey

Underwater remote sensing survey was carried out at 12 sites in MPA Lastovo Archipelago and MPA Mljet. Sites were surveyed for 30 target NIS (Annex 4), at a depth from 1 to 62.62 meters (Figure 9; Table 2).

The system consisted of the underwater and control unit. The depth sensor, light bulbs and video cameras were attached to the underwater unit. Remoted operated vehicle (ROV) was descended vertically and hovered freely over the bottom. Its altitude was regulated manually from the video stream to ensure the best video quality. The average altitude was 1.28 m above the sea bottom. The video from the underwater unit was transmitted in real-time into the control unit that contained GPS antenna and an overlay block. The total duration of the video transects is 4.88 hours (Table 3). The frame rate of the video recording was 25 frames per second. Benthic communities were analysed using video mosaics (still images made from adjacent frames of video), which were kindly produced by Dr. Aleksej Šaškov, based on the video material collected during the field study. Each video mosaic was comprised of approximately 1000 frames (40 seconds of video transect) and represented one assessment unit. The number of analysed assessment units per each site is shown in Table 3.

Table 3. Total video length, the number of assessment units and the depth range of the video taken at 12 sites in MPA Lastovo Archipelago and MPA Mljet.

Site No.	Sites	Total video length (min:sec)	No. of assessment units	Min. depth (m)	Max. depth (m)
1	Bijelac	10:58	12	16.58	55.08
2	Crnac	28:10	26	8.24	32.66
3	Prežba (NW)	11:25	27	1.88	19.48
6	Zaklopatica Bay	18:06	6	8.88	11.58
8	Lukovac Mali	31:29	21	2.18	14.13
9	Mrkjenta	28:15	20	2.61	9.35
10	Goli Rat	50:29	31	1	47.85
11	Pomeštak	30:58	34	3.34	50.38
12	Korizmeni Rat	28:20	10	15.48	62.62
13	Mljet (SW)	19:48	21	8.58	41.38
14	Gonoturska	12:27	15	3.21	51.78
15	Grabova	22:57	30	1.28	49.87

Invaded and non-invaded sites were grouped with respect to dominant substratum type (Table 4), in order to make a comparison between the sites with the same environmental conditions. The depth range of the group 'sandy substrate sites' was from 3.34 to 62.62 m. The 'rocky substrate sites' and the 'mixed-type substrate sites' (sand/rocks/gravel) depth ranges were from 1 to 47.85 m, and from 1.28 to 51.78 m, respectively.

Table 4. The groups of sites where underwater remote sensing survey was conducted. The sites were grouped according to substrate type. The number of the site is indicated in brackets. The sites invaded by *Caulerpa cylindracea* are shown in bold.

Sandy substrate sites	Rocky substrate sites	Mixed-type substrate sites
Prežba NW (3)	**Mrkjenta (9)**	**Lukovac Mali (8)**
Zaklopatica Bay (6)	**Crnac (2)**	**Gonoturska (14)**
Mljet SW (13)	Bijelac (1)	Grabova (15)
Pomeštak (11)	Goli Rat (10)	
Korizmeni Rat (12)		

3.2.2.2. *Video mosaics analysis*

Macrophytes and macroinvertebrates were identified to the lowest possible taxonomic level. The abundance was assessed using three categories: high, moderate and low. Abundance was considered to be *'low'* where species makes up only a small part of a community. A *'moderate'* level was assessed when a taxon is frequent but comprises less than half of the abundance of the community. A *'high'* level was assessed when the species exceeds half of the overall abundance, being clearly dominant (Olenin et al. 2007).

3.2.2.3. Visual assessment of fish assemblages

The abundance of 29 taxa (Annex 5) was assessed by a visual census at 12 sites (Figure 9; Table 2), which involves swimming along 30 m transect at constant speed and recording all target species seen within a 4 meter belt (total surface area=120 m^2). Transects were placed at a depth of approximately 10 m.

3.2.2.4. Sampling for stable isotope analysis

Macrophytes and macroinvertebrates were sampled manually at 12 sites (Figure 9; Table 2), along three transects (20 m of length) located at a depth of approximately10 meters, on a rocky substrate covered with photophilic macrophytes.

Six sites were invaded by *C. cylindracea*, five being located in MPA Lastovo Archipelago (2, 5, 6, 8 and 9) and one in MPA Mljet (14). Three control sites (no *C. cylindracea* present) were selected in MPA Lastovo Archipelago (1, 4 and 7), and three in MPA Mljet (11, 12 and 15) (Figure 9).

3.3. Stable isotope analysis

Samples were processed according to Levin and Currin (2012). Macroalgae were cleaned of epiphytes, animals were dissected and only the soft body tissues were taken for analysis. Fish tissue samples were taken from white muscle, with all skin and scales removed. All samples were rinsed in distilled water, oven dried (48 h at 60 °C), and homogenized by grinding. Samples of macrophytes were acidified by adding 10% HCl in order to remove inorganic carbonate.

Isotopic ratios were determined using continuous-flow isotope mass spectrometry (PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer) at the UC Davis stable isotope facility.

Carbon and nitrogen stable isotopic ratios were expressed as parts per mil deviations from the standards according to the following equation:

$$\delta X = \left[\left(R_{sample}/R_{reference}\right) - 1\right] \times 10^3$$

where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratio (Peterson and Fry 1987). The standard reference material was V-PDB (Vienna PeeDee Belemnite) and air for carbon and nitrogen, respectively. Standard deviations of the standard replicates were 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

3.4. Statistical analysis

3.4.1. Stable isotope data analysis

Differences in isotope values between sources, consumers and study sites were tested with PERMANOVA (Anderson, 2001) based on Euclidean distance (separately for $\delta^{13}C$ and $\delta^{15}N$ values). Multivariate analyses were performed using PRIMER v6 (Clarke and Gorley, 2006).

3.4.1.1. Trophic position assessment

Trophic positions (TP) were calculated by the equation proposed by Post (2002):

$$TP = 1 + (\delta^{15}N_{consumer} - \delta^{15}N_{baseline)})/2.3$$

The δ^{15}N values of primary producers were averaged to obtain the benthic isotopic baseline. Trophic enrichment factor (TEF) was assumed to be 2.3±0.28‰ (McCutchan et al. 2003).

3.4.1.2. Standard ellipse areas

On Lampedusa Island, the standard ellipse area (SEA) was used as a bivariate measure of the mean core isotopic niche of five trophic groups (Table 5) which contains 40% of the data, allowing robust comparison of datasets with different sample sizes. SEA_c (sample-size corrected version of the standard ellipse area) was used to avoid the bias induced by small sample size (Jackson et al. 2011). In MPA Lastovo Archipelago and MPA Mljet, SEA_c were used to compare the isotopic niches of invertebrates from sites invaded by *C. cylindracea* and non-invaded sites.

Table 5. Trophic groups based on previous studies (Deudero et al., 2011; Carefoot, 1991; Puccio et al., 2006).

Species	Trophic group	Species origin	Trophic group code
Aplysia dactylomela, *Percnon gibbesi*	Herbivores	NIS	NIH
Arbacia lixula, *Paracentrotus lividus*	Herbivores	Indigenous	IH
Chondrilla nucula, *Chondrosia reniformis*	Filter feeders	Indigenous	FLF

Species	**Trophic group**	**Species origin**	**Trophic group code**
Felimare picta	Benthic invertebrate feeders	Indigenous	BIF
Diplodus annularis, Serranus scriba, Spondyliosoma cantharus	Omnivorous/carnivorous fishes	Indigenous	BIF/FF/O

3.4.1.3. Bayesian mixing models

On Lampedusa Island, probability distributions for the contributions of the sources to the diet of indigenous and non-indigenous herbivores were estimated using the Bayesian stable isotope mixing model (MixSIAR). Sources were included in the model following previous studies on the diet of the species under study (Boudouresque and Verlaque 2001, Carefoot et al. 2000; Mannino et al. 2014; Puccio et al. 2006).

The models were run for *Paracentrotus lividus, Arbacia lixula* and *P. gibbesi* using seven most abundant macrophytes: *Halopteris scoparia, Dictyota* sp., *Posidonia oceanica, Padina pavonica, Cystoseira* sp., *C. cylindracea* and *A. taxiformis.* Stable isotope values of *Cystoseira* sp., *Dictyota* sp. and *Halopteris scoparia*, that belong to the class Phaeophyceae, were combined in order to reduce the number of sources (Phillips et al. 2005).

In addition to the most abundant sources, *Laurencia* sp. was included in the model for *A. dactylomela* based on observations reported in Mannino et al. (2014) and personal observation during the collecting of samples in this study. As well as *A. dactylomela, Laurencia* sp. was present only at Cala Maluk.

In MPA Lastovo Archipelago and MPA Mljet, the Bayesian stable isotope mixing models were used for estimation of probability distributions for the contributions of the sources to the diet of sea urchins. The models were run for *Paracentrotus lividus, Arbacia lixula* and *Sphaerechinus granularis* using the six most abundant macrophytes in the study sites: *Amphiroa rigida, Codium bursa, Cystoseira* sp., *Halimeda tuna, Padina pavonica, Posidonia oceanica.* In addition to the six most abundant macrophytes, *C. cylindracea* was included in the mixing models for invaded sites.

In both study areas, all sources used in mixing models had significantly different isotope values. TEF was assumed to be 1.3±0.3‰ for $\delta^{13}C$ and 2.2±0.3‰ for $\delta^{15}N$ (McCutchan et al. 2003). We ran length "long" for the number of Monte Carlo Markov Chain simulations. Mixing models were calculated using MixSIAR GUI and standard ellipse areas were calculated using SIBER (Stock and Semmens 2013; Jackson et al. 2011) in R statistical software (R CoreTeam, 2015).

3.4.2. Community data analysis

Differences in assemblage structure between the sites were assessed using the Bray-Curtis similarity matrices followed by non-metric multi-dimensional scaling (MDS) ordination and ANOSIM (Clarke and Warwick 1994). Differences in species richness were tested with PERMANOVA (Anderson, 2001). While both statistical tests use permutation in order to obtain p value, R test statistics is obtained by ANOSIM, while the pseudo-F test statistics is computed by PERMANOVA. When the differences in macrophyte assemblages between the sites invaded by *C. cylindracea* and non-invaded sites were assessed, *C. cylindracea* was excluded from the analysis.

Macrophyte, macroinvertebrate and fish taxa abundance data from Lampedusa, and fish abundance data from Croatian MPAs, was square root-transformed prior to analysis. To examine the contributions of individual taxa to the differences between the sites, SIMPER (similarity percentages) analysis (Clarke, 1993) was also carried out (the cut off for low contributions was set at 90%).

All multivariate analyses were conducted using PRIMER v6 (Clarke and Gorley, 2006).

3.5. Summary of the materials and methods

In total, four different menthods were used in this study, which provided 316 samples surveyed by rapid assessment methods, 253 assessment units obtained by underwater remote sensing, and 433 samples selected for SIA (Table 6).

Table 6. Sampling methods and numbers of samples obtained in each study area.

Study area	Rapid assessments		Underwater remote sensing	Sampling for stable isotope analysis
	Underwater visual census	Mooring lines sampling		
Lampedusa Island (MPA Pelagie Islands)	120 quadrats; 48 transects			149 samples
Eastern Adriatic coast		81 line		
MPAs Lastovo Archipelago and Mljet	36 transects	31 line	253 assessment units	284 samples

4. Results

4.1. Case study 1: Lampedusa Island (inside and outside of Marine Protected Area Pelagie Islands)

4.1.1. Assessment of the benthic community and non-indigenous species abundance and distribution on Lampedusa Island

4.1.1.1. Macrophytes assemblages

The substrate at three study sites was mainly rocky and not completely colonised by algae. *Posidonia oceanica* meadows were mainly inhabiting sandy bottoms. The macrophyte assemblages were dominated by *H. scoparia* (24.1±25.8% per m^2), *Dictyota* sp. (14.9±23.7% per m^2), *P. oceanica* (11.7±28.9% per m^2) and *P. pavonica* (11.4±19% per m^2), followed by *Cystoseira* sp. (2.4±7.1% per m^2).

The cover of the two NIS, *C. cylindracea* and *A. taxiformis,* was 1.7±3.4% and 0.7±5.8%, respectively (Figure 10; Table 7). PERMANOVA test showed that there were no significant differences in *C. cylindracea* and *A. taxiformis* cover between the study sites (pseudo-F=1.5172; p(perm)=0.221 and pseudo-F=0.82462; p(perm)=0.506, respectively).

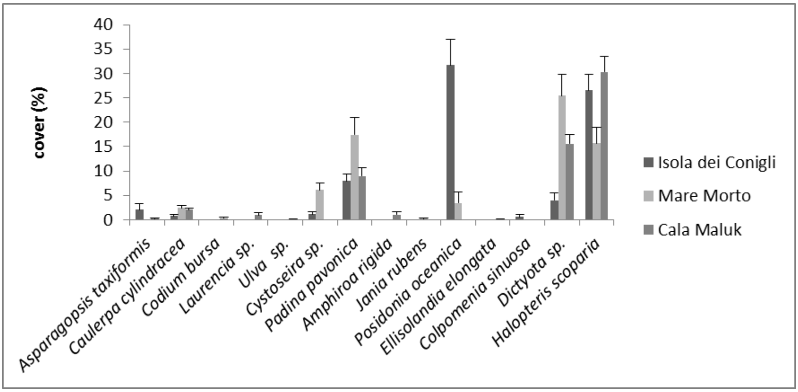


Figure 10: Mean abundances (±SE) of macrophyte taxa (target taxa are listed in Annex 1) recorded at Isola dei Conigli, Mare Morto and Cala Maluk.

Table 7. Macrophyte taxa percent cover (per m^2) at Isola dei Conigli, Mare Morto and Cala Maluk.

Taxa	Isola dei Conigli		Mare Morto		Cala Maluk		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chlorophyta								
Caulerpa cylindracea	0.8	1.9	2.4	4.55	2.0	3.1	1.7	3.4
Codium bursa	-	-	-	-	0.1	1	0.1	0.9
Ulva sp.	-	-	-	-	0.1	0.5	0.02	0.3
Ochrophyta								
Colpomenia sinuosa	0.5	4.1	-	-	-	-	0.2	2.4
Cystoseira sp.	1.1	3.7	6.1	10.9	-	-	2.4	7.1
Dyctiota sp.	4.0	11.6	25.4	33.7	15.5	14.5	14.9	23.7
Halopteris scoparia	26.5	25.9	15.7	25.5	30.3	24.3	24.1	25.8
Padina pavonica	7.9	11	17.4	27.5	8.9	12.9	11.4	19
Rhodophyta								
Amphiroa rigida	-	-	-	-	1.0	4.4	0.4	2.6
Asparagopsis taxiformis	2.0	10.1	-	-	0.1	1	0.7	5.9
Ellisolandia elongata	-	-	-	-	0.1	0.5	0.0	0.3

Taxa	**Isola dei Conigli**		**Mare Morto**		**Cala Maluk**		**Total**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Jania rubens	-	-	0.1	1	-	-	0.0	0.6
Laurencia sp.	-	-	-	-	0.9	4.4	0.3	2.6
Tracheophyta								
Posidonia oceanica	31.7	40.1	3.4	18.1	-	-	11.7	28.9

Although the global one-way ANOSIM test was significant (p = 0.1%), the low value of R (0.211) and nMDS (Figure 11) indicated low dissimilarity between the three study sites. All pairwise tests were likewise significant (all p=0.1%), but they had a low R value (<0.275). SIMPER analysis revealed that the average Bray–Curtis dissimilarity was the highest between Isola dei Conigli and Mare Morto (74.15%). *Posidonia oceanica* contributed the most (22.46%) to the dissimilarity between the sites. Similarly, *P. oceanica* contributed the most (22.87%) to the average dissimilarity between Isola dei Conigli and Cala Maluk (66.7%). SIMPER showed that the lowest dissimilarity was between Mare Morto and Cala Maluk (65.56%), where the native alga *H. scoparia* showed the highest contribution to dissimilarity.

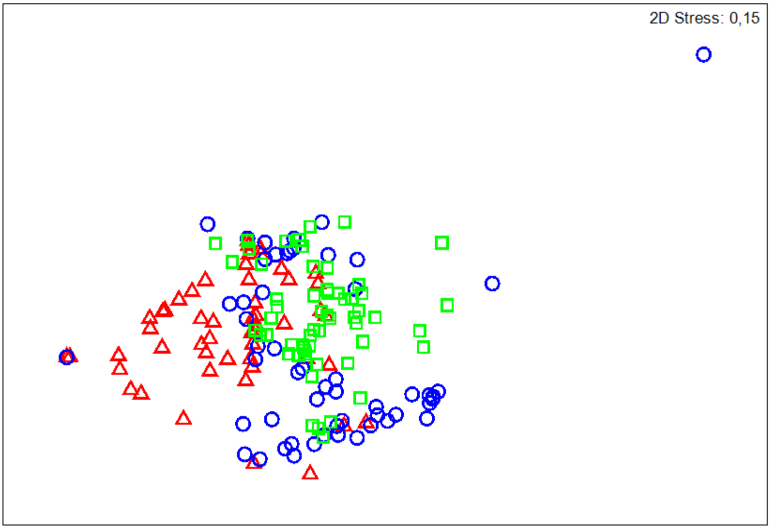


Figure 11. Non-metric MDS plot based on Bray-Curtis similarity of square root-transformed macrophytes cover data. The study sites are represented by different symbols: Isola dei Conigli - triangle, Mare Morto - circle and Cala Maluk - square.

