Title: The vulnerability of fish and macroinvertebrate species with bioactive potential in a Mediterranean marine protected area

Arnau Carreño[1] and Josep Lloret[1]

1. Universitat de Girona. Faculty of sciences. C/Maria Aurelia Capmany, 69, 17003 Girona, Spain

Abstract

- A high percentage of marine animals produce bioactive compounds that may play a leading role in the discovery of future compounds and drugs of marine origin. However, commercial fishing and other human activities leading to sea warming and pollution may affect these marine animals, even putting them in danger of extinction.
- To date, no comprehensive studies have evaluated the conservation status of Mediterranean species with bioactive potential, which is crucial to better understanding how these species cope with the impacts of human activity.
- The study reviewed the bioactive potential and vulnerability of 833 fish and macro-invertebrate species inhabiting the marine protected area of Cap de Creus and surrounding areas. The most active taxa found were Porifera (49 out of 59 species; 83.0%) and Tunicata (17 out of 27 species; 63.0%). The most vulnerable species were Chondrichthyes (8 out of 9 species) and Porifera (9 out of 12 species), which together account for over 75% of species classified as such.
- Results emphasize the need to introduce specific management measures which protect vulnerable species with bioactive potential as this is a valuable component of marine ecosystems, as well as a potential source of molecules with pharmacological properties beneficial for human health.
- Marine protected areas can contribute to preserving marine species of medical interest and achieving their sustainable use in the marine biotechnology industry.

Key words:

vulnerability, marine drugs, Mediterranean, marine protected areas, medicine

1. Introduction

Marine organisms, which account for approximately 2 million species, are highly competitive and complex species which are forced to share limited space and compete for habitats (Simmons et al., 2005). As a result, a high percentage of species have adapted to these conditions by producing chemical compounds, often referred to as “bioactive compounds,” to defend themselves against predators, overgrowth of competing species, or conversely, to subdue prey (Ercolano et al., 2019; Williams et al., 1989). Bioactive compounds are complex molecules produced by an array of organisms, ranging from bacteria, fungi, and microalgae to complex organisms such as macroalgae, plants, and animals. These compounds are present in a wide range of molecules, including anticancer peptides, which are characterized by their cytotoxic and anti-tumor action against different lines of cancer cells; antibacterial and antiviral secondary metabolites; toxins (and antitoxins); and even essential oils, to which popular culture attributes numerous curative and therapeutic properties (Dhinakaran & Lipton, 2014; Malve, 2016; Swanson et al., 2012). The literature highlights the value of biodiversity for human health (Grifo and Rosenthal, 1997), one of its most obvious benefits being the large proportion of pharmaceutical compounds derived from the natural world (Alves & Rosa, 2007). However, in some circumstances, both biotechnological research and traditional (or alternative) medicine are contributing to the decline of plant and animal species such as rhinos, tigers, monkeys, pangolins and crabs in both terrestrial and marine ecosystems (Alves & Rosa, 2007, Anderson et al., 2013). Thus, many plants and animals may become extinct before scientists have the opportunity to fully discover their medicinal properties (Brower, 2008).

A number of animal species found in the Mediterranean Sea are known to have bioactive potential (Grienke et al., 2014; Ngo et al., 2012; Uriz et al., 1991), the majority of which are benthic organisms such as tunicates, sponges, bryozoans, and cnidarians. These species produce a wide variety of chemical compounds that act as a means of defense against predators, competing organisms, and parasites or invasive micro-organisms (Uriz et al., 1991; Menna, 2009; Ioannou et al., 2009; Ismail et al., 2008).

Despite a growing interest in the bioactive potential of these marine animal species for the discovery of future compounds and drugs, they are being affected by human activity, some to the point that they are in danger of extinction. Marine pollution (e.g., chemical compound spillage) damages populations of sponges and other slow growing organisms as it limits their filtering capacity (Zahn et al., 1977). Maritime recreational activities can also have a negative impact on species with bioactive potential. For example, anchors of moored boats damage algae and benthic organisms (Milazzo et al., 2004; Natalotto et al., 2015), and spearfishing, swimming, and scuba diving can harm sessile organisms inhabiting the rocky bottom (Hammerton, 2018). Sea warming caused by climate change can cause mass mortality of species due to the proliferation of thermophile opportunistic pathogenic organisms (Lloret, 2010). Finally, some professional fishing methods, such as trawling, profoundly affect marine biota (Dayton et al., 1995; Graham et al., 2001; Pipitone et al., 2000). However, to date, no in-depth studies have been carried out to evaluate the conservation status of Mediterranean species with bioactive potential. This research is crucial in order to better understand how these species cope with the impacts of human activities.

Thus, the aim of this paper is to evaluate the bioactive potential of animal species inhabiting the coastal waters of the NW Mediterranean, and relate this to their conservation status. Then using this analysis as a basis, we propose specific management measures that could be enforced in order to protect species with bioactive potential, and which are particularly vulnerable to human activities. The study focuses on a marine protected area (MPA) and its surrounding waters. Recreational and fishing activities are important here; yet so far no measures have been taken to protect these species with a potential role in the future development of new drugs. The main focus of the paper is benthic macro-invertebrates previously studied in other areas of the NW Mediterranean by Uriz et al. (1991), as well as other taxa not included in previous studies such as benthic and pelagic fish, cnidarians, and mollusks.

2. Material and methods

Study area

The study area is the marine protected area (MPA) of Cap de Creus, located in the north-east corner of the Iberian Peninsula, in the north-western Mediterranean (Figure 1). This MPA has a wide diversity of marine habitats (coralligenous, rocky, sandy, seagrass meadows, maërl, etc.) inhabited by a large variety of animal species. Despite the protected status of the area, it is used for a wide range of anthropogenic activities such as scuba diving, artisanal fishing, angling, shellfish collection and leisure boating, all of which impact the Cap de Creus flora and fauna (reviewed by Lloret & Riera, 2008).

Data sources (species)

This study focuses on marine fish and macro-invertebrates. Although marine micro-invertebrates may also present compounds with bioactive potential, these have not been included in this study as the existing list of micro-invertebrates in the study area is not sufficiently complete. The following databases were used to assess the macro-invertebrate and fish species inhabiting the natural park:

• The Joan Ortensi Marine Zoological Collection (Mallol, 2010). This collection comprises 16,000 specimens of 549 different marine species gathered between 1975 and 1991 from the surrounding waters of the Cap de Creus peninsula, including the Gulf of Lion.

• Data from the SafeNet Project, retrieved from the MEDITS trawl survey around the MPA, initiated in 1994.

• Data from monitoring macro-invertebrates and fish species caught through artisanal fishing in Cap de Creus in the years 2009, 2010, and 2015 (Lloret, 2015; Lloret et al., 2009, 2010).

• Species guide on the Catalan Opisthobranch Research Group website (GROC/Grup de Recerca d'Opistobranquis de Catalunya, 2009).

• Catalogue of the fauna of Cap de Creus (Parc Natural de Cap de Creus, 1999), compiled from the literature, and research published on the Iberian Fauna Project web page (CSIC, 1997).

Using the data sources listed above, 833 marine macro-invertebrates and fish were identified (Table 1).

Information related to the bioactive potential of the selected species

The literature was reviewed to gather information on the bioactive potential of the macro-invertebrate and fish species under study. Any study on that particular species carried out anywhere in the world was considered in the review. The databases of ScienceDirect, PubMed, and PlosOne (among others) were scrutinized using the following search criteria: name of species + type of bioactive potential (anti-tumor, antibacterial, antiviral, cytotoxic, antifungal, toxin, antioxidant, anti-inflammatory, or anticoagulant).

The species were then classified according to their bioactive potential type. The macroinvertebrate species were classified according to their phylum following the criteria used by Uriz et al., (1991): Bryozoa, Chordata, Cnidaria, Crustacea, Echinodermata, Mollusca, and Porifera. If the phylum contained over 150 species, they were separated into classes.

Vulnerability of species with bioactive potential

We used the framework established by Lloret et al. (2019) to assess the vulnerability of species with bioactive potential. Within this framework, the IUCN Red List - Mediterranean regional assessment (www.iucnredlist.org) was reviewed to check for those species classified as Threatened (i.e., Critically Endangered-CR, Endangered-EN, and Vulnerable-VU) and Near Threatened-NT. The IUCN Red List is recognized as one of the most authoritative sources of information on the global conservation status of species (Rodrigues et al., 2006) and classifies species at high risk of global extinction under various categories following well-established criteria (IUCN, 2020).

Species included in the IUCN Red List as Least Concern (LC), but with an index of vulnerability (IV) higher than 60 (i.e., high to very high vulnerability), were selected. The IV defines the intrinsic vulnerability of marine fishes to fishing and is calculated using fuzzy logic expert systems. It is based on the life history traits and ecological characteristics of the species, such as maximum body length, age at first maturity, the von Bertalanffy growth parameter K, natural mortality rate, maximum age, geographical range, annual fecundity, and the strength of aggregation behavior (Cheung et al., 2005; Cheung & Pitcher, 2008). Generally, the most vulnerable species are those with slow growth, low reproductive potential, larger body size, higher longevity, and a narrow geographical range. The index values range from 1 to 100, with 100 being the most vulnerable. Four levels of vulnerability were used: very high vulnerability to extinction (>70); high vulnerability (50–70); moderate vulnerability (30–50); and low vulnerability (<30). These IV values were obtained from FishBase (Froese and Pauly, 2019) for fishes, and from the SealifeBase (Palomares & Pauly, 2019) for macro-invertebrates. Finally, we also considered vulnerable those species with bioactive potential included in the Barcelona, Bern, or CITES conventions, and/or in the EU Habitats Directive, even though they appear on the IUCN Red List as LC or Data Deficient (DD) and having an IV index below 60.

Statistical analyses

Correspondence analyses (Legendre & Legendre, 1979) and graphics were performed on a species-activity matrix to ascertain relationships between activities using RStudio statistical software (RStudio, Inc.).

3. Results

Bioactive potential of the species

Of the 833 macro-invertebrate and fish species documented in the study area, 166 (19.9%) were found to have some kind of bioactive potential. The presence of bioactive potential varied depending on the main taxonomic group. The most documented active taxa were Agnatha (100%) (but only one species was documented); Porifera (49 out of 59 species; 83.0%); Tunicata (17 out of 27 species; 63.0%); and Cnidaria (19 out of 34 species (55.9%). (See Table 1 for aggregated data, and supplementary table 1 for species-specific information.)

The species studied were also classified into sessile and vagile animals in order to compare the proportion of species with bioactive potential within these categories. The proportion of sessile species with bioactive potential (40.4%) is much higher than that of vagile species with bioactive potential (12.1%) (See Table 2).

Activity and taxonomy

The species reviewed show different types of bioactive potential including antibacterial, antifungal, antioxidant, anti-tumor, cytotoxic, anti-inflammatory, and anticoagulant activities, and the capacity to produce biotoxins, which is distributed differently depending on the taxa.

The phylum with the highest antibacterial bioactivity was Porifera (37 out of 59 species; 62.7%), followed by Tunicata (6 out of 27;22.2%). Anti-tumor and cytotoxic bioactivities were mainly found in Porifera and Tunicata, and to a lesser extent, Cnidaria and Bryozoa. A low proportion of anti-tumor activity was also present in Chondrichthyes (4 out of 35; 11.4%), which had the same proportion of species with antioxidant potential. The highest antioxidant bioactive potential was found in Cephalopoda (2 out of 12; 16.7%) and Osteichthyes, (12 out of 152; 7.9%). Antiviral and anticoagulant bioactive potentials were less frequent (less than 3% in Cnidaria, Chondrichthyes, Gastropoda, and Bivalvia (excluding the Agnatha group, of which only one species has been found in Cap de Creus). Anti-inflammatory activities and biotoxins were mainly found in Cnidaria (Table 3).

Relationship between bioactive potential and taxa

The spatial ordination of bioactive potentials obtained from correspondence analysis (Figure 2) shows associations between antibacterial and antifungal potentials, and anti-inflammatory and anti-tumor potentials, as both pairs are on the same factorial axis. Cytotoxic bioactive potential was found between anti-inflammatory, anti-tumor, anticoagulant bioactive potentials, and biotoxins. However, antiviral and anticoagulant activity, and the presence of biotoxins was found to be unrelated to any other activity. For the most part, Porifera appear to have antifungal and antibacterial bioactive potential, and most of the cnidarians and opisthobranches show cytotoxic potential, or have biotoxins. Most Chondrichthyes and Osteichthyes with bioactive potential appear to correlate with antioxidant bioactive potential. No other clear associations were found between taxa and the presence of bioactive potentials.

Vulnerability of species with bioactive potential

Of the 166 species with bioactive potential documented in the study area, 80 were assessed for vulnerability by the IUCN Red List, IV, the Bern and Barcelona conventions and CITES. Of these 80 species, 32 (i.e., 40% of the total), are classified as vulnerable. Excluding agnate fish, the vulnerability of Chondrichthyes and Porifera with bioactive potential is of particular importance, as 8 of the 9 species of Chondrichthyes (88.9%) and 9 of the 12 species of Porifera (75%) are classified as vulnerable. It is also worth noting that the highest number of species (25) assessed for vulnerability were Osteichthyes, 9 of which have been classified as vulnerable (36%) (Figure 3).