4.1.1.2. Macroinvertebrates assemblages

The sea urchin *Arbacia lixula* was the most abundant invertebrate taxa with an average abundance of 37.7±29.9 per 120m^2. The species had the highest abundance at Cala Maluk (46.8±19.4 per 120m^2). The sea anemone *Anemonia viridis* was the second most abundant species, with 24.2±34.2 per 120m^2. The sea urchin *Paracentrotus lividus* was the next most abundant taxa (6.2±10.8 per 120m^2) (Figure 12).

Percnon gibbesi was common at all sites, with a mean abundance of 1.5±0.7 per 120 m^2. At Isola dei Conigli, the abundance of *P. gibbesi* was the highest (15.7±19.7 per 120m^2) (Figure 12; Table

8). There was no significant difference in *P. gibbesi* abundance between the study sites (pseudo-F=0.45297; p(perm)=0.648). *Aplysia dactylomela* was present only at Cala Maluk, where eight individuals were observed.

Table 8. Abundances of macroinvertebrate taxa (mean number of individuals per 120 m^2; target taxa listed in Annex 2) at Isola dei Conigli, Mare Morto and Cala Maluk.

Taxa	**Isola dei Conigli**		**Mare Morto**		**Cala Maluk**		**Total**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Actinia equina	-	-	-	-	1.3	0.6	1.3	0.6
Aiptasia sp.	3.0	0.0	-	-	11.3	7.5	9.3	7.4
Anemonia viridis	4.0	0.0	10.0	0.0	32.8	40.6	24.2	34.2
Aplysia dactylomela	-	-	-	-	3.5	0.7	3.5	0.7
Arbacia lixula	22.3	9.3	44.9	47.4	46.8	19.4	37.7	29.9
Chiton sp.	2.5	0.7	-	-	10.3	7.8	7.7	7.3
Chondrosia reniformis	2.0	-	1.0	0.0	-	-	1.0	0.0
Echinaster sepositus	-	-	1.0	0.0	-	-	4.7	3.8
Hermodice carunculata	-	-	4.7	3.8	-	-	2.8	1.5
Holoturia sp.	2.5	0.7	2.8	1.5	2.8	1.9	3.5	1.6
Ircinia sp.	2.7	2.9	3.6	1.5	4.0	1.7	1.6	0.8

Taxa	Isola dei Conigli		Mare Morto		Cala Maluk		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pachygrapsus marmoratus	2	-	-	0.0	1.4	0.9	5.9	3.3
Paracentrotus lividus	3.5	1.9	7.7	3.1	6.3	3.4	6.3	10.8
Percnon gibbesi	15.7	19.7	3.7	1.5	2.0	1.7	1.5	0.7

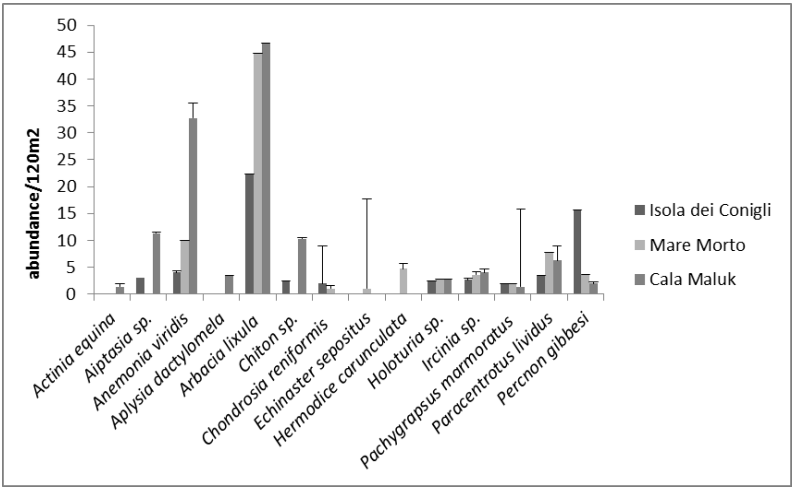


Figure 12. Mean abundances (±SE) of macroinvertebrate taxa at Isola dei Conigli, Mare Morto and Cala Maluk.

The one-way ANOSIM test and nMDS (Figure 13) showed that there were no significant differences between the three study sites (R = 0.106, P = 1.8%).

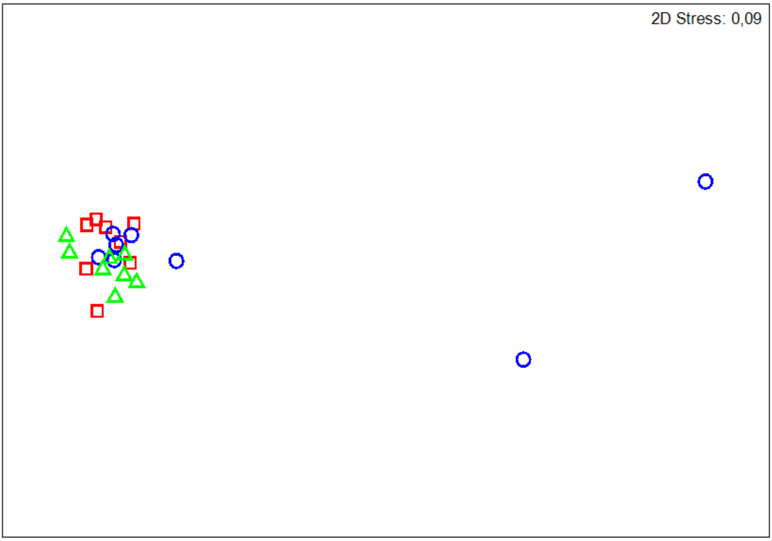


Figure 13. Non-metric MDS plot based on Bray-Curtis similarity of square root-transformed invertebrate abundance data. The study sites are represented by different symbols: Isola dei Conigli - triangle, Mare Morto - circle and Cala Maluk - square.

4.1.1.3. Fish assemblages

Sarpa salpa was the most abundant species with the average abundance of 16.7±34.8 per 120m^2. The second most abundant species was *Thalassoma pavo* (6.3±4.3 per 120m^2), followed by *Diplodus vulgaris* (3.3±4.2 per 120m^2). The non-indigenous fish *Siganus luridus* was recorded at Mare Morto, where 2 individuals were observed (Figure 14; Table 9).

Table 9. Abundances of fish taxa (mean number of individuals per 120 m^2) at Isola dei Conigli, Mare Morto and Cala Maluk.

Taxa	Isola dei Conigli		Mare Morto		Cala Maluk		Total	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Coris julis	3.3	2.3	1.3	2.3	4.6	6.6	2.6	4.1
Diplodus annularis	2.7	2.1	0.7	1.2	2.6	2	1.8	1.9
Diplodus puntazzo	-	-	0.1	0.2	-	-	0.0	0.2
Diplodus sargus	3.1	3.3	0.3	0.7	3.3	2.3	1.1	2
Diplodus vulgaris	6.3	6	2.7	4.4	3.6	2.8	3.3	4.2
Labrus sp.	1.0	-	-	-	-	-	0.0	0.2
Mullus sp.	2.0	0.0	0.1	0.4	1.5	0.7	0.3	0.6
Oblada melanura	1.3	0.5	0.3	0.9	1.0	0.0	0.5	0.9
Sarpa salpa	15.4	16.4	16.8	46.7	17.9	15.8	16.7	34.8
Seriola fasciata	1.0	0.0	-	-	-	-	0.0	0.2
Serranus cabrilla	1.0	0.0	0.1	0.3	1.3	0.5	0.3	0.5
Serranus scriba	1.2	0.4	0.4	0.7	1.4	0.7	0.7	0.8
Siganus luridus	-	-	0.1	0.3	-	-	0.1	0.3
Sparisoma cretense	3.7	1.4	1.2	2.1	6.4	9.5	2.4	3.9
Sparus aurata	1.0	0.0	0.1	0.2	1.0	0.0	0.2	0.4
Spicara smaris	1.0	0.0	1.3	4.9	19.7	26.5	3.1	9.9

Taxa	Isola dei Conigli		Mare Morto		Cala Maluk		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Spondyliosoma cantharus	1.0	0.0	-	-	3.5	3.4	0.6	1.9
Symphodus sp.	2.8	2.3	3	2	5.6	2.8	3.8	2.6
Thalassoma pavo	4.4	3.3	6.3	4.4	7.9	4.4	6.3	4.3

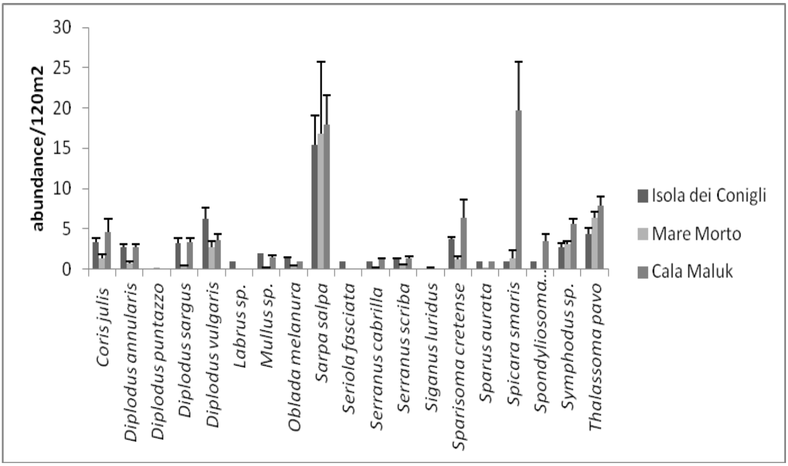


Figure 14: Mean abundances (±SE) of fish taxa recorded at Isola dei Conigli, Mare Morto and Cala Maluk.

Similarly to the macrophytes assemblages, the ANOSIM test was significant (p = 0.1%), but the low value of R (0.141) indicated low separation between the three study sites (Figure 15). Pairwise tests showed that there was no significant dissimilarity between Mare Morto and Cala Maluk (R=0.05; p=9.5%), while the difference between Isola dei Conigli and Mare Morto was significant (p=0.1), but with a low R value (0.215). Similarly, the difference between Isola dei Conigli and Cala Maluk was also significant (p=0.4), with a low R value (0.16).

SIMPER analysis revealed that the average Bray–Curtis dissimilarity was the highest between Isola dei Conigli and Mare Morto (65.64%). *Sarpa salpa* contributed the most to the dissimilarity between the sites in all three cases (from 17.76% to 21.51%). The average dissimilarity between Isola dei Conigli and Cala Maluk was 62.16%, and between Mare Morto and Cala Maluk 57.94%.

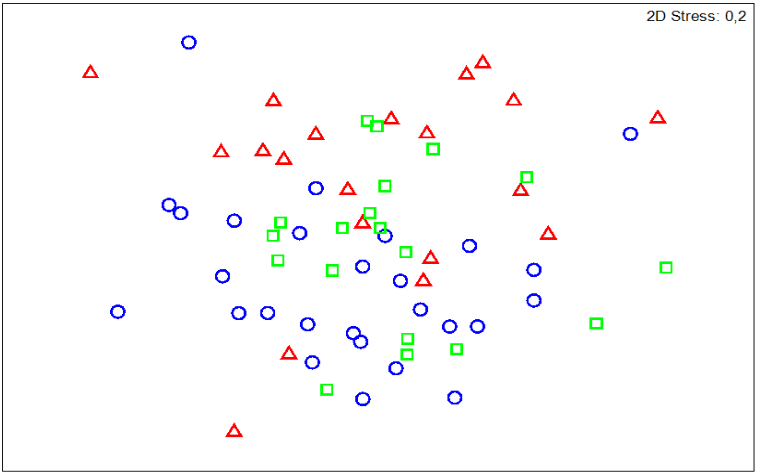


Figure 15. Non-metric MDS plot based on Bray-Curtis similarity of square root-transformed fish abundance data. The study sites are represented by different symbols: Isola dei Conigli - triangle, Mare Morto - circle and Cala Maluk - square.

4.1.2. Trophic interactions between indigenous and non-indigenous species from Lampedusa Island

4.1.2.1. Carbon and nitrogen stable isotope values and trophic positions

Stable isotope values of each species did not differ between the sites (all p(perm)>0.05). Therefore, the samples were pooled per species for further analyses (Table 10). Moreover, when all stable isotope values were pooled, they did not differ between the sites (pseudo-F=0.342, p(perm)=0.799).

Mean $\delta^{13}C$ values of macrophyta ranged from -8.4‰ (*Cymodocea nodosa*) to -31.9‰ (*A. taxiformis*). The red alga *Laurencia* sp. had the highest $\delta^{15}N$ values (6.6‰) and *Peyssonnelia* sp. the lowest $\delta^{15}N$ values (0.7‰).

Mean $\delta^{13}C$ values of invertebrates ranged from -20.6‰ (*Felimare picta*) to -11.7‰ (*Arbacia lixula*). The nudibranch mollusc *F. picta* had the most enriched $\delta^{15}N$ values among the invertebrates analysed (6.9‰) and the sponge *Chondrosia reniformis* (3.2‰) was the most depleted in $\delta^{15}N$ values. $\delta^{15}N$ values of *P. gibbesi* and *A. dactylomela* were 6.2‰ and 6.1‰, respectively. They were more enriched than those of the indigenous herbivores *A. lixula* (4.8‰) and *P. lividus* (4.4‰).

Carnivorous and omnivorous fishes had considerably higher $\delta^{15}N$ values than invertebrates (from 8.5‰ to 10‰), thus having the highest trophic positions among the sampled fauna. *P. gibbesi* and *A. dactylomela* had the same trophic position (2.6±1.7), while the trophic positions of *A. lixula* and *P. lividus* were lower (2±1.9 and 1.8±1.7, respectively) (Figure 16; Table 10).

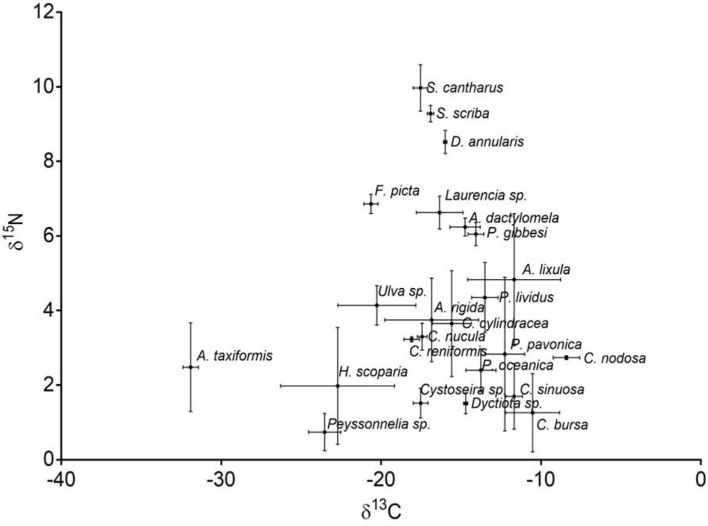


Figure 16. Mean values (± SD) of stable isotope values of carbon and nitrogen of macrophyta, invertebrates and fish in Lampedusa Island. Corresponding number of specimens analysed: see Table 10.

Table 10. Species names, number of replicates (pooled), sites where species were sampled: Isola dei Conigli (1), Cala Maluk (2), and Mare Morto (3), trophic group codes, stable-nitrogen and stable-carbon isotope ratios (mean and SD) and corresponding trophic positions (TP) (mean and SD) of primary producers, invertebrates and fish. Trophic group codes are: primary producer = PP, filter-feeder = FLF, non-indigenous herbivore = NIH, indigenous herbivore= IH, benthic invertebrate feeder = BIF, omnivore=O, fish feeder = FF. Trophic groups are based on previous studies (Deudero et al. 2011; Carefoot, 1991; Puccio et al. 2006). Trophic positions (TP) were determined using average $\delta^{15}N$ values of benthic primary producers as isotopic baseline (2.47±1.70) (see section 2. Materials and methods). Standard deviations (SD) of TP were determined by first-order error propagation of uncertainties:

$SD_{combined} = \sqrt{SD_1^2 + SD_2^2}$, where SD_1 is the standard deviation of TP of consumer, and SD_2 is the standard deviation of isotopic baseline.