The vulnerability of species with bioactive potential was also assessed, and then grouped by bioactive potential (Figure 4).

13 out of 78 species with antibacterial potential are classified as vulnerable; for example, *E. marginatus* or *D. dentex* have an IV index >60, and are under threat according to the IUCN Red List (*E. marginatus*: EN and *D. dentex*: VU). Furthermore, the sponge species *S. officinallis* and *A. polypoides* are listed in Annex III of the Barcelona and/or Bern conventions.

3 out of 10 species with antiviral potential were classified as vulnerable: *G. savaglia* (IV = 86; Annex II of Barcelona and Bern conventions, *S. acanthias* (IV = 74; Annex II of the Bonn Convention) and *P. marinus*, which is included in Annex III of the Barcelona and Bern conventions.

8 out of 28 species with antioxidant potential were classified as vulnerable. *S. canicula* and *M. mustelus* are the species with the highest IV (>70). Interesting to note is the blue shark *P. glauca* (IV = 69), which is critically endangered in the Mediterranean according to the IUCN Red List and Annex III of the Barcelona and Bern Conventions.

12 of 48 species with antitumoral potential have been classified as vulnerable (Figure 3). Examples which stand out are the basking shark (*C. maximus*) (IV = 73), which is listed as endangered in the Mediterranean by the IUCN, CITES, the Barcelona and Bern Conventions (Annex II), and the Bonn Convention (Annex I & II); the Bluefin tuna (*T. thynnus*) (IV = 74), listed as endangered in the Mediterranean by the IUCN and the Barcelona Convention (Annex III); and the sponge *Geodia cydonium*, (IV = 82), listed as endangered by the Bern Convention (annex II) and the Spanish List of Species Under Special Protection (Royal decree 139/2011).

Only 2 out of 42 species with cytotoxic bioactive potential have been classified as vulnerable: *Spongia agaricina*, listed in the Barcelona and Bern Conventions (annex III); and *Sarcotragus foetidus*, which is listed in the Barcelona convention (annex II).

Of the 18 species with anti-inflammatory potential, 6 have been listed as vulnerable. Examples are short-snouted seahorse (*H. hippocampus*) (IV = 66), which is near threatened according to the IUCN, and also listed in the CITES, Barcelona and Bern Conventions (annex II) and in the Spanish List of Species Under Special Protection (Royal Decree 139/2011). The sea urchin (*P. lividus*) (IV = 79) is also listed in the Barcelona and Bern Conventions (Annex III).

3 out of 5 species with anticoagulant potential are classified as vulnerable. The sea lamprey *P. marinus* stands out because although it is classified as least concern in the Mediterranean, with an IV of 40, it is listed in the Barcelona and Bern Conventions (Annex III) and Habitats Directive.

6 out of 36 species with antifungal potential are classified as vulnerable. Two examples are *Dasyatis pastinaca*, classified as vulnerable by the IUCN, and *Hippospongia communis*, included within the Barcelona convention (Annex III).

Finally, 8 out of 29 species with other types of bioactive potential were classified as vulnerable. Among these there are condrychtians *S. canicula* (IV = 72) and *M. mustelus* (IV = 77), listed in the Barcelona Convention (Annex III), and *G. galeus* (IV=74) classified as vulnerable by the IUCN and listed in the Barcelona Convention (Annex II), and the osteichthyan *L. piscatorius* (IV = 72).

4. Discussion

The results provide new insights into the bioactive potential of marine fish and macro-invertebrates and their conservation status in a Mediterranean MPA. In view of the importance of these species in the discovery of new marine drugs, the results also envisage management measures to protect them. The pharmacological importance of the taxa reviewed is highlighted by the fact that of the 833 marine fish and macro-invertebrates studied, 166 have bioactive potential (19.93%). The number of (benthic and pelagic) species reviewed in the present study is higher than that of Uriz et al. (1991), who analyzed 238 species of marine benthic organisms (algae, phanerogams, and invertebrates) for bioactive potential in the Balearic Sea.

Agnatha, Porifera, Tunicata, and Cnidaria are the taxa with the highest percentage of species showing bioactive potential. The majority of studies conducted worldwide to find molecules with bioactive potential currently focus on these three groups of marine benthic invertebrates (Suarez-Jimenez et al., 2012). This suggests that these taxa are also good candidates for the discovery of new sources of medicines in the study area. Nevertheless, our study also shows that vagile species such as groups of Chondrichthyes and Osteichthyes, cephalopods and vagile cnidarians could also be good candidates.

It is worth noting that almost 40% of the species documented with bioactive potential in Cap de Creus were considered vulnerable, indicating their fragility. Chondrichthyes and Porifera were the most vulnerable, with over 75% of these taxa with bioactive potential classified as vulnerable. For example, fractions isolated from blue shark (*Prionace glauca*) skin gelatin hydrolases (Weng et al., 2014) show strong antioxidant properties. The blue shark is also one of the most heavily exploited shark species. It is mainly caught as bycatch in longlines and driftnets while fishing for tuna and swordfish, but is also targeted by commercial and recreational fisheries. Despite being reported as Near Threatened worldwide on the IUCN Red List, its population is decreasing in the Mediterranean (classified as Critically Endangered), and it has an Intrinsic Vulnerability Index value of 77. Although recreational fishermen in Spain are unable to fish pelagic sharks such as the blue shark, they are not obliged to report bycatch, and therefore the impact on them is difficult to quantify (Lloret et al., 2019). Furthermore, almost half of species with antitumor potential, such as the bluefin tuna (*Thunnus thynnus*) and the common smooth-hound (*Mustelus mustelus*), are classified as vulnerable.

To date, MPA is the best-known and most effective management and conservation tool to protect marine ecosystems in the Mediterranean. Most of these protected areas are historical fishing spots along the coast, and particularly attractive destinations for small-scale fishing and recreational marine activities such as scuba diving or leisure boating (Font & Lloret, 2011; Venturini et al., 2016; Lucrezi et al., 2017). Various monitoring studies carried out in the study area show that many of the vulnerable species with bioactive potential are threatened by a number of anthropogenic factors such as fishing, tourism, pollution, and climate change (Lloret & Riera, 2008). For example, the warming of Mediterranean waters due to climate change is negatively affecting the growth and survival of gorgonian species such as *Paramuricea clavata*, and many other species such as red coral (*Corallium rubrum*) (Linares et al., 2005; Verdura et al., 2019). Populations of fish with bioactive potential are being threatened by the actions of small-scale, recreational fishing, both offshore and in MPAs, where large, slow growing species such as *E. marginatus* or *D. dentex* are especially targeted (Lloret et al., 2019; Lloret et al., 2008; Prato et al., 2016). In addition, populations of bryozoans, cnidarians, and sponges (e.g., *Pentapora fascialis* or *Reteporella grimaldii*) are damaged by anthropogenic activities such as scuba diving (Luna et al., 2009; Casoli et al., 2017) and fishing, where fishing lines and tackle damage sessile species (abrasion, strangulation, etc.) (Lloret et al., 2014). However, even though these species are all considered vulnerable, and there is considerable evidence which proves they are being affected by human activities and climate change, this does not mean that they are legally protected; even in an MPA such as the one in this study.

In comparison with marine ecosystems, the richest sources of bioactive compounds in terrestrial ecosystems are found in habitats with high complexity and biodiversity such as mangroves or rainforests (Zhou & Guo, 2012; Cândido et al., 2015; Avila-Sosa et al., 2019). However, a great many of these habitats are severely affected by climate change, and this together with a lack of national or international regulations on human activity in these areas puts the species inhabiting them under extreme threat (Osorio, Wingfield, & Roux, 2016; Roque et al., 2018), particularly animal species used in traditional medicine. Endangered mammals such as lions, elephants, hippopotamus, tigers, pandas, monkeys or pangolins are still being exploited to obtain products used in traditional medicines, and plant extinctions are occurring at a rate unmatched in geological history, leaving entire ecosystems impoverished and incomplete (Costa-Neto, 2005; Alves & Rosa, 2007). Several species of fauna and flora have become extinct and therefore potential sources of bioactive compounds lost (Brower, 2008; May, 2011). Several studies and reports provide recommendations on managing the exploitation of species with bioactive potential and securing the long-term survival of wild populations and their associated habitats. These studies highlight the contribution protected areas make to human health through their ecosystem services and the biodiversity inhabiting them (Alves & Rosa, 2007; Chivian, 2002; Solton et al., 2010; WHO, 2003). Despite the growing concern around protecting bioactive species and their habitats in order to preserve human health, most studies go no further than providing optional guidelines (i.e., good practices); they fail to propose mandatory legal regulations for governments, and as a result, these species and habitats continue to be endangered. Moreover, they only refer to terrestrial ecosystems, and it is in marine ecosystems where these guidelines are severely lacking. The discovery of drugs in both terrestrial and marine environments is in danger of species with bioactive potential becoming extinct (Brower, 2008; Leal & Calado, 2015). This has led the growing recognition of marine protected areas as sites where researchers can look for new medicines; for example, Kiunga Marine National Reserve (Kenya) (Solton et al., 2010).

MPAs can also contribute positively to protecting species with bioactive potential in a similar way to terrestrial ecosystems, and this should be part of their role as quality blue places of important recreational value. Thus, they can contribute to improving people's physical and mental health (Solton et al., 2010). However, specific management measures are required in order to protect marine species with bioactive potential within MPAs, particularly those listed as vulnerable. These measures should include monitoring vulnerable bioactive species populations and establishing specific measures to manage and protect them, for instance introducing a ban on fishing for *Epinephelus marginatus* or *Dentex dentex,* both of which have antibacterial bioactive potential. Several measures could be implemented to protect marine habitats inhabited by species with bioactive potential; for example, limit access for boats with high levels of pollution and prohibit anchoring on Posidonia beds and coralligenous assemblages as these are the habitat for several species with bioactive potential, e.g., *Pinna nobilis* and *Syngnathus acus*.

The goals set out by MPAs generally center around the protection of depleted, threatened, rare or endangered species, populations, habitats and the preservation of fishery resources (Kelleher & Kenchington, 1992; Di Franco et al., 2016). However, MPAs also need to be considered as tools for the conservation of wildlife species of medical interest, and as places where these species can be harvested in a sustainable way. To achieve these objectives, all stakeholders involved in developing, maintaining and using MPAs need to cooperate (Table 4), much in the same way Hawkins (2008) suggested for terrestrial medical plants.

Therefore, we propose that international bodies take into account the bioactive potential of species (as a complementary character of a species) to be used as a new indicator of goods and services marine ecosystems can provide to human health. However, the ultimate goal of the pharmaceutical industry should be to isolate and chemically synthesize molecules with bioactive potential, and thus, the continuous exploitation of the species need not occur.

It is also crucial to raise awareness of the importance of protecting species with bioactive potential that are vulnerable to human activities by informing the population in general, as well as MPA users, fishermen, and companies dealing with maritime recreational activities (diving, leisure boating, etc.). Dissemination activities should contribute to the idea that these species must be protected as they are valuable components of marine ecosystems; because they present a potential source of molecules with pharmacological properties which are beneficial for human health; and in the future, could potentially be used to discover new antibiotic, antifungal, antiviral, and antitumor drugs (Chivian, 2002; WHO, 2003).

Our results support the idea that marine biotechnology companies must sustainably harvest vulnerable species with bioactive potential. However, this is not always the case, the case of the Atlantic horseshoe crab (*Limulus polyphemus)* illustrates. This key species in the Nord-western Atlantic trophic chain is classified as vulnerable (VU) by the IUCN; however, in certain north-western Atlantic coasts it is decreasing because of fragmented habitats and overharvesting (Smith et al., 2017). Horseshoe crab blood is a crucial component of vaccines as it is used to detect endotoxins. In the context of the recent worldwide SARS-CoV2 pandemic, the search for a new vaccine means that this species will be even more overexploited (Krisfalusi-Gannon et al., 2018). Numbers of *Limulus polyphemus* are decreasing alarming due to the harvesting process, with mortality rates 10–30%, rising to 18% after bleeding, and the mortality rate after release as yet unknown (James-Pirri, Veillette, & Leschen, 2012; Anderson, Watson, & Chabot, 2013). Despite several studies validating the efficacy of synthetic alternatives to horseshoe crab blood, which would reduce the need to bleed this species by 90%, the pharmaceutical industry has yet to adopt any recombinant options (Maloney, Phelan, & Simmons, 2018). This example illustrates the urgent need for international agreements for the protection of vulnerable species such as horseshoe crabs.

Furthermore, according to the methodology criteria, approximately 80% of the macroinvertebrate and fish species with bioactive potential at Cap de Creus have not been classified as vulnerable. Therefore, it would also be advisable to cover these species under an agreement and/or regulation as they could be a source of new medicinal compounds of pharmacological interest.