Taxa	**N**	**Site**	**Trophic group code**	**δ13C**		**δ15N**		**TP**	
				mean	**SD**	**mean**	**SD**	**mean**	**SD**
Chlorophyta									
Caulerpa cylindracea	10	1,2,3	PP	-15.6	1.2	3.7	1.4		
Codium bursa	10	1,2,3	PP	-10.5	1.7	1.3	1.1		
Ulva sp.	6	1,2	PP	-20.3	2.4	4.1	0.5		
Ochrophyta									
Colpomenia sinuosa	7	1,3	PP	-11.7	0.5	1.7	0.9		
Cystoseira sp.	6	2,3	PP	-17.5	0.5	1.5	0.4		
Dictyota sp.	7	2,3	PP	-14.7	0.1	1.5	0.3		
Halopteris scoparia	9	1,2,3	PP	-22.7	3.6	2	1.6		
Padina pavonica	8	1,2,3	PP	-12.3	1.3	2.8	2.1		
Rhodophyta									
Amphiroa rigida	3	2	PP	-16.8	2.9	3.8	1.1		
Asparagopsis taxiformis	8	1,2,3	PP	-31.9	0.5	2.5	1.2		

Taxa	N	Site	Trophic group code	$\delta^{13}C$		$\delta^{15}N$		TP	
				mean	SD	mean	SD	mean	SD
Laurencia sp.	3	2	PP	-16.3	1.5	6.6	0.4		
Peyssonnelia sp.	6	1,3	PP	-23.5	1	0.7	0.5		
Tracheophyta									
Cymodocea nodosa	3	2	PP	-8.4	0.8	2.7	0.1		
Posidonia oceanica	10	1,2,3	PP	-13.8	0.9	2.4	0.6		
Porifera									
Chondrilla nucula	4	3	FLF	-17.4	0.3	3.3	0.4	1.4	1.7
Chondrosia reniformis	3	1	FLF	-18.1	0.5	3.2	0.1	1.3	1.7
Mollusca									
Aplysia dactylomela	8	2	NIH	-14.1	0.5	6.1	0.3	2.6	1.7
Felimare picta	4	3	BIF	-20.6	0.4	6.9	0.3	2.9	1.7
Arthropoda									
Percnon gibbesi	4	1,3	NIH	-14.8	1	6.2	0.2	2.6	1.7
Echinodermata									
Arbacia lixula	7	1,2,3	IH	-11.7	2.9	4.8	1.8	2	1.9

Taxa	N	Site	Trophic group code	δ13C		δ15N		TP	
				mean	SD	mean	SD	mean	SD
Paracentrotus lividus	5	2,3	IH	-13.5	0.8	4.4	0.9	1.8	1.7
Chordata									
Diplodus annularis	3	1,3	BIF	-16	0.1	8.5	0.3	3.6	1.7
Serranus scriba	12	1,2,3	FF/BIF	-16.9	0.2	9.3	0.2	4	1.7
Spondylioso-ma cantharus	3	1,2	O	-17.5	0.5	10	0.6	4.3	1.7

4.1.2.2. Trophic groups

The PERMANOVA pair-wise test showed significant variations in the isotopic composition between all *a priori* established trophic groups (Table 11).

Table 11. Results of PERMANOVA pair-wise tests for trophic groups, after 9999 permutations. Trophic groups are defined in Table 5.

		δ13C		δ15N	
Trophic group codes	**df**	**t**	**p (perm)**	**t**	**p (perm)**
NIH, IH	21	3.5057	0.002	3.2224	0.004
NIH, FLF	15	9.4779	0.001	19.521	0.001
NIH, BIF	14	16.458	0.001	4.5281	0.002
NIH, BIF/FF/O	22	12.205	0.001	30.133	0.001

		$\delta^{13}C$		$\delta^{15}N$	
Trophic group codes	**df**	**t**	**p (perm)**	**t**	**p (perm)**
IH, FLF	14	5.8445	0.001	2.3139	0.034
IH, BIF	13	7.9659	0.002	2.8827	0.017
IH, BIF/FF/O	21	8.0368	0.001	11.052	0.001
FLF, BIF	7	8.7713	0.008	22.75	0.009
FLF, BIF/FF/O	15	4.4861	0.001	51.701	0.001
BIF, BIF/FF/O	14	23.857	0.002	18.146	0.001

IH had the largest isotopic trophic niche of the five groups (6.53) followed by FLF (4.73). SEA_c of NIH (0.62) was similar to that of BIF/FF/O (0.71). There was no overlap between the isotopic niches of the groups (Figure 17).

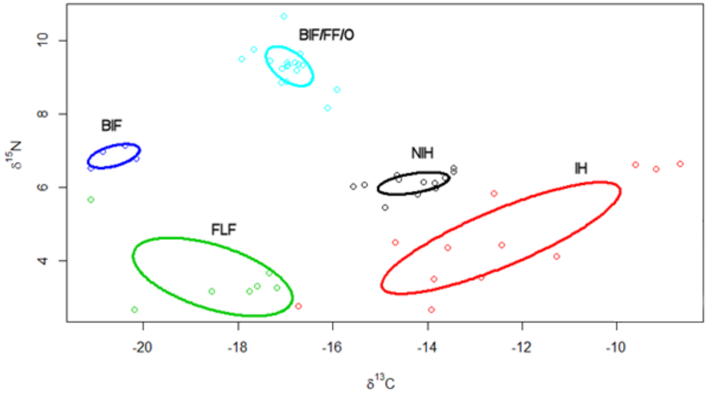


Figure 17. Stable isotope bi-plot illustrating the isotopic niches. Lines enclose standard ellipse areas (SEA_c) for the five trophic groups: NIH (black), IH (red), FLF (green), BIF (blue) and BIF/FF/O (cyan).

4.1.2.3. Mixing models

Bayesian mixing models indicated that *C. cylindracea* had the largest contribution to the diet of *P. gibbesi* (0.431-1), while the proportional contributions of the remaining sources ranged between 0 and 0.383, but overlapped considerably. The dietary contribution estimates overlapped considerably for all sources of *A. dactylomela, A. lixula* and *P. lividus* (Table 12).

Table 12. Median (±SD) indigenous and non-indigenous herbivores diet proportion and 95% credible interval (2.5% and 97.5% quantiles, in brackets) estimated using the Bayesian stable isotope mixing model (MixSIAR).

	A. dactylomela	***P. gibbesi***	***A.lixula***	***P. lividus***
A. taxiformis	0.013±0.018 (0,0.064)	0.013±0.027 (0,0.094)	0.006±0.014 (0,0.051)	0.017±0.031 (0,0.108)
C. cylindracea	0.151±0.229 (0,0.911)	0.906±0.158 (0.431,1.000)	0.020±0.046 (0,0.171)	0.062±0.159 (0,0.579)
Cystoseira* sp./ *Dictyota* sp./ *H. scoparia	0.033±0.049 (0,0.170)	0.017±0.045 (0,0.164)	0.053±0.178 (0,0.996)	0.044±0.100 (0,0.340)
P. pavonica	0.052±0.076 (0,0.261)	0.037±0.099 (0,0.383)	0.885±0.242 (0,1.000)	0.117±0.235 (0,0.819)
P. oceanica	0.438±0.181 (0,0.682)	0.027±0.064 (0,0.226)	0.037±0.159 (0,0.777)	0.760±0.346 (0,1.000)
***Laurencia* sp.**	0.313±0.104 (0,0.458)	n/a	n/a	n/a

4.2. Case study 2: eastern Adriatic Sea

4.2.1. Rapid assessment of marine non-indigenous species on mooring lines of leisure craft

Four NIS were found during the survey: the serpulid *Hydroides elegans* (Haswell, 1883), the bryozoans *Amathia verticillata* (Delle Chiaje, 1822) and *Celleporaria brunnea* (Hinks, 1884), and the tunicate *Styela plicata* (Leseur, 1823). Three cryptogenic species were also recorded: the barnacle *Amphibalanus amphitrite* (Darwin, 1854), the amphipod *Elasmopus rapax* Costa, 1853 and the bryozoan *Watersipora subtorquata* (d'Orbigny, 1852). The latter belongs to a problematic genus, which has undergone several historical misidentifications; hereby it was identified following the most recent morphometric and morphological taxonomic key provided by Vieira et al. (2014). All NIS and cryptogenic species were recorded in Biograd na Moru. In addition, *S. plicata* was also recorded in Zadar. No NIS or cryptogenic species were found in Rogoznica, Pasadur, Ubli and Pomena.

Filter-feeding fauna dominated the fouling community on 'lazy' lines at mainland sites. Bryozoans, tubeworms and tunicates were the most abundant taxa. Native bryozoan species included *Aetea truncata* (Landsborough, 1852), *Amathia gracilis* (Leidy, 1855), *Bugula neritina* (Linnaeus, 1758), *Cryptosula pallasiana* (Moll, 1803) and *Schizoporella errata* (Waters, 1878). *Bugula neritina* and *S. errata* were dominant taxa at Rogoznica Quay.

Native species of tubeworms included *Hydroides pseudouncinatus* Zibrowius, 1968, *Serpula concharum* Langerhans, 1880, *Serpula vermicularis* Linnaeus, 1767, *Spirobranchus* *polytrema* (Philippi, 1884), *Spirobranchus triqueter* (Linnaeus, 1758), *Simplaria pseudomilitaris* (Thiriot – Quievreux, 1965) and *Janua* sp. (Saint-Joseph, 1894). Other polychaete families were present: Sabellidae, Syllidae, Terebellidae and Nereididae. Bivalves included *Mytilus galloprovincialis* Lamarck, 1819, *Hiatella arctica* (Linnaeus, 1767), *Mytilaster* sp. Monterosato, 1884, *Modiolus barbatus* (Linnaeus, 1758), *Musculus* sp. Röding, 1798 and *Ostrea edulis* Linnaeus, 1758. Samples also contained crustaceans: *Apocorophium acutum* (Chevreux, 1908), *Dynamene edwardsi* (Lucas, 1849), *Leptochelia savignyi* (Krøyer, 1842) and *Zeuxo* sp. Templeton, 1840. A single juvenile of *Caprella* sp. was also found.

The only NIS that could be recognised in the field with reasonable certainty, for which visual abundance estimation was performed, were *S. plicata* and *A. verticillata*. The tunicate *S. plicata* was seen within two marinas on the Croatian mainland, in Biograd na Moru and Zadar, and was found on the majority of examined 'lazy' lines (80% and 97%, respectively), with low or moderate abundance (Table 13). Other tunicate taxa obtained in the Biograd na Moru and Zadar marinas included *Clavelina* sp. Savigny, 1816, *Didemnum* sp. Savigny, 1816, *Diplosoma* sp. Macdonald, 1859 and *Microcosmus* sp. Heller, 1877. No tunicates were found at offshore sites and Rogoznica Quay.

Table 13. The numbers of lines with 'low', 'moderate' and 'high' abundances of *A. verticillata* and *S. plicata*.

	Biograd na Moru *A. verticillata*	Biograd na Moru *S. plicata*	Zadar *S. plicata*
Low	1	25	25
Moderate	2	7	4
High	1	0	0
Total No. of lines examined	40	40	30

Amathia verticillata was confined to the inner sheltered part of the marina in Biograd na Moru occurring on 10% of the examined lines, in low to high abundances (Table 13). Colonies were dark green due to the presence of a film of diatoms and sediment. It was likewise present on the hull of several craft at the same location.

While algae were rare at mainland sites, Dictyotales dominated the fouling community at three offshore sites. Among them, *Padina pavonica* (Linnaeus) Thivy, 1960 was the most common species, followed by *Dictyota* sp. The green alga *Acetabularia acetabulum* (Linnaeus) P.C. Silva, 1952 and the red encrusting coralline algae were also common.

Fouling fauna at offshore sites included the bryozoans *Crisia* sp. Lamouroux, 1812, *Cradoscrupocellaria bertholletii* (Audouin, 1826), *B. neritina*, *A. gracilis* and *S. errata*, polychaetes *S. pseudomilitaris*, the bivalves *M. barbatus* and *M. galloprovincialis*, and the crustaceans *Caprella* sp. and *L. savignyi*.

4.2.2. The role of *Caulerpa cylindracea* in macrophyte and macroinvertebrate assemblages in Marine Protected Areas Lastovo Archipelago and Mljet

Out of a total of 12 locations, seven locations (five in MPA Lastovo Archipelago, two in MPA Mljet) were colonized by *C. cylindracea* (Table 14). *C. cylindracea* abundance was significantly different between three groups of sites with different substrate types (pseudo-F=28.059, p(perm)=0.001). Sandy substrate sites had the highest *C. cylindracea* abundance, followed by mixed-type substrate sites (Tables 15, 17 and 19).

Table 14. The upper and lower depth limits (m) and the numbers of assessment units with 'low', 'moderate' and 'high' abundances of *C. cylindracea.*

Site No.	Sites	Substrate type	*Caulerpa cylindracea* upper and lower depth limits (m)	Low	Moderate	High
1	Bijelac	rocky	-	-	-	-
2	Crnac	rocky	5-20	12	3	-
3	Prežba (NW)	sandy	7	8	12	8
6	Zaklopatica Bay	sandy	8-10	2	1	3
8	Lukovac Mali	mixed	6-10	3	2	-
9	Mrkjenta	rocky	6-27	9	6	-
10	Goli Rat	rocky	-	-	-	-

Site No.	Sites	Substrate type	*Caulerpa cylindracea* upper and lower depth limits (m)	Low	Moderate	High
11	Pomeštak	sandy	-	-	-	-
12	Korizmeni Rat	sandy	-	-	-	-
13	Mljet (SW)	sandy	10-41	7	13	-
14	Gonoturska	mixed	10-30	8	4	1
15	Grabova	mixed	-	-	-	-

4.2.2.1. Sandy substrate sites

At the sandy substrate sites, a community of coastal detritus bottoms and *P. oceanica* patches was present.

C. cylindracea was present in 51 out of a total of 55 assessment units from the invaded sites where sand was the dominant substrate (Zaklopatica Bay, Prežba NW and Mljet SW). Other frequent macrophyte taxa at invaded sites were *H. scoparia* and *Osmundaria volubilis*, while turf algae, *H. tuna*, *P. oceanica* and maerl had the highest occurrence in assessment units from the non-invaded sites (Pomeštak and Korizmeni Rat) (Table 15).

Table 15. The numbers of assessment units with 'low', 'moderate' and 'high' abundances of macrophytes and macroinvertebrate taxa at invaded and non-invaded sites with dominant sandy substrate

Taxa	Invaded			Non-invaded		
	low	moderate	high	low	moderate	high
Chlorophyta						
Acetabularia acetabulum	4	-	-	1	-	-
Caulerpa cylindracea	15	24	10	-	-	-
Codium bursa	-	-	-	1	-	-
Halimeda tuna	-	-	-	4	5	-
Ochrophyta						
Halopteris scoparia	11	11	1	-	-	-
Padina pavonica	2	-	-	-	-	-
Rhodophyta						
Amphiroa rigida	3	-	-	-	-	-
Lithophyllum sp.	-	-	-	4	-	-
Peyssonnelia sp.	-	-	-	3	-	-
Osmundaria volubilis	2	7	9	-	-	-
maerl	-	-	-	2	3	2
Macroalgae functional group						
Turf algae	4	1	1	4	7	1
Tracheophyta						

Taxa	Invaded			Non-invaded		
	low	moderate	high	low	moderate	high
Posidonia oceanica	4	-	-	3	3	4
Cnidaria						
Eunicella cavolini	-	-	-	1	-	-
Echinodermata						
Echinaster sepositus	-	-	-	2	-	-

The global one-way ANOSIM test showed significant variations in macrophyte assemblages between invaded and non-invaded sites (R=0.245; p = 0.1%) (Figure 18).

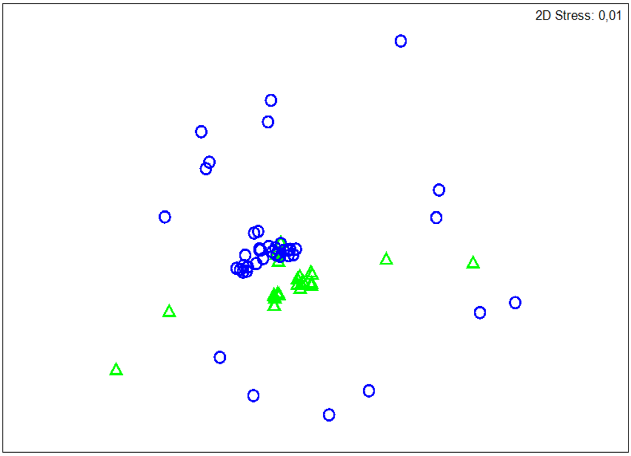


Figure 18. Non-metric MDS of sandy substrate sites based on untransformed macrophytes abundance data per assessment unit. Invaded sites are represented by triangles (Zaklopatica Bay, Prežba NW and Mljet SW) and non-invaded sites by circles (Pomeštak and Korizmeni Rat).

Similarity Percentages analysis (SIMPER) of macrophyte assemblages indicated an average dissimilarity of 98.08% between invaded and non-invaded sites. *O. volubilis* contributed most to these differences (25.91%), followed by *H. scoparia* (25.27%) (Table 16).

The brown alga *H. scoparium* had the largest contribution to the similarity between invaded sites (52.21%), followed by *O. volubilis* (43.89%). The average similarity was 25.37%.

The average similarity in the non-invaded sites was 9.47%. The following species accounted for the similarities: turf algae (29.30%), *H. tuna* (23.89%), *P. oceanica* (23.47%) and maerl (19.26%).

Table 16. Percentage contribution of the taxa/functional group contributing the most to the Bray–Curtis dissimilarity between non-invaded and invaded sandy substrate sites.

Taxa/functional group	Contribution (%)
Osmundaria volubilis	25.91
Halopteris scoparium	25.27
turf algae	13.99
Posidonia oceanica	9.93
maerl	8.28
Halimeda tuna	7.92

ANOSIM and MDS plot indicated that macroinvertebrate assemblages did not differ between invaded and non-invaded sandy substrate sites (R=0.008; p = 9.9%) (Figure 19).

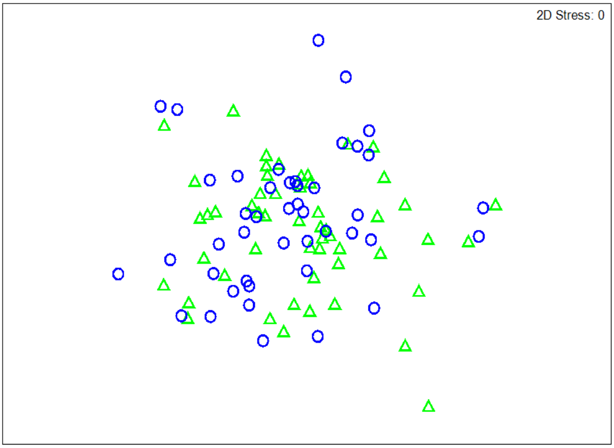


Figure 19. Non-metric MDS of sandy substrate sites based on untransformed macroinvertebrates abundance data per assessment unit. Invaded sites are represented by triangles (Zaklopatica Bay, Prežba NW and Mljet SW) and non-invaded by circles (Pomeštak and Korizmeni Rat).