Finally, it should be noted that although bioactive potential was not found in many (667) of the species in Cap de Creus, it may still exist in them, and could be detected if they were studied in greater depth. Further studies should therefore be conducted on species that are phylogenetically close to species that have documented bioactive potential in search of similar properties due to evolutionary proximity. Examples include *Sepia officinalis* and *S. pharaonis.* Both species have documented antibacterial properties in their ink and internal skeleton (Hajji et al., 2015; Karthik et al., 2017), as well as other antifungal and anti-tumor properties attributed to their salivary glands. Two species of cuttlefish inhabiting the study area (*S. elegans* and *S. orbignyana*) have not yet been studied; however, their phylogenetic proximity to species mentioned previously with antibacterial potential suggests that they could also possess some type of similar molecule, in a similar location. These, therefore, would be suitable potential candidates for future study.

Other possible candidates for future research are the tunicates *Clavelina lepadiformis*, *C. oblonga,* and *C. phlegraea*, which present anticoagulant, antifungal, and cytotoxic potential respectively (Aiello et al., 2007; Jugé et al., 2001; Kossuga et al., 2004). Moreover, to date, no studies have been carried out on *C. nana*. This is another species of the same genus and would be another good candidate for research due to its phylogenetic proximity.

Conclusions

The increasing pressure exerted on marine ecosystems underscores the importance of recognizing MPAs as sites where marine species with bioactive potential can be safeguarded. MPAs can contribute to preserving marine species with bioactive potential and protecting the habitats these species inhabit, while also regulating harvesting by the marine biotechnology pharmaceutical industry.

The interdependence between the sustainability of the marine environment and the sustainability of the marine biotechnology industry needs full recognition, and should be translated into policies and actions leading to the sustainable use of marine species with bioactive potential in pharmacological research.

Diverse stakeholders need to be involved in designing regulatory measures to avoid marine biodiversity losses that may be important for the discovery of new drugs of marine origin. These stakeholders include MPA managers, policy makers and the pharmaceutical industry, all of whom must be made aware of the need to conserve marine resources in order to secure their sustainable exploitation for biomedical research.

Acknowledgements

This research has benefited from a PhD fellowship awarded to A. Carreño by Tossa de Mar Town Council and the Oceans & Human Health Chair. The Oceans & Human Health Chair is supported by the University of Girona, Roses Town Council, the Catalan Fishmongers Association and the Roses Fishermen's Guild. This study was funded by La Caixa Foundation/Cap de Creus Natural Park and the Interreg Pharos4MPAs project.

The authors would like to thank Raül Golo for help with the statistical analyses and Peter Redmond and Kate Wiggins for language editing and proofreading.

Bibliography

Aiello A., Fattorusso E., Giordano A., Menna M., Navarrete C., & Muñoz E. 2007. Clavaminols A–F, novel cytotoxic 2-amino-3-alkanols from the ascidian Clavelina phlegraea. *Bioorganic & Medicinal Chemistry* 15: 2920–2926.

Alves R.R.N., & Rosa I.M.L. 2007. Biodiversity, traditional medicine and public health: Where do they meet? *Journal of Ethnobiology and Ethnomedicine* 3: 14.

Anderson R.L., Watson W.H., & Chabot CC. 2013. Sublethal behavioral and physiological effects of the biomedical bleeding process on the american horseshoe crab, Limulus polyphemus. *Biological Bulletin* 225: 137–151.

Avila-Sosa R., Montero-Rodríguez A.F., Aguilar-Alonso P., Vera-López O., Lazcano-Hernández M., Morales-Medina JC., & Navarro-Cruz AR. 2019. Antioxidant Properties of Amazonian Fruits: A Mini Review of In Vivo and In Vitro Studies. *Oxidative Medicine and Cellular Longevity* 2019.

Brower V. 2008. Back to nature: Extinction of medicinal plants threatens drug discovery. *Journal of the National Cancer Institute* 100: 838–839.

Cândido T.L.N., Silva M.R., & Agostini-Costa TS. 2015. Bioactive compounds and antioxidant capacity of buriti (Mauritia flexuosa L.f.) from the Cerrado and Amazon biomes. *Food Chemistry* 177: 313–319.

Casoli E., Nicoletti L., Mastrantonio G., Jona-Lasinio G., Belluscio A., & Ardizzone G.D. 2017. Scuba diving damage on coralligenous builders: Bryozoan species as an indicator of stress. *Ecological Indicators* 74: 441–450.

~~Center for Health and the Global Environment. 2002. Biodiversity: Its Importance to Human Health~~

Cheung WWL., & Pitcher TJ. 2008. Evaluating the status of exploited taxa in the northern South China Sea using intrinsic vulnerability and spatially explicit catch-per-unit-effort data. *Fisheries Research* 92: 28–40.

Cheung WWL., Pitcher TJ., & Pauly D. 2005. A fuzzy logic expert system to estimate intrinsic extinction vulnerabilities of marine fishes to fishing. *Biological Conservation* 124: 97–111.

Chivian E. 2002. Biodiversity: Its Importance to Human Health. Center for Health and the Global Environment. Harvard Medical School.

Costa-Neto EM. 2005. Animal-based medicines: Biological prospection and the sustainable use of zootherapeutic resources. *Anais da Academia Brasileira de Ciencias* 77: 33–43.

CSIC. 1997. Fauna Ibérica.

Dayton PK., Thrush SF., Agardy MT., & Hofman RJ. 1995. Environmental effects of marine fishing. *Aquatic Conservation: Marine and Freshwater Ecosystems* 5: 205–232.

Dhinakaran DI., & Lipton AP. 2014. Bioactive compounds from Holothuria atra of Indian ocean. *SpringerPlus* 3: 673.

Ercolano G., De Cicco P., & Ianaro A. 2019. New Drugs from the Sea: Pro-Apoptotic Activity of Sponges and Algae Derived Compounds. *Marine drugs* 17.

Font T., & Lloret J. 2011. Biological implications of recreational shore angling and harvest in a marine reserve: the case of Cape Creus. *Aquatic Conservation: Marine and Freshwater Ecosystems* 21: 210–217.

Froese, R. and D. Pauly. Editors. 2019. *FishBase. World Wide Web electronic publication.* Available at: www.fishbase.org [version (12/2019)]. [Accessed 17 September 2020]

Di Franco A., Thiriet P., Di Carlo G., Dimitriadis C., Francour P., Gutiérrez N.L., De Grissac A.J., Koutsoubas D., Milazzo M., Otero M., et al. 2016. Five key attributes can increase marine protected areas performance for small-scale fisheries management. *Scientific Reports*: 1–9.

Graham K.J., Andrew N.L., & Hodgson K.E. 2001. Changes in relative abundance of sharks and rays on Australian South East Fishery trawl grounds after twenty years of fishing. *Marine and Freshwater Research* 52: 549.

Grienke U., Silke J., & Tasdemir D. 2014. Bioactive compounds from marine mussels and their effects on human health. *Food Chemistry* 142: 48–60.

Hajji S., Younes I., Rinaudo M., Jellouli K., & Nasri M. 2015. Characterization and In Vitro Evaluation of Cytotoxicity, Antimicrobial and Antioxidant Activities of Chitosans Extracted from Three Different Marine Sources. *Applied Biochemistry and Biotechnology* 177: 18–35.

Hammerton Z. 2018. Risk assessment of SCUBA diver contacts on subtropical benthic taxa. *Ocean & Coastal Management* 158: 176–185.

Hawkins B. 2008. Plants for life: Medicinal plant conservation and botanic gardens. Botanic Gardens Conservation International, Richmond, U.K

Ioannou E., Abdel-Razik AF., Zervou M., Christofidis D., Alexi X., Vagias C., Alexis MN., & Roussis V. 2009. 5alpha,8alpha-Epidioxysterols from the gorgonian Eunicella cavolini and the ascidian Trididemnum inarmatum: isolation and evaluation of their antiproliferative activity. *Steroids* 74: 73–80.

Ismail H., Lemriss S., Ben Aoun Z., Mhadhebi L., Dellai A., Kacem Y., Boiron P., & Bouraoui A. 2008. Antifungal activity of aqueous and methanolic extracts from the Mediterranean sea cucumber, Holothuria polii. *Journal de Mycologie Médicale* 18: 23–26.

IUCN. 2020. *IUCN Red List of Threatened Species.* Available at: https://www.iucn.org/resources/conservation-tools/iucn-red-list-threatened-species. [Accessed 17 September 2020]

James-Pirri M.J., Veillette P.A., & Leschen A.S. 2012. Selected hemolymph constituents of captive, biomedically bled, and wild caught adult female American horseshoe crabs (Limulus polyphemus). *Marine and Freshwater Behaviour and Physiology* 45: 281–289.

Jugé M., Grimaud N., Biard JF., Sauviat MP., Nabil M., Verbist JF., & Petit JY. 2001. Cardiovascular effects of lepadiformine, an alkaloid isolated from the ascidians Clavelina lepadiformis (Müller) and C. moluccensis (Sluiter). *Toxicon : official journal of the International Society on Toxinology* 39: 1231–7.

Karthik R., Manigandan V., Ebenezar K., Vijayashree R., & Saravanan R. 2017. Data supporting the anticancer activity of posterior salivary gland (PSG) toxin from the cuttlefish Sepia pharaonis Ehrenberg (1831). *Data in brief* 13: 295–300.

Kelleher G., & Kenchington R. 1992. Guidelines for Establishing Marine Protected Areas. A Marine Conservation and Development Report. IUCN, Gland, Switzerland. : vii+ 79 pp.

Kossuga MH., MacMillan JB., Rogers EW., Molinski TF., Nascimento GGF., Rocha RM., & Berlinck RGS. 2004. (2 *S* ,3 *R*)-2-Aminododecan-3-ol, a New Antifungal Agent from the

Ascidian *Clavelina o blonga*. *Journal of Natural Products* 67: 1879–1881.

Krisfalusi-Gannon J., Ali W., Dellinger K., Robertson L., Brady T.E., Goddard MKM., Tinker-Kulberg R., Kepley C.L., & Dellinger A.L. 2018. The Role of Horseshoe Crabs in the Biomedical Industry and Recent Trends Impacting Species Sustainability. *Frontiers in Marine Science* 5: 185.

Leal MC., & Calado R. 2015. Marine Natural Products: Biodiscovery, Biodiversity, and Bioproduction. In *Bioactive Natural Products* Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany; 473–490.

Legendre, L., Legendre, P. (1979). I. Le traitement multiple des donnees ecologiques. 11. La structure des donnees ecologiques. *Ecologie numerique.* 1st Paris: Masson.

Linares C., Coma R., Diaz D., Zabala M., Hereu B., & Dantart L. 2005. Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. *Marine Ecology Progress Series* 305: 127–137.

Lloret J. 2015. Seguiment de la pesca artesanal al Parc Natural de Cap de Creus. Any 2015

Lloret J., & Riera V. 2008. Evolution of a Mediterranean Coastal Zone: Human Impacts on the Marine Environment of Cape Creus. *Environmental Management* 42: 977–988.

Lloret J., Casadevall M., & Muñoz M. 2009. Seguiment d'espècies d'interès pesquer a les aigües del Parc Natural de Cap de Creus. Informe anual Abril 2008-Març 2009.

Lloret J., Casadevall M., & Muñoz M. 2010. Seguiment de la pesca artesanal al Parc Natural de Cap de Creus. Estudis 2008-2010.

Lloret J., Zaragoza N., Caballero D., Font T., Casadevall M., & Riera V. 2008. Spearfishing pressure on fish communities in rocky coastal habitats in a Mediterranean marine protected area. *Fisheries Research* 94: 84–91.

Lloret J., Garrote A., Balasch N., & Font T. 2014. Estimating recreational fishing tackle loss in Mediterranean coastal areas: Potential impacts on wildlife. *Aquatic Ecosystem Health & Management* 17: 179–185.

Lloret J., Biton-Porsmoguer S., Carreño A., Franco A Di., Sahyoun R., Melia P., Claudet J., Sève C., Ligas A., Belharet M., et al. 2019. Recreational and small-scale fisheries may pose a threat to vulnerable species in coastal and offshore waters of the western Mediterranean. *ICES Journal of Marine Science*.

Lucrezi S., Milanese M., Markantonatou V., Cerrano C., Sarà A., Palma M., & Saayman M. 2017. Scuba diving tourism systems and sustainability: Perceptions by the scuba diving industry in two Marine Protected Areas. *Tourism Management* 59: 385–403.

Luna B., Pérez CV., Luis J., Luna S-L., Pérez V., Sánchez-Lizaso C., Luna B., Pérez C V., & Sánchez-Lizaso JL. 2009. Benthic impacts of recreational divers in a Mediterranean Marine Protected Area. – *ICES Journal of Marine Science* 66: 517–523.

Maloney T., Phelan R., & Simmons N. 2018. Saving the horseshoe crab: A synthetic alternative to horseshoe crab blood for endotoxin detection. *PLoS Biology* 16.

Malve H. 2016. Exploring the ocean for new drug developments: Marine pharmacology. *Journal of Pharmacy & Bioallied Sciences* 8: 83.

May RM. 2011. Why should we be concerned about loss of biodiversity. *Comptes Rendus -*

Biologies 334: 346–350.

Milazzo M., Badalamenti F., Ceccherelli G., & Chemello R. 2004. Boat anchoring on Posidonia oceanica beds in a marine protected area (Italy, western Mediterranean): effect of anchor types in different anchoring stages. *Journal of Experimental Marine Biology and Ecology* 299: 51–62.