4.2.2.2. Rocky substrate sites

The sites with a rocky substrate were characterized by the infralittoral photophilic algae community, coralligenous community and patches of *P. oceanica*. At the invaded sites (Crnac and Mrkjenta), *C. cylindracea* was present in 28 out of a total of 46 assessment units. The turf algae, *P. pavonica* and *C. cylindracea* were the most common algae at the invaded sites. The most common macrophyte taxa at non-invaded sites (Bijelac and Goli rat) were *P. oceanica* and *P. pavonica* (Table 17).

The cnidarian *Eunicella cavolini* was the most abundant macroinvertebrate at non-invaded sites, and the sponge *Ircinia* sp. at invaded sites (Table 17).

The global one-way ANOSIM test showed significant variations in macrophyte assemblages between invaded and non-invaded sites (R=0.461; p = 0.1%). Non-metric MDS plot affirmed that the macrophyte assemblages of the assessment units from non-invaded sites Bijelac and Goli rat were clearly separated, while the invaded sites Mrkjenta and Crnac were not distinguishable (Figure 20).

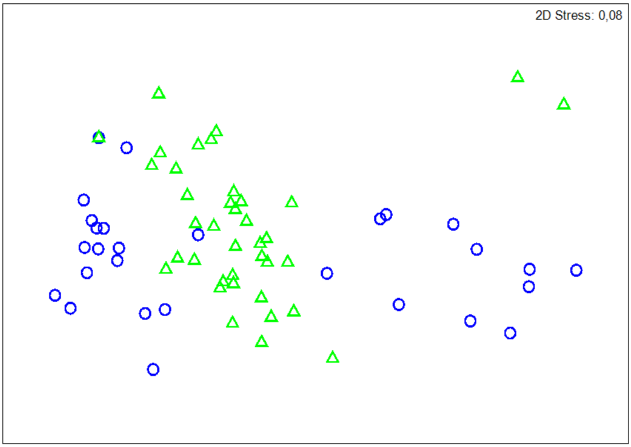


Figure 20. Non-metric MDS of rocky substrate sites based on untransformed macrophytes abundance data per assessment unit. Invaded sites are represented by triangles (Crnac and Mrkjenta) and non-invaded by circles (Bijelac and Goli rat).

Table 17. The numbers of assessment units with 'low', 'moderate' and 'high' abundances of macrophytes and macroinvertebrate taxa at invaded and non-invaded sites with dominant rocky substrate.

Taxa	Invaded			Non-invaded		
	low	moderate	high	low	moderate	high
Chlorophyta						
Acetabularia acetabulum	9	-	-	-	-	-
Caulerpa cylindracea	20	8	-	-	-	-
Codium bursa	16	1	-	5	3	-
Codium effusum	-	-	-	-	3	1
Flabelia petiolata	3	-	-	2	4	-
Halimeda tuna	5	1	-	-	-	-
Ochrophyta						
Cystoseira sp.	6	11	8	1	-	-
Dyctiota sp.	6	3	-	1	1	-
Padina pavonica	14	15	-	7	6	2
Sargassum sp.	1	-	-	-	-	-
Rhodophyta						
Amphiroa rigida	2	-	-	1	-	-
Lithophyllum sp.		-	-	3	6	3
Peyssonnelia sp.	2	-	-	1	2	-
Macroalgae functional group						

Taxa	Invaded			Non-invaded		
	low	moderate	high	low	moderate	high
Turf algae	2	12	18	1	3	1
Tracheophyta						
Posidonia oceanica	3	2	1	4	7	14
Porifera						
Ircinia sp.	7	2	-	-	-	-
Cnidaria						
Eunicella cavolini	1	-	-	6	5	-
Echinodermata						
Arbacia lixula	-	3	-	-	-	-
Paracentrotus lividus	1	-	-	1	-	-
Sphaerechinus granularis	-	-	2	-	-	-
Echinaster sepositus	2	-	-	-	-	-
Polychaeta						
Sabella pavonina	1	-	-	1	-	-

Similarity Percentages analysis (SIMPER) of the macrophyte assemblages from rocky substrate sites indicated an average dissimilarity of 87.13% between invaded and non-invaded sites. Turf algae contributed the most to these differences (22.40%), followed by *P. oceanica* (20.45%) (Table 18).

The average similarity between the invaded sites was 42.30%. Turf algae had the largest contribution to the similarity between invaded sites (47.51%), followed by *Cystoseira* sp. (21.88%) and *P. pavonica* (21.47%).

Posidonia oceanica had the largest contribution to the similarity between the non-invaded sites (72.32%). Other species that accounted for the similarities at the non-invaded sites were *P. pavonica* (14.21%) and *Lithophyllum* sp. (8.53%). The average similarity between the non-invaded sites was 31.48%.

Table 18. Percentage contribution of the taxa/functional group contributing the most to the Bray–Curtis dissimilarity between non-invaded and invaded rocky substrate sites.

Taxa/functional group	Contribution (%)
turf algae	22.40
Posidonia oceanica	20.45
Cystoseira sp.	14.50
Padina pavonica	13.01
Lithophyllum sp.	6.90
Codium bursa	6.10
Dictyota sp.	3.32
Flabelia petiolata	3.30
Codium effusum	2.40

Macroinvertebrate assemblages from invaded and non-invaded rocky substrate sites were not distinguishable (R=0.104; p = 0.1%) (Figure 21).

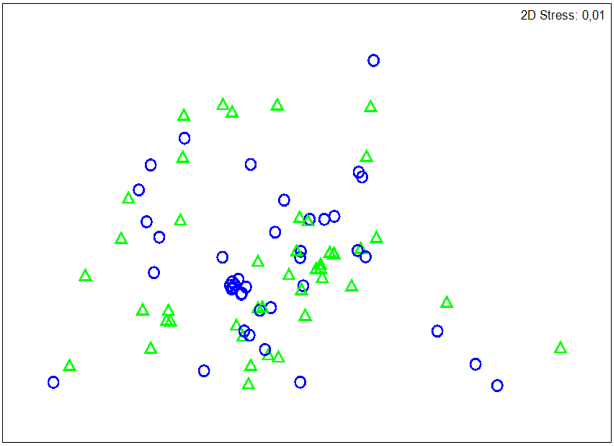


Figure 21. Non-metric MDS of rocky substrate sites based on macroinvertebrates abundance data per assessment unit. Invaded sites are represented by triangles (Crnac and Mrkjenta) and non-invaded by circles (Bijelac and Goli rat).

4.2.2.3. Mixed-type substrate sites

The turf algae were the most common macrophyte taxa at invaded sites with mixed-type substrate sites (Mali Lukovac and Gonoturska), where they were present in 32 assessment units, while at the non-invaded site (Grabova), the turf algae were present in 12 assessment units. *Caulerpa cylindracea* was present in 16 out of a total of 37 assessment units at invaded sites. *Codium bursa* and *P. oceanica* were also common at invaded sites (present in 23 and 16 assessment units, respectively). The most common taxa at non-invaded sites were *Peyssonnelia* sp. and *H. tuna* (present in 19 and 17 assessment units, respectively) (Table 19).

Macroinvertebrates were present in 11 assessment units at non-invaded sites, and in 3 assessment units at invaded sites.

The sea urchin *Sphaerechinus granularis* occurred in 6 assessment units at non-invaded sites, while it was not recorded at invaded sites (Table 19).

Table 19. The numbers of assessment units with 'low', 'moderate' and 'high' abundances of macrophytes and macroinvertebrate taxa at invaded and non-invaded sites with dominant mixed-type substrate.

Taxa	**Invaded**			**Non-invaded**		
	low	**moderate**	**high**	**low**	**moderate**	**high**
Chlorophyta						
Acetabularia acetabulum	8	-	-	3	-	-
Caulerpa cylindracea	9	6	1	-	-	-
Codium bursa	20	3	-	12	-	-
Flabelia petiolata	-	-	-	3	-	-
Halimeda tuna	9	1	-	12	5	-
Ochrophyta						
Cystoseira sp.	4	7	3	2	-	-
Dyctiota sp.	2	-	-	-	-	-
Halopteris scoparia	-	-	-	-	-	-
Padina pavonica	10	-	-	2	-	-
Rhodophyta						
Amphiroa rigida	-	-	-	2	-	-

Taxa	**Invaded**			**Non-invaded**		
	low	**moderate**	**high**	**low**	**moderate**	**high**
Lithophyllum sp.	-	-	-	1	-	-
Peyssonnelia sp.	1	-	-	7	10	2
Osmundaria volubilis	4	-	-	4	7	5
Macroalgae functional group						
Turf algae	9	13	10	3	1	8
Tracheophyta						
Posidonia oceanica	7	2	7	7	5	-
Cnidaria						
Eunicella cavolini	1	-	-	-	-	-
Echinodermata						
Arbacia lixula	1	-	-	1	-	-
Sphaerechinus granularis	-	-	-	6	-	-
Echinaster sepositus	1	-	-	3	-	-
Polychaeta						
Sabella pavonina	-	-	-	1	-	-

Although the non-metric MDS plot did not clearly separate macrophyte assemblages from invaded and non-invaded sites with a mixed-type substrate (Figure 22), the global one-way

ANOSIM test showed significant variations between them (R=0.403; p = 0.1%).

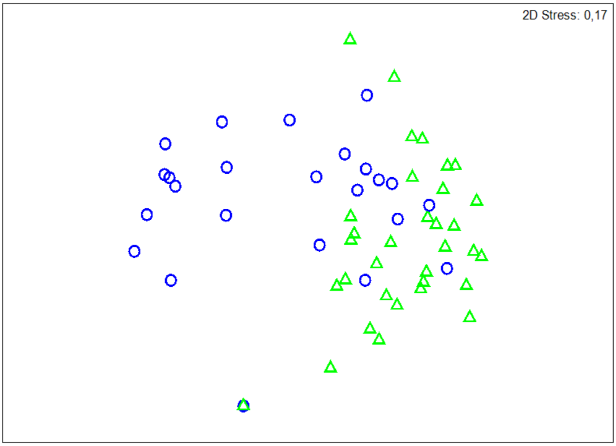


Figure 22. Non-metric MDS of mixed-type substrate sites based on untransformed macrophytes abundance data per assessment unit. Invaded sites are represented by triangles (Mali Lukovac and Gonoturska) and non-invaded (Grabova) by circles.

Similarity Percentages analysis (SIMPER) of macrophyte assemblages indicated an average dissimilarity of 77.66% between invaded and non-invaded sites (Table 20). The turf algae contributed the most to these differences (21.63%), followed by *O. volubilis* (16.27%) (Table 20).

The average similarity between the invaded sites was 44.04%. Turf algae had the largest contribution to the similarity between invaded sites (60.43%), followed by *C. bursa* (16.17%), *P. oceanica* (9.93%) and *Cystoseira* sp. (6.61%).

The average similarity between the non-invaded sites was 36.25%. The following species accounted for the similarities at the non-invaded sites: *Peyssonnelia* sp. (32.46%), *O. volubilis* (29.94%), *H. tuna* (13.39%), turf algae (10.39%), and *P. oceanica* (6.81%).

Table 20. Percentage contribution of the taxa/functional group contributing the most to the Bray–Curtis dissimilarity between non-invaded and invaded sites.

Taxa/functional group	Contribution (%)
turf algae	21.63
Osmundaria volubilis	16.27
Peyssonnelia sp.	15.16
Posidonia oceanica	12.42
Halimeda tuna	8.36
Cystoseira sp.	8.08
Codium bursa	8.05
Acetabularia acetabulum	3.31

The ANOSIM test and MDS plot did not show significant variations in macroinvertebrate assemblages between invaded and non-invaded sites (R=0.043; p = 0.8%) (Figure 23).

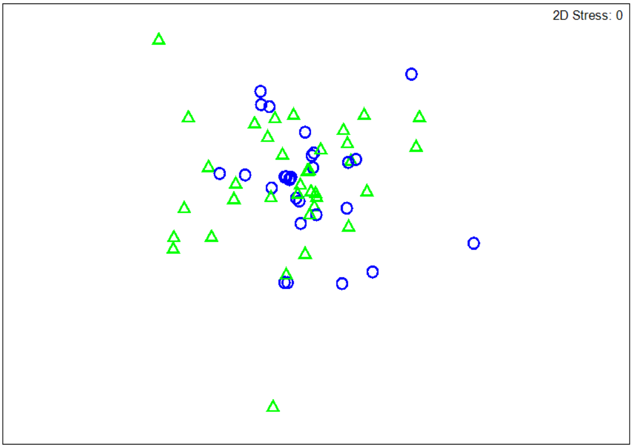


Figure 23. Non-metric MDS of mixed-type substrate sites based on untransformed macroinvertebrates abundance data per assessment unit. Invaded sites (Mali Lukovac and Gonoturska) are represented by triengles and non-invaded (Grabova) by circles.

4.2.2.4. Comparison of native species richness between invaded and non-invaded sites

Rocky substrate sites were the group with the highest species richness, with the total of 21 species/higher taxa/functional groups of macroalgae and macroinvertebrates. Mixed-type substrate sites had 17 taxa present, while sandy substrate sites had the lowest species richness (14 in total).

Sandy substrate sites

Out of a total of 14 species and higher taxa recorded from the sandy substrate sites, 7 were recorded at the invaded sites, and 10 at the non-invaded sites.

There were no significant differences in species richness between invaded and non-invaded sites (pseudo-F=2.787E-2; p(perm)=0.908).

There were significant differences in the species richness across assessment units with different abundance of *C. cylindracea* (pseudo-F=11.457; p(perm)=0.001). The richness was the lowest in the assessment units with moderate *C. cylindracea* abundance (Figure 24).

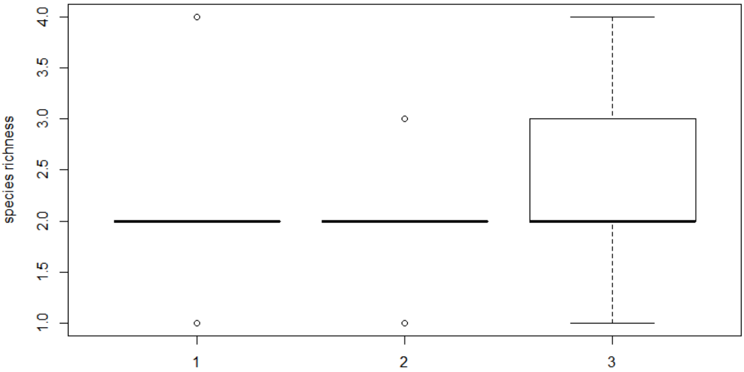


Figure 24. Boxplots of the species richness in assessment units with low (1), moderate (2) and high (3) abundance of *C. cylindracea* at invaded sandy substrate sites. Mid lines, boxes and whiskers represent median, lower and upper quartiles, and smallest and largest observation within an interquartile range of 1.5, respectively. Circles indicate observations beyond an interquartile range of 1.5.

Rocky substrate sites

Out of a total of 21 species and higher taxa recorded from the rocky substrate sites, 19 were recorded at the invaded sites, and 14 at the non-invaded sites. Species richness was significantly higher at invaded than at the non-invaded sites (pseudo-F=24.712; p(perm)=0.001) (Figure 25).

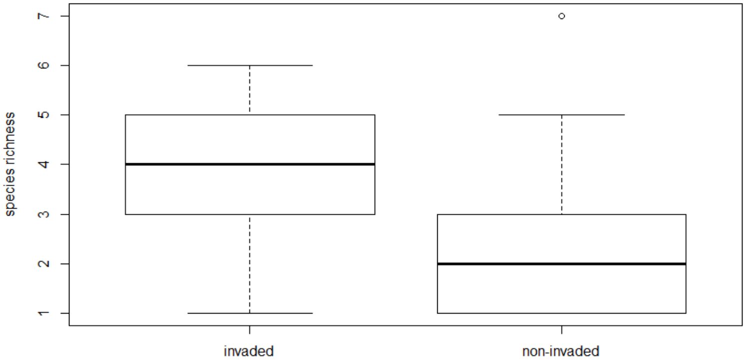


Figure 25. Boxplots of the species richness at invaded and non-invaded rocky substrate sites. Mid lines, boxes and whiskers represent median, lower and upper quartiles, and smallest and largest observation within an interquartile range of 1.5, respectively. Circles indicate observations beyond an interquartile range of 1.5.

There were significant differences in the species richness across assessment units with different abundance of *C. cylindracea* (pseudo-F=23.786; p(perm)=0.001). The median richness was the same in the assessment units with low and moderate *C. cylindracea* abundance, but the assessment units with lower abundance had a larger inter-quartile range (Figure 26).

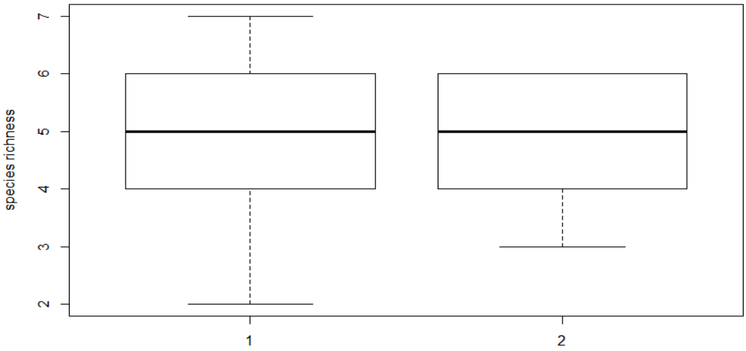


Figure 26. Boxplots of the species richness in assessment units with low (1) and moderate (2) abundance of *C. cylindracea* at invaded rocky substrate sites. Mid lines, boxes and whiskers represent median, lower and upper quartiles, and smallest and largest observation within an interquartile range of 1.5, respectively. Circles indicate observations beyond an interquartile range of 1.5.

Mixed-type substrate sites

Out of a total of 18 species and higher taxa recorded from the mixed-type substrate sites, 13 were recorded at the invaded sites, and 18 at the non-invaded sites.

Species richness between invaded and non-invaded sites did not differ (pseudo-F=0.4579; p(perm)=0.557), but there were significant differences in the species richness across assessment units with different abundance of *C. cylindracea* (pseudo-F=27.489; p(perm)=0.001). The only assessment unit with a high abundance of *C. cylindracea* had six species present. The lowest richness was in the assessment units where *C. cylindracea* had moderate abundance (Figure 27).

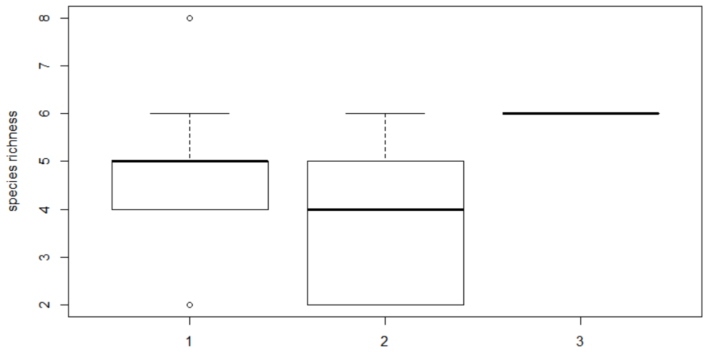


Figure 27. Boxplots of the species richness in assessment units with low (1), moderate (2) and high (3) abundance of *C. cylindracea* at invaded mixed-type substrate sites. Mid lines, boxes and whiskers represent median, lower and upper quartiles, and smallest and largest observation within an interquartile range of 1.5, respectively. Circles indicate observations beyond an interquartile range of 1.5.

4.2.3. Impacts of *Caulerpa cylindracea* on fish assemblages in Marine Protected Areas Lastovo Archipelago and Mljet

The difference between fish assemblages at invaded and non-invaded sites was not significant (R=0.096; p=3%) (Figure 28). SIMPER analysis revealed that the average Bray–Curtis dissimilarity between invaded and non-invaded sites was 57.52%. *O. melanura* contributed the most (9.95%) to the dissimilarity between the sites.

The herbivore *Sarpa salpa* was the most abundant species at both invaded and non-invaded sites, with the average abundance of 7.2±4.4 and 9.3±5.4 per 120m^2, respectively. The second most abundant species at non-invaded sites was *Oblada melanura* (8.8±6.7 per 120m^2), followed by *Thalassoma pavo* (5.8±4.1 per 120m^2). At invaded sites, the second most abundant species was *Apogon imberbis* (7.0±7.4 per 120m^2), followed by *O. melanura* (4.7±5.0 per 120m^2) (Figure 29; Table 21).

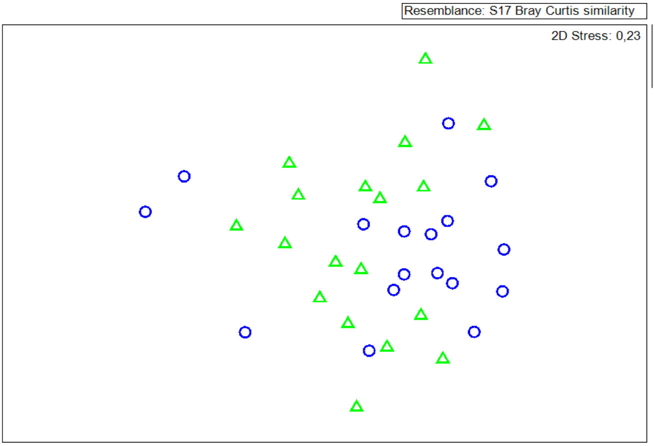


Figure 28. Non-metric MDS based on square root transformed fish abundance data per transect. Transects from non-invaded sites are represented by circles and triangles represent transects from the sites invaded by *Caulerpa cylindracea*.

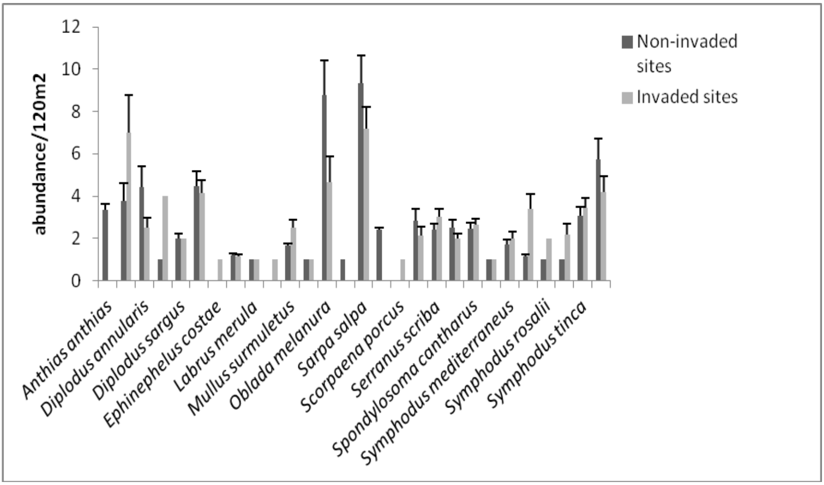


Figure 29. Comparison of mean abundance (±SE) of fish between the sites invaded by *C. cylindracea* and non-invaded sites.

Table 21. Species and abundance (mean individuals number per 120 m^2) of fish at the sites invaded by *C. cylindracea* and non-invaded sites.

Species	Invaded sites		Non-invaded sites	
	mean	SD	mean	SD
Anthias anthias	-	-	3.3	1.2
Apogon imberbis	7.0	7.4	3.8	3.5
Diplodus annularis	2.5	1.9	4.4	4.1
Diplodus puntazzo	4.0	0.0	1.0	0.0
Diplodus sargus	2.0	0.0	2.0	1.0
Diplodus vulgaris	4.1	2.5	4.5	3.0
Epinephelus costae	1.0	0.0	-	-

Species	**Invaded sites**		**Non-invaded sites**	
	mean	**SD**	**mean**	**SD**
Epinephelus marginatus	1.2	0.4	1.2	0.4
Labrus merula	1.0	0.0	1.0	0.0
Labrus viridis	1.0	0.0	-	-
Mullus surmuletus	2.5	1.5	1.7	0.5
Muraena helena	1.0	0.0	1.0	0.0
Oblada melanura	4.7	5.0	8.8	6.7
Phycis phycis	-	-	1.0	0.0
Sarpa salpa	7.2	4.4	9.3	5.4
Scorpaena notata	-	-	2.4	0.5
Scorpaena porcus	1.0	0.0	-	-
Serranus cabrilla	2.1	1.7	2.9	2.3
Serranus scriba	3.0	1.6	2.4	1.1
Sparisoma cretense	2.0	0.9	2.5	1.7
Spondylosoma cantharus	2.6	1.2	2.4	1.3
Symphodus doderlaini	1.0	0.0	1.0	0.0
Symphodus mediterraneus	2.0	1.3	1.7	0.9
Symphodus melanocercus	3.4	2.8	1.2	0.4
Symphodus rosalii	2.0	0.0	1.0	0.0
Symphodus rostratus	2.2	2.2	1.0	0.0
Symphodus tinca	3.4	2.0	3.1	1.7
Thalassoma pavo	4.2	3.1	5.8	4.1

4.2.4. Impacts of *Caulerpa cylindracea* on trophic interactions in Marine Protected Areas Lastovo Archipelago and Mljet

Stable isotope values of macrophytes and macroinvertebrates did not differ between invaded sites. The difference between non-invaded sites was also not significant (Table 22).

Table 22. PERMANOVA testing for differences in stable isotope values macrophytes and macroinvertebrates between sampling sites in MPA Lastovo Archipelago and MPA Mljet.

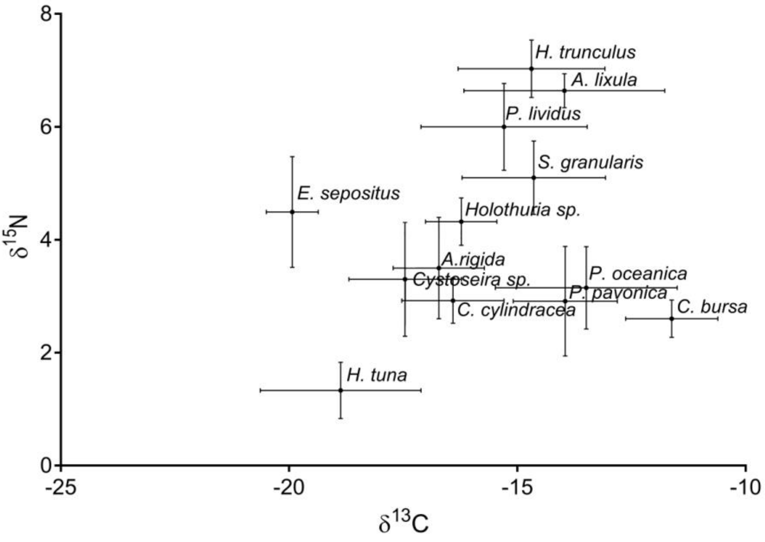
	Invaded sites				**Non-invaded sites**			
	δ^{13}C		**δ^{15}N**		**δ^{13}C**		**δ^{15}N**	
	pseudo-F	**p(perm)**	**pseudo-F**	**p(perm)**	**pseudo-F**	**p(perm)**	**pseudo-F**	**p(perm)**
Macrophytes	0.83749	0.541	1.0028	0.427	0.72669	0.546	1.1937	0.328
Macroinvertebrates	0.70057	0.601	0.76519	0.539	2.3552	0.093	1.9298	0.138

Pair-wise tests also showed no differences between MPA Lastovo Archipelago and MPA Mljet for both invaded and non-invaded sites (all p(perm)>0.05). Therefore, all samples were pooled for invaded and non-invaded sites (Table 23).

Pooled isotope values of macrobenthic organisms from invaded sites differed significantly from pooled values from non-invaded sites (pseudo-F=10.89; p(perm)=0.001). Mean δ^{13}C values of macrophyta ranged from –11.6‰ (*Codium bursa*) to -18.8‰ (*Halimeda tuna*) at invaded sites. Similarly, they ranged from –11.7‰ (*Codium bursa*) to -18.9‰ (*Halimeda tuna*) at non-invaded sites (Figure 30; Table 23).

Mean δ^{13}C values of macroinvertebrates ranged from -19.9‰ (*E. sepositus*) to -13.9‰ (*A. lixula*) at invaded sites, and from -18.5‰ (*E. sepositus*) to -13.9‰ (*S. granularis*). *Hexaplex trunculus* had the most enriched δ^{15}N values, and thus the highest trophic position among the macroinvertebrates analysed (7.0‰ at invaded sites and 6.8‰ at non-invaded sites). The macroinvertebrates with the most depleted δ^{15}N values and the lowest trophic position were *Holothuria* sp. at both invaded (4.3‰) and non-invaded sites (3.9‰) (Figure 30; Table 23).

a) Invaded sites



b) Non-invaded sites

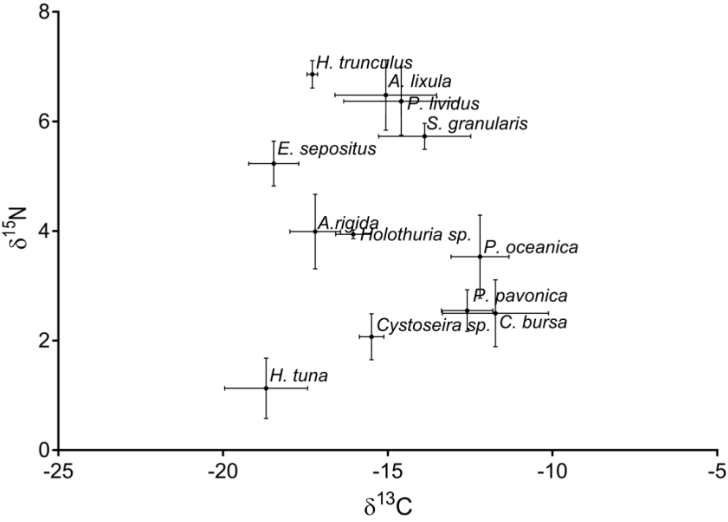


Figure 30. Mean values (± SD) of stable isotope values of carbon and nitrogen of macrophytes and macroinvertebrates from a) invaded and b) non-invaded sites in MPA Lastovo Archipelago and MPA Mljet. Names of taxa and the corresponding number of specimens analysed: see Table 23.

Table 23. Trophic group codes, stable-nitrogen and stable-carbon isotope ratios (mean and SD) and corresponding trophic positions (TP) (mean and SD) of macrophytes and invertebrates from a) invaded and b) non-invaded sites in MPA Lastovo Archipelago and MPA Mljet. Trophic group codes are: Primary producer = PP, deposit-feeder = DF, herbivore = HE, benthic invertebrate feeder = BIF, omnivore= O. Trophic groups are based on previous studies (Deudero et al. 2011; Carefoot, 1991; Puccio et al. 2006). Trophic

positions (TP) were determined using average $\delta^{15}N$ values of benthic primary producers as isotopic baseline. Isotopic baseline was 2.82±1.009 for invaded sites, and 2.56±1.14 for non-invaded sites (see section 2. Materials and methods). Standard deviations (SD) of TP were determined by first-order error propagation of uncertainties: $SD_{combined} = \sqrt{SD_1^2 + SD_2^2}$, where SD_1 is the standard deviation of TP of consumer, and SD_2 is the standard deviation of isotopic baseline.

a) Invaded sites

Taxa	N	Trophic group	$\delta^{13}C$		$\delta^{15}N$		TP	
			mean	SD	mean	SD	mean	SD
Chlorophyta								
Caulerpa cylindracea	16	PP	-16.4	1.1	2.9	0.4		
Codium bursa	9	PP	-11.6	1.0	2.6	0.3		
Halimeda tuna	16	PP	-18.8	1.7	1.3	0.5		
Ochrophyta								
Cystoseira sp.	24	PP	-17.4	1.2	3.3	1.0		
Padina pavonica	17	PP	-13.9	1.1	2.9	0.9		
Rhodophyta								
Amphiroa rigida	10	PP	-16.7	1.0	3.5	0.9		
Tracheophyta								
Posidonia oceanica	16	PP	-13.4	1.9	3.1	0.7		
Mollusca								

Taxa	N	Trophic group	$\delta^{13}C$		$\delta^{15}N$		TP	
			mean	SD	mean	SD	mean	SD
Hexaplex trunculus	4	BIF	-14.6	1.6	7.0	0.5	2.8	1.0
Echinodermata								
Arbacia lixula	17	HE	-13.9	2.2	6.6	0.3	2.6	1.0
Paracentrotus lividus	22	HE	-15.2	1.8	6.0	0.7	2.3	1.1
Sphaerechinus granularis	16	HE	-14.6	1.5	5.1	0.6	1.9	1.0
Echinaster sepositus	6	O	-19.9	0.5	4.4	0.9	1.7	1.1
Holothuria sp.	3	DF	-16.2	0.7	4.3	0.4	1.6	1.2

b) Non-invaded sites

Taxa	N	Trophic group code	δ13C		δ15N		TL	
			mean	SD	mean	SD	mean	SD
Chlorophyta								
Codium bursa	12	PP	-11.7	1.6	2.5	0.61		
Halimeda tuna	11	PP	-18.7	1.2	1.1	0.55		
Ochrophyta								
Cystoseira sp.	3	PP	-15.5	0.3	2.0	0.4		

Padina pavonica	3	PP	-12.6	0.7	2.5	0.3		
Rhodophyta								
Amphiroa rigida	6	PP	-17.2	0.7	3.9	0.6		
Tracheophyta								
Posidonia oceanica	6	PP	-12.2	0.8	3.5	0.7		
Mollusca								
Hexaplex trunculus	3	BIF	-17.3	0.1	6.8	0.2	2.9	1.0
Echinodermata								
Arbacia lixula	13	HE	-15.1	1.5	6.4	0.6	2.7	1.0
Paracentrotus lividus	15	HE	-14.6	1.7	6.3	0.6	2.6	1.0
Sphaerechinus granularis	9	HE	-13.9	1.4	5.7	0.2	2.3	1.0
Echinaster sepositus	8	O	-18.5	0.7	5.2	0.4	2.2	1.0
Holothuria sp.	3	DF	-16.1	0.5	3.9	0.1	1.6	1.1

PERMANOVA showed that the trophic positions of *E. sepositus, P. lividus* and *S. granularis* at non-invaded sites were significantly higher than at the invaded sites (Table 24).

Table 24. PERMANOVA testing for differences in trophic positions of invertebrates between invaded and non-invaded sites in MPA Lastovo Archipelago and MPA Mljet.