Natalotto A., Sureda A., Maisano M., Spanò N., Mauceri A., & Deudero S. 2015. Biomarkers of environmental stress in gills of Pinna nobilis (Linnaeus 1758) from Balearic Island. *Ecotoxicology and Environmental Safety* 122: 9–16.

Ngo D.H., Vo T.S., Ngo D.N., Wijesekara I., & Kim S.K. 2012. Biological activities and potential health benefits of bioactive peptides derived from marine organisms. *International Journal of Biological Macromolecules* 51: 378–383.

Osorio J.A., Wingfield M.J., & Roux J. 2016. A review of factors associated with decline and death of mangroves, with particular reference to fungal pathogens. *South African Journal of Botany* 103: 295–301.

Palomares M.L.D., & Pauly D. 2019. SeaLifeBase. *World Wide Web electronic publication.*

Parc Natural de Cap de Creus. 1999. Catàleg de la fauna de Cap de Creus. : 1–50.

Pipitone C., Badalamenti F., D'Anna G., & Patti B. 2000. Fish biomass increase after a four-year trawl ban in the Gulf of Castellammare (NW Sicily, Mediterranean Sea). *Fisheries Research* 48: 23–30.

Prato G., Barrier C., Francour P., Cappanera V., Markantonatou V., Guidetti P., Mangialajo L., Cattaneo-Vietti R., & Gascuel D. 2016. Assessing interacting impacts of artisanal and recreational fisheries in a small Marine Protected Area (Portofino, NW Mediterranean Sea). *Ecosphere* 7.

Rodrigues ASL., Pilgrim JD., Lamoreux JF., Hoffmann M., & Brooks TM. 2006. The value of the IUCN Red List for conservation. *Trends in Ecology and Evolution* 21: 71–76.

Roque Fdo., Menezes J.F.S., Northfield T., Ochoa-Quintero J.M., Campbell M.J., & Laurance W.F. 2018. Warning signals of biodiversity collapse across gradients of tropical forest loss. *Scientific Reports* 8.

Simmons T.L., Andrianasolo E., McPhail K., Flatt P., & Gerwick W.H. 2005. Marine natural products as anticancer drugs. *Molecular cancer therapeutics* 4: 333–42.

Smith D.R., Brockmann H.J., Beekey MA., King T.L., Millard M.J., & Zaldívar-Rae J. 2017. Conservation status of the American horseshoe crab, (Limulus polyphemus): a regional assessment. *Reviews in Fish Biology and Fisheries* 27: 135–175.

Solton S., Dudley N., & Equilibrium research. 2010. Vital Sites: The contribution of protected areas to human health

Suarez-Jimenez G-M., Burgos-Hernandez A., & Ezquerra-Brauer J-M. 2012. Bioactive peptides and depsipeptides with anticancer potential: sources from marine animals. *Marine drugs* 10: 963–86.

Swanson D., Block R., & Mousa SA. 2012. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in nutrition (Bethesda, Md.)* 3: 1–7.

Uriz M.J., Martin D., Turon X., Ballasteros E., Hughes R., & Acebal C. 1991. An approach to the ecological significance of chemically mediated bioactivity in Mediterranean benthic communities. *Marine Ecology Progress Series* 70: 175–188.

Venturini S., Massa F., Castellano M., Costa S., Lavarello I., Olivari E., & Povero P. 2016. Recreational Boating in Ligurian Marine Protected Areas (Italy): A Quantitative Evaluation for a Sustainable Management. *Environmental Management* 57: 163–175.

Verdura J., Linares C., Ballesteros E., Coma R., Uriz M.J., Bensoussan N., & Cebrian E. 2019. Biodiversity loss in a Mediterranean ecosystem due to an extreme warming event unveils the role of an engineering gorgonian species. *Nature Scientific Reports* 9.

Weng W., Tang L., Wang B., Chen J., Su W., Osako K., & Tanaka M. 2014. Antioxidant properties of fractions isolated from blue shark (Prionace glauca) skin gelatin hydrolysates. *Journal of Functional Foods* 11: 342–351.

WHO. 2003. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants., Geneva.

Williams D.H., Stone M.J., Hauck P.R., & Rahman S.K. 1989. Why Are Secondary Metabolites (Natural Products) Biosynthesized? *Journal of Natural Products* 52: 1189–1208.

Zahn R.K., Zahn G., Müller W.E.G., Müller I., Beyer R., Müller-berger U., Kurelec B., Rijavec M., & Britvić S. 1977. Consequences of detergent pollution of the sea: Effects on regenerating sponge cubes of Geodia cydonium. *Science of The Total Environment* 8: 109–151.

Zhou Z.F., & Guo Y.W. 2012. Bioactive natural products from Chinese marine flora and fauna. *Acta Pharmacologica Sinica* 33: 1159–1169.

Tables

Table 1. Summary of the number of species in the Cap de Creus MPA distributed per taxa (macroinvertebrates and fish) found in the consulted data sources.

Group	No. of species	Relative frequency (%)	No. species with Bioactive Potential	Relative freq. (%)
Tunicata	27	3.241	17	62.9
Bivalvia	101	12.125	9	8.9
Gastropoda	107	12.845	7	6.5
Cephalopoda	12	1.441	2	16.7
Opisthobranchia	132	15.846	8	6.1
Poliplacophora	2	0.240	0	0.0
Scaphopoda	2	0.240	0	0.0
Bryozoa	12	1.441	5	41.7
Cnidaria	34	4.082	19	55.9
Agnatha	1	0.120	1	100.0
Chondrichthyes	35	4.202	9	25.7
Osteichthyes	152	18.247	25	16.4
Crustacea	108	12.965	8	7.4
Porifera	59	7.083	49	83.1
Echinodermata	49	5.882	7	14.3
TOTAL	833	100.000	166	19.9

Table 2: Proportion of bioactive vagile and sessile species with bioactive potential in Cap de Creus.

Category	No. of species with bioactive potential	Total no. of species reviewed	Relative frequency (%)
Sessile	93	230	40.4%
Vagile	73	603	12.1%
Total	166	833	19.9%

Table 3. Percentages of species, grouped by their taxa, reported with bioactive potential per taxa.