Taxa	df	Pseudo-F	p(perm)
Arbacia lixula	1	0.282	0.627
Paracentrotus lividus	1	7.44	0.007
Sphaerechinus granularis	1	13.416	0.002
Echinaster sepositus	1	6.908	0.022
Hexaplex trunculus	1	7.84E-2	0.787
Holothuria sp.	1	0.247	0.91

4.2.4.1. Functional changes in trophic structure of macroinvertebrates between invaded and non-invaded sites

There was a considerably higher niche overlap between *A. lixula* and *P. lividus* at non-invaded sites (2.44) than at invaded sites (0.37). The percentage of *A. lixula* niche area (SEAc) which overlaps with *P. lividus* was considerably higher at non-invaded sites (77.7%) than at invaded sites (16.92%). There was no overlap of *A. lixula* and *S. granularis* niches at invaded sites, while 23% of the *A. lixula* niche area overlapped with the *S. granularis* isotopic niche at non-invaded sites. The niches of *S. granularis* and *P. lividus* did not overlap at invaded sites, but at non-invaded sites 25.99% of *P. lividus* niche overlapped with the niche of *S. granularis*. Interestingly, the percentage of the *A. lixula* niche which overlaps with *H. trunculus* in invaded sites was 57.88%, while there was no overlap in non-invaded sites (Figure 31).

Hexaplex trunculus and *Holothuria* sp. sampled at non-invaded sites had significantly smaller isotopic niches than those from invaded sites (p=99.97% and p=98.12%, respectively) (Figure 31; Table 25). Trophic niche widths did not significantly differ between invaded and non-invaded sites for other consumers (Figure 32).

Table 25. Standard ellipse areas (SEA_c) invertebrates from invaded and non-invaded sites in MPA Lastovo Archipelago and MPA Mljet

Taxa	Invaded	Non-invaded
Arbacia lixula	2.23	3.14
Paracentrotus lividus	5.10	3.53
Sphaerechinus granularis	1.93	2.73
Echinaster sepositus	2.14	1.07
Hexaplex trunculus	3.78	0.18
Holothuria sp.	1.10	0.23

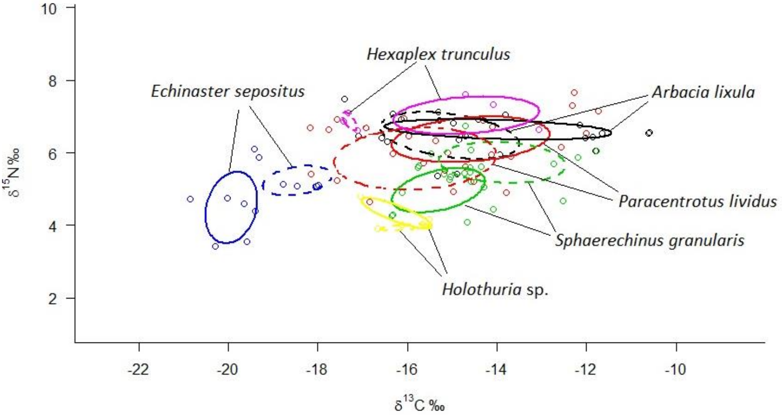
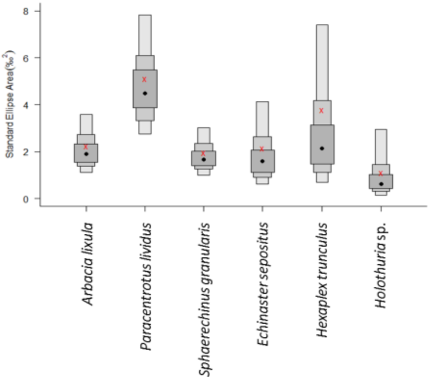


Figure 31. Stable isotope bi-plot illustrating the isotopic niches in invaded (solid line) and non-invaded sites (dashed line). Lines enclose standard ellipse areas (SEAc) for six species: *Arbacia lixula* (black), *Paracentrotus lividus* (red), *Sphaerechinus granularis* (green), *Echinaster sepositus* (blue), *Hexaplex trunculus* (pink) and *Holothuria* sp. (yellow)

a) Invaded sites



b) Non-invaded sites

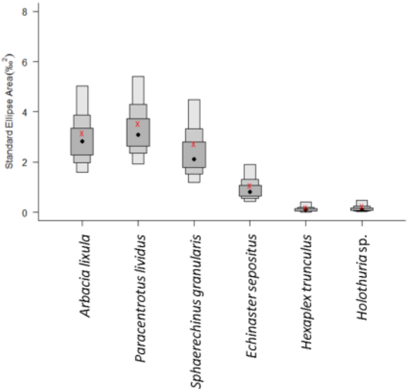


Figure 32. Measures of uncertainty and central tendency (black symbols = mode) of Bayesian standard ellipse areas (SEAC, corrected for small samples) based on 100 000 posterior draws of parameters showing 95, 75 and 50% credibility intervals from light to dark grey, respectively.

4.2.4.2. Comparison of the diet of sea urchins from invaded and non-invaded sites

Bayesian mixing models indicated that in both invaded and non-invaded sites the dietary contribution estimates overlapped considerably for all sources of *A. lixula, P. lividus* and *S. granularis* (Table 26).

Table 26. Mean (±SD) indigenous and non-indigenous herbivores diet proportion in a) invaded sites and b) non-invaded sites, and 95% credible interval (2.5% and 97.5% quantiles, in brackets) estimated using the Bayesian stable isotope mixing model (MixSIAR).

a) Invaded sites

	A. lixula	*P. lividus*	*S. granularis*
Amphiroa rigida	0.337±0.172 (0, 0.651)	0.038±0.106 (0, 0.407)	0.012±0.030 (0, 0.087)
Caulerpa cylindracea	0.273±0.320 (0, 0.999)	0.096±0.240 (0, 0.995)	0.017±0.041 (0, 0.133)
Codium bursa	0.066±0.105 (0, 0.359)	0.040±0.067 (0, 0.233)	0.134±0.145 (0, 0.420)
Cystoseira sp.	0.038±0.072 (0, 0.259)	0.668±0.291 (0, 1.000)	0.023±0.072 (0, 0.180)
Halimeda tuna	0.019±0.036 (0, 0.131)	0.042±0.101 (0, 0.416)	0.595±0.123 (0.326, 0.815)
Padina pavonica	0.180±0.219 (0, 0.663)	0.047±0.092 (0, 0.315)	0.078±0.142 (0, 0.482)
Posidonia oceanica	0.087±0.153 (0, 0.543)	0.069±0.133 (0, 0.469)	0.142±0.194 (0, 0.583)

b) Non-invaded sites

	A. lixula	*P. lividus*	*S. granularis*
Amphiroa rigida	0.631±0.144 (0.386, 0.984)	0.549±0.130 (0.320, 0.893)	0.203±0.133 (0, 0.472)
Codium bursa	0.054±0.059 (0, 0.216)	0.078±0.084 (0, 0.291)	0.098±0.115 (0, 0.403)
***Cystoseira* sp.**	0.124±0.125 (0, 0.441)	0.139±0.141 (0, 0.498)	0.364±0.310 (0.002, 0.999)
Halimeda tuna	0.073±0.070 (0, 0.239)	0.075±0.070 (0, 0.237)	0.127±0.117 (0, 0.385)
Padina pavonica	0.063±0.072 (0, 0.256)	0.088±0.094 (0, 0.333)	0.118±0.140 (0, 0.493)
Posidonia oceanica	0.055±0.063 (0, 0.221)	0.071±0.082 (0, 0.287)	0.090±0.111 (0, 0.405)

5. Discussion

5.1. Are Marine Protected Areas protected against non-indigenous species introductions? The Mediterranean case study in a global context

Although stressed environments are more sensitive to invasion than pristine ones (Occhipinti-Ambrogi and Savini 2003; Zaiko et al. 2007), the vulnerability of MPAs to NIS is related to several community parameters such as species richness, habitat complexity, interactions between species and NIS characteristics (Francour et al. 2010). The number of NIS in MPAs may be lower due to increased native biodiversity and biomass, or increased due to protection from harvesting, increased fishing pressure in surrounding areas and increased propagule pressure as a result of a higher number of visitors (Burfeind et al. 2013).

Mediterranean MPAs, in which this study was conducted, have some differences according to their protection regime, accessibility, etc. The Croatian MPAs Lastovo Archipelago and Mljet have smaller distance to the nearest mainland than MPA Pelagie Islands, and are thus more easily accessible by ferry or leisure craft. Moreover, MPA Pelagie Islands is comprised of zones with different levels of protection (the study sites were located in the integral and general reserve), while Croatian MPAs are not divided into zones and there is a lack of integral reserves.

Using a visual census, rapid assessment of macroalgae, macroinvertebrates and fish was conducted at three sites on Lampedusa Island, two of them being inside the MPA and one outside. This study provides occurrence and abundance data of some NIS that were previously recorded on Lampedusa Island (Alongi et al. 1993; Trainito 2003; Pipitone et al., 2001). There was no evidence of the protection regime on the abundance and distribution of NIS *C. cylindracea*, *A. taxiformis* and *P. gibbesi*. These results are consistent with previous studies that described MPAs as inefficient in stopping NIS introductions (Guidetti et al. 2014, Fridley et al. 2007; Mannino et al. 2016). Generally, the introduction of these species have been attributed to vessels and natural spread from neighbouring countries (AquaNIS and references therein). Their arrival to Lampedusa island, most probably, is the result of secondary spread following the primary introduction elsewhere.

The benthic macroalgae *A. taxiformis* and *C. cylindracea* were present in all three study sites, from the surface to 20 m of depth. *C. cylindracea* was widely spread both in the macroalgal community on rocky bottoms and in the *P. oceanica* meadows on sandy bottom, while *A. taxiformis* had a considerably lower abundance than *C. cylindracea* and it was present only on the rocky substrate covered with macroalgae.

The sea hare *Aplysia dactylomela* was found only outside the MPA, in Cala Maluk. Since there was no difference between the macrobenthic assemblages structure at the sites with different levels of protection, the possibility that the protection may have a role in the *A. dactylomela* distribution is unlikely. Interestingly, in comparison with the other two study sites, Cala Maluk is located considerably closer to the Lampedusa port, where *A. dactylomela* was recorded for the first time in the Mediterranean Sea in April 2002 (Trainito 2003; Crocetta et al. 2009). The first specimen was observed on the sandy/rocky bottom with *C. cylindracea* (Crocetta et al. 2009). Moreover, *A. dactylomela* has been observed feeding on the red alga *Laurencia* sp. (Yokeş, 2006; Mannino et al., 2014; personal observation during collection of samples in this study) (Figure 33). As well as *A. dactylomela*, *Laurencia* sp. was present only at Cala Maluk, thus the distribution of *A. dactylomela* may be related with the distribution of its preferred food item.

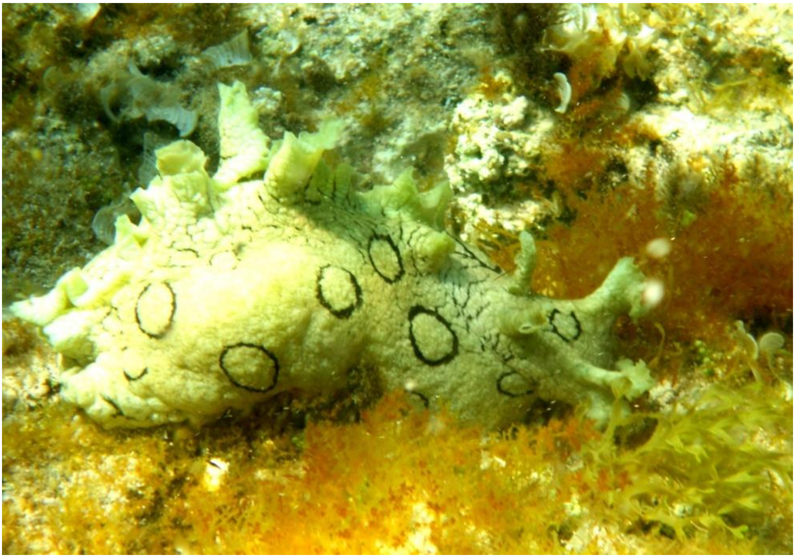


Figure 33. The sea hare *Aplysia dactylomela* feeding on the red alga *Laurencia* sp. in Cala Maluk, Lampedusa Island (Photo: MARECO Summer School).

Shortly after the first record of *P. gibbesi* from Linosa Island in 1999 (Relini et al. 2000), the species was recorded on Lampedusa and its surrounding islands (Pipitone et al., 2001). In this study, *P. gibbesi* was observed in all three sites, where it was widely spread along the upper infralittoral rocks, mostly feeding near crevices and between boulders.

Conversely, the invasive fish *S. luridus* was not widely spread at study sites. Two specimens were observed only at one site, Mare Morto. Other target non-indigenous fish, which are invasive elsewhere in the Mediterranean Sea (*Fistularia commersonii, Siganus rivulatus, Seriola fasciata, Seriola carpenteri* and *Sphoeroides pachygaster*) (Otero et al. 2013), were not observed in this study.

This study confirmed the successful acclimatization of *C. cylindracea, A. taxiformis, A. dactylomela, P. gibbesi* and *S. luridus* on Lampedusa Island. Moreover, visual censuses revealed no detectable disturbance of macrobenthic communities due to anthropogenic impact.

In the study of the presence of NIS on mooring lines in berthing areas, all fouling NIS and cryptogenic species were recorded outside of MPAs. Shipping and leisure craft are considered the most likely vectors for their introduction along the eastern Adriatic coast. Some studies suggested that marinas hosting small leisure vessels can be an important factor for the secondary spread of NIS in the Mediterranean Sea, accelerating the rate of NIS introductions in coastal areas (Savini et al. 2006). The lack of NIS and the predominance of the native algal species on mooring lines in MPAs may reflect the reduced levels of boat activity and anthropogenic pollution. However, ferries regularly cruise from the mainland ports to the MPAs once or twice a day and the number of leisure craft is increasing (Jakl et al. 2009). Therefore, the risk of NIS introduction within MPAs is certain, unless a regulation on the fouling is implemented strictly.

C. brunnea, S. plicata, and *W. subtorquata* are new records for the Croatian coast and have not been reported in either Slovenia (Lipej et al. 2012), Montenegro or Albania (Katsanevakis et al. 2011). *A. verticillata, H. elegans*, *E. rapax* and *A. amphitrite* have been recorded in Croatia previously (Zimmermann 1907; Pallaoro et al. 2006; Zavodnik and Kovačić 2000). However, they were not considered in the recent Croatian NIS/cryptogenic species list (Pećarević et al. 2013). This is either because of overlooked literature records, or because these are examples of species introduced a long time ago and thus sometimes perceived as components of the native biota (Marchini et al. 2015b).

In the Mediterranean Sea, *C. brunnea* has been reported in the Levantine Sea in 2003 (Harmelin 2014), in the Aegean Sea in 2004 (Kocak 2007) and, more recently (2010-2014), in several central and western Mediterranean localities (Lodola et al. 2015; Lezzi et al. 2015). The Croatian record is the first one from the Adriatic Sea. The species is native to the East Pacific, occurring on the western coast of the Americas from British Columbia to Ecuador (Soule et al. 1997) and Panama (Hastings 1929).

The tunicate *S. plicata* was distributed at two marina sites, in moderate to low abundances. Specimens were likewise found attached to boat hulls, close to the waterline, at the Biograd na Moru and Zadar marinas, as well as being present on ‘lazy’ lines. The native origin of this species is thought to be the Northwest Pacific (Hewitt et al. 2004; Barros et al. 2009; Carlton 2009; Airoldi et al. 2015), although Lambert (2011) has expressed uncertainty as to its native range. In the Mediterranean Sea, the species is known since the mid 19th century, and a molecular study (Maltagliati et al. 2015) has shown that *S. plicata* has experienced multiple introduction events, being distributed as hull fouling in the early years of sea transport. Because of the continuous reshuffle of populations due to vessel-mediated transport, the reconstruction of its invasive pathway has high uncertainty (Maltagliati et al. 2015). Pineda et al. (2016) confirmed its non-indigenous status in the Mediterranean Sea. *Styela plicata* is the first non-indigenous ascidian recorded in Croatia.

The bryozoan *W. subtorquata* is common in tropical to temperate waters worldwide, specifically the West Atlantic, West and South Africa, the Pacific Ocean, and the Mediterranean Sea (Vieira et al. 2014). This species forms orange to brownish-purple or black encrusting colonies, which can overgrow other sessile invertebrates. Due to the absence of certain morphological features, such as external ovicells, avicularia and orificial spines, which are used to identify other bryozoan species (Ryland et al. 2009), *W. subtorquata* has often been misidentified. Historically, the genus was supposed to be represented in the Mediterranean Sea by only two species: *Watersipora subovoidea* (d'Orbigny, 1852) and *Watersipora complanata* (Norman, 1864). However, after a recent revision by Vieira et al. (2014), the Mediterranean samples of *W. subovoidea* were assigned to either *W. subtorquata* (so far only in the Western basin) or *Watersipora cucullata* (Busk, 1854). The material of *W. subovoidea* from Croatia (Hayward and McKinney 2002) belonged to *W. cucullata* (Vieira et al. 2014). Therefore, the present record of *W. subtorquata* is the first one of this cryptogenic bryozoan for the Adriatic Sea.

The amphipod *E. rapax* Costa, 1853 was first described in Italy. Hughes and Lowry (2010) redescribed the species and confirmed it as an invasive species in Australian ports. Although the type locality is in the Mediterranean Sea, its region of origin could be the Pacific Ocean, considering the high number of its congeneric species in the Hawaiian and North Pacific amphipod fauna. Therefore, it should be considered as a cryptogenic species (Hughes and Lowry 2010). Pallaoro et al. (2006) recorded *E. rapax* in the stomach contents of the fish *Diplodus vulgaris*, sampled from ten stations along the eastern Adriatic coast.

The barnacle *A. amphitrite*, native to Indo-West Pacific, is a widespread species of barnacle that has been transported by ships for centuries (Carlton et al. 2011). Although it is considered an introduced species elsewhere in Europe (Chainho et al. 2015), the archaeological finding of *A. amphitrite* in a "Carthaginian naval base near Tunis" in North Africa, dated to the second century B.C., may indicate that this barnacle is one of the Tethyan relicts and therefore native to the Mediterranean Sea. Due to the uncertain provenance of the vessel that was brought to that naval base (Carlton et al. 2011), the species is hereby considered cryptogenic in the Mediterranean Sea. *A. amphitrite* was already known in Croatia (Rijeka Bay; Zavodnik and Kovačić 2000).

The bryozoan *A. verticillata* was originally considered to be native to the Mediterranean Sea but has since been described as being pseudoindigenous (Galil and Gevili 2014) on the account of its associate nudibranch *Okenia zoobotryon* (Smallwood 1910) known in the Caribbean Sea (Ortea et al. 2009). Furthermore, the species inhabits natural habitats in the Caribbean Sea, whereas in the Mediterranean region it occurs mainly in artificial environments and continues to expand its range in the Mediterranean Sea and Macaronesia (Marchini et al. 2015b). Although this bryozoan is currently widespread in the Mediterranean Sea, it has been known from Egypt since the early 1800s (Ehrenberg 1828). It most likely arrived as hull fouling to the Mediterranean Sea on wooden vessels, since ballast water was not a developed technology and aquaculture, involving trans-oceanic trade, had not been developed at that time. In this study, it was found only within a small area of the marina in Biograd na Moru.

The first record of *A. verticillata* from the Adriatic Sea was in the nineteenth century in the Gulf of Trieste, Italy (Reichert 1867). Other records from the Italian Adriatic coast are: San Cataldo (Condorelli 1898), the Lagoon of Venice (Neviani 1937), Brindisi (Chimenz and Faraglia 1993) and Manfredonia (Gherardi et al. 1974). *Amathia verticillata* has been known in Croatia only in the northern part of the Adriatic Sea, Rovinj (Zimmermann 1907; Vatova 1928) and Rijeka Bay (Zavodnik and Kovačić 2000).

Serpulids formed extensive crusts on 'lazy' lines. There were eight identified species of serpulids present in samples. The only species that could be confirmed NIS was *H. elegans.*

H. elegans was recorded in ports of the eastern Adriatic coast (Igić 1984, 1995; Slišković et al. 2003). It is thought to have arrived to the Mediterranean Sea as ship hull fouling and has a native origin in the Indo-Pacific (ten Hove 1974). It has been one of the dominant fouling species at Naples harbour as early as 1888 (Zibrowius 1991) and is known to be widely distributed in Mediterranean harbours and lagoons (Relini et al. 1980; Çinar 2006; Pettengill et al. 2007). The species also occurs in northern Europe (ten Hove 1974).

All recorded fouling NIS and cryptogenic species in this study are species associated with vessel vectors (AquaNIS 2016 and references therein). Since the publication of Pećarević et al. (2013) inventory of Croatian NIS, that listed five records of vessel-associated NIS from the middle Adriatic Sea, the cnidarian *Oculina patagonica* (De Angelis 1908) was recorded from Split (Cvitković et al. 2013a), the sponge *Paraleucillla magna* (Klautau, Monteiro & Borojevic 2004) from Ploče harbour (Cvitković et al. 2013b), and the crustacean *Caprella scaura* (Templeton 1836) from fish farms located off Ugljan Island and Brač Island (Fernandez-Gonzalez and P. Sanchez-Jerez 2014).

The low number of marine NIS records from the middle Adriatic Sea is probably due to limited research efforts. It is likely that several species have been overlooked so far and may be recorded from this region in the future, if adequate monitoring surveys are carried out. The appearance of previously unrecorded NIS emphasizes the need for regular NIS surveys of leisure craft berthing areas.

The introduction of new fouling species along the eastern Adriatic coast may have serious negative impacts on native communities, ecosystems, coastal infrastructure and mariculture. The current absence of NIS in the offshore sites within MPAs in the Adriatic Sea should encourage actions for preventing possible NIS introductions in the years to come. The expansion of NIS by recreational vessels represents an actual risk for MPAs, which are popular tourist destinations. The dissemination of good practices for cleaning and maintenance habits of recreational vessels may help in avoiding new NIS introduction, therefore enhancing MPA conservation. Recorded NIS and cryptogenic species are likely to continue to expand, and further arrivals are to be expected, thus monitoring of the marine biota is of critical importance. This study may be utilised as a baseline for future assessments of the occurrence of NIS in berthing areas along the eastern Adriatic.

Burfeind et al. (2013) reviewed the effects of marine reserves on NIS and concluded that NIS do equally well or better within marine reserves, but also that there is not enough currently available data to draw general conclusions. This study revealed that the effect of MPA was species-specific: no effect was observed on the pattern of benthic NIS spread on Lampedusa Island, while the lack of high antropogenic pressure in berthing areas inside of Adriatic MPAs resulted in the absence of fouling NIS on mooring lines.

5.2. Vulnerability of benthic habitats to the invasion of the green alga *Caulerpa cylindracea*

Species composition and the structure of the macropyhte and macroinvertebrate assemblages, inhabiting three different substrate types: rocky, sandy and mixed-type bottom substrates, were analysed in order to assess the abundance and distribution of *C. cylindracea* in MPAs Lastovo Archipelago and Mljet, Croatia. Macrobenthic and fish assemblages were compared among non-invaded sites and sites invaded by *C.*

cylindracea in order to evaluate the impacts of *C. cylindracea* and vulnerability of benthic habitats to the invasion of this alga.

Rocky bottom substrate sites, where infralittoral photophilic algae community, coralligenous community and the patches of *P. oceanica* were present, had the lowest *C. cylindracea* abundance, while the sandy substrate, inhabited with turf algae *H. scoparia* and fleshy algae *O. volubilis,* seemed to be most favourable to the *C. cylindracea* invasion.

Although *C. cylindracea* has rapidly colonized both hard and soft bottoms all over the Mediterranean Sea (Piazzi et al., 2005), the study conducted in MPA Zakynthos (Ionian Sea, Greece) indicated that the rocky bottoms were more vulnerable to the *C. cylindracea* invasion than to the unvegetated sandy/muddy substrates. The margins of *P. oceanica* meadows had the highest probability of presence of *C. cylindracea,* while its lowest density was recorded within *P. oceanica* meadows (Katsanevakis et al. 2010).

Soft bottoms with coarse texture (e.g. rhodolite beds) provide suitable three-dimensional substrate for *C. cylindracea* stolon attachment (Klein and Verlaque 2009; Piazzi et al. 2016; Pacciardi et al. 2011). The invaded study sites with dominant sandy bottom in MPA Lastovo and MPA Mljet were inhabited by either turf algae or fleshy red alga *O. volubilis.* Therefore, these algae provided a more complex substrate than bare sandy bottom and enhanced the expansion of *C. cylindracea.* Moreover, the turf algae were dominant in invaded, while *P. oceanica* was dominating at non-invaded sites with rocky substrate.

The morphological traits of the assemblages could be a crucial determinant of vulnerability (Bulleri and Benedetti-Cecchi 2008;

Ceccherelli et al. 2002). Bulleri and Benedetti-Cecchi (2008) observed that regardless of the substrate type, the turf algae had a positive effect on the spreading of *C. cylindracea*. The high invasibility of the assemblages dominated by the turf algae may be caused by a lower complexity of these assemblages or by mechanical mechanisms such as trapping drifting fragments of rhizoids (Ceccherelli et al. 2002; Piazzi et al. 2001). Both turf and encrusting algae are less resistant to the invasion than the seagrass *P. oceanica* and erect macroalgae (Piazzi et al. 2001).

Ceccherelli et al. (2000) examined the effects of the presence of the native seagrass *Posidonia oceanica* on *C. cylindracea*. They showed that the invasibility of the *P. oceanica* community is related to the availability of sandy substrates, since the growth of *C. cylindracea* is influenced by seagrass density. Thus, it is not surprising that the dense *P. oceanica* patches were common in the non-invaded rocky substrate sites.

Invaded sandy bottom sites displayed the highest dissimilarity in macrophytes community structure and species composition between invaded and non-invaded sites. On the other hand, macroinvertebrate assemblages did not differ between invaded and non-invaded sites.

Previous studies of the impact of *C. cylindracea* on infralittoral macrophyte, coralligenous and detritic assemblages and dead *P. oceanica* mats, indicated a decrease in the number of species in the presence of this invasive algae (Piazzi et al. 2001; Piazzi and Balata 2008; Klein and Verlaque 2008; Katsanevakis et al. 2010; Antolić et al. 2008). In this study, sites with a rocky substrate had higher species richness at invaded sites in comparison with non-invaded sites. This could be explained by the high number of assessment units with low abundance of *C. cylindracea* and the absence of assessment units where *C. cylindracea* was dominant. When the total number of species per substrate group was considered, the highest species richness was found at the rocky substrate sites (where both infralittoral algae and coralligenous communities were present) and the lowest at the sandy substrate sites. Stachowicz et al. (1999) suggested that increased species richness is decreasing invasion success because the available space is more entirely and efficiently used by the communities with higher species richness. Thus, although it has successfully invaded the highly diverse community, *C. cylindracea* did not reach an abundance level which would cause a decline in diversity of indigenous macrobenthic organisms inhabiting the rocky substrate. These results confirmed that it is difficult to generalise the effects of invaders because they depend on the phase of invasion, the recipient biota and current environmental factors (Reise et al. 2006).

The impacts of *C. cylindracea* on macroinvertebrate assemblages have been described in several studies, such as the owergrowth of the sponge *Sarcotragus spinosulus* (Žuljević et al. 2011), the red gorgonian *Paramuricea clavata* (Cebrian et al. 2012) and the coral *Cladocora caespitosa* (Kružić et al. 2008), but no such effects were observed in this study. Baldacconi and Corriero (2009) reported that *C. cylindracea* competed with sponges for available substrate and reduced their cover, but the invasion did not change the composition of the sponge assemblage.

Similarly to the results of the previous study of the impact of *C. cylindracea* on fish assemblages (Ulas et al. 2011), the invasive alga did not have an effect on fish assemblages. The abundance of the herbivorous fish *S. salpa*, that was the most abundant fish at both invaded and non-invaded sites, was slightly lower at invaded sites, but the difference was not significant. Considering that this species has been observed feeding on *C. cylindracea* (Tomas et al. 2011a), this result is not surprising.

The study conducted in Sicilian waters indicated that the fish assemblage, at the sites colonized by *C. taxifolia*, had higher species diversity and significantly differed from those where *C. cylindracea* and the native alga *C. prolifera* were present (Gianguzza et al. 2006). Conversely, Harmelin-Vivien et al. (1999) observed a significant decrease in species richness, density and biomass of fish assemblages in habitats invaded by *C. taxifolia* during the duration of their study (i.e. 6 years).

The impacts of *C. cylindracea* may vary between different benthic components, and biotic and abiotic conditions (Ceccherelli and Campo 2002; Bulleri et al. 2010). Although *C. cylindracea* invaded all types of substrates, the abundance of the *C. cylindracea* in the Adriatic MPAs depended on the substrate type and communities present. The sites with a rocky substrate, characterized by the coralligenous community, the macroalgal assemblages with dominant erect algae and dense *P. oceanica* patches were slightly more resilient to the invasion than other communities, resulting with the lowest abundance of *C. cylindracea*. While the species richness did not differ between invaded and non-invaded sites with sandy and mixed-type substrate, sites with a rocky substrate had higher species richness at invaded sites in comparison with non-invaded sites.

5.3. Invasional meltdown hypothesis in the light of the trophic interaction analysis

Using stable isotope ratios and subsequent isotopic population metrics, the trophic structure of macrobenthos in Lampedusa Island invaded by non-indigenous algae and herbivores was assessed. The trophic ecology of indigenous and non-indigenous herbivores was the main focus in order to determine the role of NIS in invaded ecosystems and its possible impacts on indigenous consumers.

It was shown that the indigenous herbivores can incorporate NIS into their diet and can consequently contribute to invasion control (Parker et al. 2006). Previous studies displayed feeding on *C. cylindracea* by *P. lividus,* one of the most abundant herbivores in the Mediterranean Sea (Ruitton et al. 2006; Žuljević et al. 2008). However, the spread of *C. cylindracea* might not be inhibited by grazing (Bulleri and Malquori 2015). Likewise, even though it was preferred over the indigenous seagrass *P. oceanica* during the feeding preference experiments on *P. lividus,* consumption of *C. cylindracea* had negative impacts on their performance. This enemy escape mechanism contributes to the explanation of how *C. cylindracea* is not eradicated by grazing (Tomas et al. 2011b). Ruitton et al. (2006) stated that *P. lividus* exhibited a more selective feeding behaviour pattern and consumed less *C. cylindracea* than the sea urchin *S. granularis*. Moreover, Bulleri and Benedetti-Cecchi (2008) evaluated the effects of *A. lixula* and *P. lividus* on the patterns of distribution of *C. cylindracea* between habitats. Considering that the removal of sea urchins had no effect on *C. cylindracea,* they suggested that the consumption of *C.*

cylindracea by the sea urchins was rather small regardless of the type of habitat.

Although the extracts of *A. taxiformis* were found to have antibacterial, antiprotozoan, antifouling and ichthyotoxic compounds (Manilal et al. 2010; Genovese et al. 2009, 2012), this alga was among the medium preference algae in the feeding preference experiments on the rabbitfish *Siganus argenteus* (Paul et al. 1990). Conversely, it was completely avoided in the experiments on the herbivorous fish *Zebrasoma flavescens* (Wylie and Paul 1988) and one of the least preferred algae for the abalone *Haliotis asinina* (Angell et al. 2012). Verges et al. (2008) reported that male gametophytes of closely related species *Asparagopsis armata* were preferred over female gametophytes by the herbivore *Aplysia parvula* due to lower concentrations of secondary metabolites.

In this study, the mixing models did not show significant differences between food source contributions to the diets of sea urchins and *A. dactylomela*. Therefore, it is not possible to determine whether or not *C. cylindracea* and *A. taxiformis* were included in the diets of indigenous herbivores. Sea urchins may have consumed non-indigenous algae, but considering the observations from previous studies and a relatively low abundance of non-indigenous algae at the studied sites, it is unlikely that they were an important food source.

In the Mediterranean Sea, *A. dactylomela* was observed to feed on Dictyotaceae and *Laurencia* sp. It has also been observed on dense algal mats with the presence of *C. cylindracea* (Yokes 2006; Crocetta and Colamonaco 2008; Mannino et al. 2014). In addition to oceanographic changes and eutrophication, the presence of non-indigenous algae is proposed as an important factor that might have facilitated the rapid invasion of *A. dactylomela* in the eastern Mediterranean (Valdés et al. 2015).

Field observations of the food selection of *P. gibbesi* showed that this species was feeding primarily on algae (Müller 2001; Sciberras and Schembri 2008), but has also displayed opportunistic behavior, feeding on pagurids and polychaetes (Deudero et al. 2005). Canicci et al. (2004) reported that animal matter, primarily gastropods and crustaceans, constituted a large part of their stomach contents.

Puccio et al. (2006) reported that filamentous, calcareous and corticated algae make up the largest amount of stomach contents of *P. gibbesi*, together with a few fragments of bryozoans, hydrozoans and very small copepods, gastropods and decapods. Therefore, it was suggested that the ingestion of animal items was non-selective. Ferrer and Frau (2005) ranked laminar and filamentous algae as the most important dietary contributors for the crab. *P. gibbesi* was also observed to feed on *C. cylindracea* (Sciberras and Schembri 2008). In this study, the mixing models indicated that *C. cylindracea* was the most important resource in the diet of *P. gibbesi.*

According to the trophic niche theory, when an NIS establishes itself in a new environment, instead of increasing inter-specific competition which causes the decline of indigenous species, they will exploit different food sources in order to co-exist (Chesson 2000). Davis et al. (2000) suggested that species will have greater success in invading a community if it does not encounter intense competition for resources from resident species.

Furthermore, the study of the diets of the non-indigenous fish *Siganus luridus* and its two ecological indigenous analogues in the Mediterranean Sea, *Sarpa salpa* and *Sparisoma cretense*, revealed that *S. luridus* consumed *C. cylindracea*, while this alga was not found in the stomachs of the two indigenous herbivorous fishes. Furthermore, the study showed resource partitioning between *S. luridus* and *Sarpa salpa* (Azzurro et al. 2007b). The success of lessepsian fishes in the Mediterranean has been explained by the lack of competition, which is expected when the preferred resources should become limited (Golani 1994; Lundberg and Golani 1995).

Since the isotopic niche of NIS did not overlap with the niche of indigenous herbivores, they may have consumed the food sources that were underutilized by the sea urchins. Although a comparison between the sites invaded by *C. cylindracea* and non-invaded sites was not possible, there is a suggestion of invasion facilitation, since the main component of the diet of *P. gibbesi* is an NIS itself (Simberloff and Von Holle 1999).

The trophic positions of indigenous and non-indigenous taxa obtained in this study corresponded well with the trophic classification based on previous studies (Deudero et al. 2011; Carefoot 1991; Puccio et al. 2006). Thus, the species with the most enriched $\delta^{15}N$ values were carnivorous and omnivorous, while filter feeders, herbivores and benthic invertebrate feeders had lower $\delta^{15}N$ values. Although both NIS had more enriched $\delta^{15}N$ values than the sea urchins, the overall range of $\delta^{15}N$ for all herbivorous species (1.9) indicates they occupy the same trophic level, assuming the trophic enrichment of 2.3‰ (McCutchan et al. 2003). This is in line with the previous findings suggesting that *P. gibbesi* is herbivorous. NIS displayed $\delta^{15}N$ values close to those of the opistobranch *F. picta*, which could be explained by the diet of *F. picta* that consists of sponges with low trophic positions.

Despite the fact that some successful NIS displayed a broader trophic niche than indigenous ones and demonstrate a more generalist dietary behaviour (Romanuk et al. 2009; Olsson et al. 2009), this study revealed a narrower range of $\delta^{13}C$ and $\delta^{15}N$ values for NIS, compared to indigenous herbivores. This highlights that the success of these two NIS may be due to the utilization of less exploited niche components that indigenous species are avoiding to use. These results are similar to those obtained in the study on the impacts of highly invasive fish *Pseudorasbora parva* on native fishes (Tran et al. 2015). Although more data on the niche width of indigenous and non-indigenous herbivores in the Mediterranean Sea are required to understand the invasive capacity of herbivorous NIS, trophic plasticity is an essential trait for explaining the success of NIS.

The long-term ecosystem effects of the invasion of *P. gibbesi* and *A. dactylomela* are not yet well understood. Although the niches of indigenous and non-indigenous consumers did not overlap in this study, their expansion and the increase of their abundance may cause a decline in algae abundance, thus causing significant changes in ecosystem functioning. Taking into consideration the growing body of evidence pointing at the potential threats caused by NIS, this study highlights the need for more information on the trophic ecology of *P. gibbesi* and *A. dactylomela*, e.g. on possible competitive interactions with other macroinvertebrates and herbivorous fish.

During the field study in the Adriatic Sea, *P. gibbesi* was one of the target NIS, but despite a thorough search targeting its habitat, it was not observed in MPA Lastovo Archipelago and MPA Mljet during June and July 2014. Dulčić and Dragičević (2015) reported the presence of four specimens of *P. gibbesi* at Molunat Bay, located south of our study area, in April 2014. There are more records of *P. gibbesi* in the middle part of the eastern Adriatic Sea (Dubrovnik, Korčula, Vis, Lastovo), but they are still unconfirmed (IZOR, 2016). Considering that *C. cylindracea* is widespread in the Adriatic Sea (Iveša et al. 2015), and results of Bayesian mixing models, which indicated the importance of *C. cylindracea* in the diet of *P. gibbesi*, it is possible that *C. cylindracea* might have facilitated the spreading of *P. gibbesi* in the Adriatic Sea as well. Therefore, this study provides further evidence that supports the invasional meltdown hypothesis.

5.4. Mixed message from the Bayesian mixing models and standard ellipse areas: analyzing the role of the green alga *Caulerpa cylindracea* in the trophic interactions

The SIA was used to examine the trophic shifts in the community inhabiting the rocky substrate invaded by *C. cylindracea* in comparison with non-invaded sites. Although the species composition of macroinvertebrates did not differ between invaded and non-invaded sites with rocky substrate, changes in trophic niche widths and trophic positions of consumers were observed.

Previous studies on the impacts of *C. cylindracea* on the trophic structure in *P. oceanica* meadows and macroalgal communities also observed changes in food web structure and trophic guilds. The invasion of *C. cylindracea* broadened the trophic diversity of both ecosystems of rocky bottoms and *P. oceanica* meadows, probably due to the establishment of a new basal resource (Deudero et al. 2011; Alomar et al. 2016).

Other than direct consumption by herbivores, *C. cylindracea* may be incorporated into the food web in detritus form (Deudero et al. 2011). *Caulerpa cylindracea* detritus was a significant food source for some invertebrates (polychaetes, gammarids and gastropods) (Casu et al. 2008), thus having an impact on the diet of their predators.

Isotopic niche widths did not show a clear pattern for the sampled invertebrate taxa. Significant changes in trophic niche widths were evidenced for the deposit feeder *Holothuria* sp. and benthic invertebrate feeder, the gastropod *H. trunculus*. Smaller isotopic niches at non-invaded sites was observed in *P. lividus*, while the other two herbivores *A. lixula* and *S. granularis* had broader niches at non-invaded sites.

A higher overlap of trophic niche space between the sea urchins *A. lixula* and *P. lividus* at non-invaded sites indicated higher competition between these species when *C. cylindracea* was absent. The reason for lower competition in invaded areas might be the consumption of *C. cylindracea* by *P. lividus*, which the previous studies have already demonstrated (Ruitton et al., 2006; Žuljević et al., 2008).

Moreover, a niche overlap between *A. lixula* and *S. granularis* at non-invaded sites and the lack of it at invaded sites could also be due to the consumption of *C. cylindracea* by *S. granularis*. Despite that, *P. lividus* and *S. granularis* niches did not overlap at invaded sites. This could be due to the fact that *S. granularis* is less selective and consumed more *C. cylindracea* than *P. lividus* (Ruitton et al. 2006). Significantly lower trophic positions of *P. lividus* and *S. granularis* at invaded sites also indicate considerable changes in the diets of these two sea urchins. Significant differences were also found for *E. sepositus*, with lower values at the invaded sites. These results are similar to those obtained in the study by Deudero et al. (2014), where *C. cylindracea* lowered the general trophic level of the invaded *P. oceanica* dead matte, and different from the study by Alomar et al. (2016), where $\delta^{15}N$ values of primary producers and primary consumers were slightly higher in macroalgal assemblages invaded by *C. cylindracea.*

Considering the fact that the mixing models could not distinguish the dietary proportions of different sources, it is difficult to confirm whether *P. lividus* and *S. granularis* consumed *C. cylindracea*, or if the changes in their trophic niche widths are caused by the differences in resource availability.

This study showed that *C. cylindracea* caused significant changes in trophic interactions and that the impact of this alga is species specific. Considering the cascading effects of variations in trophic structure, the impacts of this invader in the Mediterranean Sea should not be underestimated (Deudero et al. 2014). Thus, further investigation is necessary to understand the consequences of *C. cylindracea* spread on food webs.

5.5. Future perspectives and and recommendations

Monitoring of marine NIS presence and impacts provides a baseline for management (Lehtiniemi et al. 2015), and the scarcity of data on marine NIS impacts is slowing down the process of policy-making and management of marine NIS (Ojaveer et al. 2015). Therefore, it is crucial to develop methods for addressing NIS in MPAs which would be appropriate to the particular conditions of each area and to the species concerned (Otero et al. 2013). Furthermore, it is recommended that regular monitoring programs conducted in MPAs should be used for detection of NIS (Whomersley et al. 2015).

One of the cost-effective methods to conduct NIS assessments in MPAs is rapid assesement of target species applied in this study. For example, the use of 'lazy' lines was effective for recording serpulids, bryozoans and tunicates. Moreover, the random sampling of assemblages fouling on lazy lines allowed us to report NIS and cryptogenic species new to Croatia and the whole Adriatic Sea. The monitoring of 'lazy' lines is also a non-invasive sampling method that overcomes the technical problems while conducting biological surveys in port areas. However, the rapid assessment of the abundance can only be applied for a limited subset of species (macroscopic, easily identifiable species). The extension to all taxa would in fact require a more structured and time-consuming stratified sampling design, which would include depth or other relevant factors.

Rapid assessment surveys of epibiota on artificial structures in harbours and rapid assessment snorkelling surveys of biota on sublittoral bedrock also proved to be an effective method for recording nine new NIS on the island of Rodos, Greece: the foraminiferan *Amphisorus hemprichii*, the polychaetes *Branchiomma bairdi*, *Dorvillea similis*, *Hydroides dirampha* and *Pseudonereis anomala*, the molluscs *Aplysia parvula*, *Chama pacifica* and *Septifer cumingii*, and the bryozoan *Hippopodina feegeensis* (Corsini-Foka et al. 2015). Since these methods allowed us to measure the abundance and distribution of easily identifiable NIS in a very rapid and cost-effective way, further use should help to fill the gaps in knowledge on these NIS and cryptogenic species in the Mediterranean Sea. Moreover, these methods might provide information for managers and policy-advisers and support the decision-making regarding planning of monitoring activities in Mediterranean marinas, thus be incorporated into national strategies on NIS.

In our study, the above method was also applied in underwater conditions. Due to very good underwater visibility, using a visual census of an easily recognisable target species was effective for the rapid assessment of abundance and distribution of non-indigenous macroalgae, macroinvertebrates and fish at the study sites in MPAs in Italy and Croatia. This method can be recommended for use in other MPAs in the Mediterranean Sea. However, it may not be suitable in other marine regions with low visibility, such as the Baltic Sea (S. Olenin, pers. comm.).

Another direction of NIS in the areas of marine conservation should be assessment of the invasive species impacts on invaded ecosystems. The reduction of impacts of non-indigenous invasive species is necessary to achieve "good environmental status", according to the European Union

Marine Strategy Framework Directive. Therefore, it is necessary to perform studies on impacts of marine NIS in order to provide the knowledge base for management (Ojaveer et al. 2015). The isotopic analyses applied in this study provided novel data which contributed to the knowledge on the role of NIS in the Mediterranean Sea. Further research on *P. gibbesi, A. dactylomela,* and other herbivorous taxa in the Mediterranean Sea, such as fish *Sarpa salpa,* using the isotopic analyses approach is recommended to obtain important information on the overall effect of their invasion on trophic interactions and consequently on biodiversity.

6. Conclusions

1. Different protection regimes ("integral reserve", "general reserve" and "outside MPA") on Lampedusa Island (Italy) had no obvious effect on the pattern of NIS spread: the abundance and distribution of green alga *Caulerpa cylindracea*, red alga *Asparagopsis taxiformis* and crab *Percnon gibbesi* were found to be similar in the zones with different level of protection. Although the sea hare *Aplysia dactylomela* was found only outside the MPA near the Lampedusa port, most probably its distribution was associated with the location of its preferred food item, the red alga *Laurencia* sp.

2. All NIS and cryptogenic species on mooring lines of leisure craft were recorded outside of MPAs in the survey in Croatia. The lack of NIS and the predominance of the native algal species on mooring lines in MPAs may reflect the reduced levels of boat activity and anthropogenic pollution (artificial substrates, habitat degradation, nutrient enrichment, etc.).

3. The rapid assessment method using mooring lines inside and outside MPAs in Croatia revealed four non-indigenous (the tunicate *Styela plicata*, the serpulid *Hydroides elegans,* and bryozoans *Celleporaria brunnea*, and *Amathia verticillata*) and three cryptogenic (the amphipod *Elasmopus rapax*, the barnacle *Amphibalanus amphitrite* and the bryozoan *Watersipora subtorquata*) species. Three species (*S. plicata, C. brunnea,* and *W. subtorquata*) represented the first records for Croatia, while the two latter ones were new to the entire Adriatic Sea.

4. The invasive green alga *C. cylindracea* occurred on all types of substrates at invaded sites within the investigated depth range (1 to 42 m) in the MPAs Lastovo Archipelago and Mljet (Croatia). The abundance of the alga was the lowest on sandy bottoms (occurrence 93%, high abundance in 18% of cases), followed by mixed substrate (occurrence 43%, high abundance in 2% of cases) and rocky outcrops (occurrence 60%, only low to moderate abundance).

5. In MPAs Lastovo Archipelago and Mljet, the sites with a rocky substrate had higher species richness at invaded sites in comparison with non-invaded sites. Macrophyte assemblages from invaded sites were significantly different compared to the non-invaded sites, while macroinvertebrate and fish assemblages were not affected by *C. cylindracea* invasion.

6. Isotopic space from Bayesian ellipses suggested that no overlap occurs between indigenous and non-indigenous consumers. Bayesian mixing models indicated that the invasive alga *C. cylindracea* was an important food source for non-indigenous crab *P. gibbesi*, thus probably facilitating the spread of the latter. These results provided further evidence that supports the invasional meltdown hypothesis.

7. Changes in isotopic niche widths and trophic positions of some macroinvertebrates between the sites invaded by *C. cylindracea* and non-invaded sites were species specific and did not show a clear pattern for the trophic groups.

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Annex

ANNEX 1

List of taxa considered for macrophytes assemblages assessment (Lampedusa Island)

Chlorophyta

- *Acetabularia acetabulum*
- *Caulerpa cylindracea*
- *Caulerpa prolifera*
- *Codium bursa*
- *Dasycladus vermicularis*
- *Halimeda tuna*
- *Ulva* sp.

Rhodophyta

- *Amphiroa rigida*
- *Asparagopsis armata*
- *Asparagopsis taxiformis*
- *Ellisolandia elongata*

- *Jania rubens*
- *Laurencia obtusa*
- *Peyssonnelia squamaria*

Ochrophyta

- *Cystoseira* sp.
- *Dictyota* sp.
- *Halopteris* sp.
- *Padina pavonica*

Tracheophyta

- *Cymodocea nodosa*
- *Posidonia oceanica*

ANNEX 2

List of taxa considered for macroinvertebrate assemblages assessment (Lampedusa Island)

Porifera

- *Chondrilla nucula*
- *Chondrosia reniformis*
- *Ircinia* sp.

Mollusca

- *Aplysia dactylomela*
- *Felimare picta*
- *Pinna nobilis*

Arthropoda

- *Pachygrapsus marmoratus*
- *Percnon gibbesi*

Echinodermata

- *Arbacia lixula*
- *Echinaster sepositus*
- *Holothuria* sp.
- *Marthasterias glacialis*
- *Ophidiaster ophidianus*
- *Paracentrotus lividus*

Cnidaria

- *Actinia equina*
- *Aiptasia* sp.
- *Anemonia viridis*
- *Cladocora caespitosa*
- *Condylactis auriantiaca*

Bryozoa

- *Miriapora truncata*
- *Schizoporella errata*

Annelida

- *Hermodice carunculata*
- *Protula* sp.
- *Serpula* sp.

Chordata

- *Halocynthia papillosa*

ANNEX 3

List of taxa considered for fish assemblages assessment (Lampedusa Island)

- *Coris julis*
- *Thalassoma pavo*
- *Symphodus* sp.
- *Labrus* sp.
- *Serranus cabrilla*
- *Serranus scriba*
- *Epinephelus marginatus*
- *Epinephelus costae*
- *Chromis chromis*
- *Sparisoma cretense*
- *Seriola carpenteri*
- *Seriola fasciata*
- *Seriola dumerili*
- *Fistularia commersonii*
- *Sphoeroides pachygaster*
- *Diplodus puntazzo*
- *Diplodus vulgaris*
- *Diplodus sargus*
- *Diplodus annularis*
- *Sarpa salpa*
- *Oblada melanura*
- *Sparus aurata*
- *Siganus luridus*
- *Scorpaena* sp.
- *Pseudocaranx* sp.
- *Dasyatis pastinaca*
- *Mullus* sp.
- *Spondyliosoma cantharus*
- *Spicara smaris*

ANNEX 4

Target non-indigenous and cryptogenic species (MPA Lastovo Archipelago and MPA Mljet)

- *Amathia verticillata*
- *Anadara inaequivalvis*
- *Aplysia dactylomela*
- *Asparagopsis armata*
- *Asparagopsis taxiformis*
- *Brachidontes pharaonis*
- *Bursatella leachi*
- *Bursatella leachii*
- *Callinectes sapidus*
- *Caulerpa cylindracea*
- *Caulerpa taxifolia*
- *Codium fragile*
- *Colpomenia peregrina*
- *Crassostrea gigas*
- *Crassostrea gigas*
- *Cyclopterus lumpus*
- *Equulites klunzingeri*
- *Ficopomatus enigmaticus*
- *Fistularia commersoni*
- *Hemigrapsus sanguineus*
- *Megabalanus tinntinnabulum*
- *Melibe fimbriata*
- *Musculista senhousia*
- *Percnon gibbesi*
- *Pinctada radiata*
- *Ruditapes philippinarum*
- *Siganus luridus*
- *Siganus rivulatus*
- *Sphyraena chrysotaenia*

ANNEX 5

List of fish taxa considered for fish assemblages assessment (Marine Protected Areas Lastovo Archipelago and Mljet)

- *Anthias anthias*
- *Apogon imberbis*
- *Diplodus annularis*
- *Diplodus puntazzo*
- *Diplodus sargus*
- *Diplodus vulgaris*
- *Epinephelus costae*
- *Epinephelus marginatus*
- *Labrus merula*
- *Labrus viridis*
- *Mullus surmuletus*
- *Muraena helena*
- *Scorpaena notata*
- *Scorpaena porcus*
- *Serranus cabrilla*
- *Serranus scriba*
- *Sparisoma cretense*
- *Spondylosoma cantharus*
- *Symphodus doderlaini*
- *Symphodus mediterraneus*
- *Symphodus melanocercus*
- *Symphodus rosalii*
- *Symphodus rostratus*
- *Symphodus tinca*

- *Oblada melanura*
- *Phycis phycis*
- *Sarpa salpa*
- *Thalassoma pavo*

Klaipėdos universiteto leidykla

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