Group/Potential	Antibacterial	Antiviral	Antioxidant	Anti-tumor	Cytotoxic	Anti-inflammatory	Anticoagulant	Antifungal	Biotoxin	Others
Tunicata	22.22	0.00	0.00	29.63	40.74	0.00	0.00	7.41	0.00	7.41
Bivalvia	3.96	1.98	1.98	1.98	0.00	1.98	0.00	0.99	3.96	0.00
Bryozoa	16.67	0.00	8.33	8.33	16.67	0.00	0.00	0.00	0.00	8.33
Gastropoda	2.80	2.80	0.00	0.00	0.93	0.00	0.00	0.00	3.74	0.93
Cephalopoda	16.67	0.00	16.67	8.33	0.00	0.00	0.00	8.33	0.00	16.67

Opisthobranchia	0.76	0.76	0.00	0.76	2.27	1.52	0.00	0.76	3.03	0.00
Cnidaria	11.76	2.94	5.88	14.71	20.59	14.71	2.94	0.00	26.47	8.82
Agnatha	0.00	100.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
Chondrichthyes	2.86	2.86	11.43	11.43	0.00	0.00	2.86	2.86	0.00	8.57
Osteichthyes	5.92	0.00	7.89	2.63	0.66	1.97	0.00	0.00	0.66	5.26
Crustacea	4.63	0.00	3.70	2.78	0.93	0.00	0.93	0.00	0.93	2.78
Porifera	62.71	1.69	1.69	25.42	27.12	5.08	0.00	47.46	0.00	6.78
Echinodermata	8.16	0.00	0.00	8.16	0.00	6.12	0.00	4.08	0.00	2.04

Table 4. Stakeholders involved in the creation, maintenance, use and protection of the Marine Protected Areas (MPAs) and their relationship with marine species with bioactive potential. Adapted from Hawkins (2008).

Ecologists	To understand the complex relations between bioactive species and marine ecosystems they live in.
Health policy makers	To include conservation and utilization of marine species with bioactive potential in marine policies and planning
Legal experts	To develop effective legal mechanisms to ensure sustainable harvesting of marine species with bioactive potential
MPA managers	To conserve species with bioactive potential within MPAs
MPA planners	To ensure that the MPA contains the maximum diversity of marine species with bioactive potential
Pharmacologists	To study the application of marine bioactive species
Resource economists	To evaluate patterns of use and value of marine bioactive species
Taxonomists	To accurately identify marine organisms with bioactive potential
Biotechnology industry	To ensure that recombinant options are produced after isolation of the bioactive compound from the marine species

Figure legends

Figure 1. The Marine Protected Area of Cap de Creus and its boundaries.

Figure 2. Spatial representation of the correspondence analysis on a species-activities matrix.

Figure 3. Proportion of species of Cap de Creus with bioactive potential classified as vulnerable, grouped by taxa.

Figure 4. Percentage of bioactive species classified as vulnerable, grouped by their bioactive potential.

Appendices

Supplementary table 1: Bioactive potential and vulnerability of the species with bioactive potential reviewed (166). AB: antibacterial; AV: antiviral; AO: antioxidant; AT: antitumoral; C: cytotoxic; AI: anti-inflammatory; AC: anticoagulant; AF: antifungal; B: biotoxin; O: Other; IV: Index of vulnerability; IUCN: Classification in the IUCN red list, Mediterranean assessment; VUL: Species classified as vulnerable; LC: Least Concern; NT: Near threatened; VU: Vulnerable; EN: Endangered; CE: Critically endangered.

Taxa	Species	Mobility	AB	AV	AO	AT	C	AI	AC	AF	B	O	IV	IUCN	Convention	VUL
Agnatha	*Petromyzon marinus*	vagile		X					X				40	LC	Barcelona & Bern Conventions (Annex III); Habitats Directive; CNEA	YES
Bivalvia	*Acanthocardia tuberculata*	sessile									X		10	NA		NO
	Aequipecten opercularis	sessile									X		22	NA		NO
	Cerastoderma edule	sessile	X	X									24	NA		NO
	Modiolus modiolus	sessile	X			X		X					60	NA		YES
	Mytilus edulis	sessile	X			X		X	X	X			20	NA	Law Generalitat de Catalunya	YES
	Mytilus galloprovincialis	sessile			X						X		10	NA		NO
	Ostrea edulis	sessile	X	X									NA	NA		NA
	Pinna nobilis	sessile			X								32	NA	Habitats Directive (Annex IV); Barcelona Convention (Annex II); CNEA	YES
	Solen marginatus	sessile									X		NA	NA		NA

Bryozoa	*Bugula neritina*	sessile				X							10	NA		NO
	Myriapora truncata	sessile	X				X						NA	NA		NA
	Pentapora fascialis	sessile	X									X	NA	NA		NA
	Schizomavella mamillata	sessile			X								NA	NA		NA
	Schizoporella errata	sessile					X						NA	NA		NA
Cephalopoda	*Sepia officinalis*	vagile	X		X	X				X		X	30	LC		NO
	Loligo vulgaris	vagile	X		X							X	19	NA		NO
Chondrichthyes	*Galeorhinus galeus*	vagile										X	36	VU	Barcelona Convention (Annex II)	YES
	Raja clavata	vagile			X	X							60	NT		YES
	Raja montagui	vagile							X				57	LC		NO
	Prionace glauca	vagile			X								69	CE	Barcelona & Bern Conventions (Annex III)	YES
	Scyliorhinus canicula	vagile			X							X	72	LC		YES
	Cetorhinus maximus	vagile				X							73	EN	CITES, Barcelona & Bern Conventions (Annex II); Bonn Convention (Annex I & II); Royal Decree of Wild Species (BOE ESP)	YES
	Squalus acanthias	vagile		X									74	EN		YES
	Mustelus mustelus	vagile			X	X						X	77	VU	Barcelona Convention (Annex III)	YES
	Dasyatis pastinaca	vagile	X			X				X			NA	VU		YES

Cnidaria	*Gerardia savaglia*	sessile		X									86	NA	Barcelona & Bern conventions (annex II); List of species with special protection in Spain (Royal Decree 139/2011)	YES
	Eunicella verrucosa	sessile					X						NA	NT		NO
	Eunicella singularis	sessile			X	X		X				X	NA	NA		NA
	Leptogorgia sarmentosa	sessile					X						NA	NA		*NA*
	Aequorea forskalea	vagile										X	10	NA		NO
	Aurelia aurita	vagile	X								X		25	NA		NO
	Chrysaora hysoscella	vagile									X		16	NA		NO
	Rhizostoma pulmo	vagile					X		X		X		NA	NA		NA
	Pelagia noctiluca	vagile				X		X			X		10	NA		NO
	Veretillum cynomorium	sessile										X	NA	NA		NA
	Pennatula phosphorea	sessile	X										NA	NA		NA
	Pteroeides spinosum	sessile	X										NA	NA		NA
	Pennatula aculeata	sessile				X		X					NA	NA		NA
	Actinia equina	sessile	X			X	X	X			X		10	NA		NO
	Actinia cari	sessile					X				X		10	DD		NO
	Actinia fragacea	sessile									X		NA	NA		NA
	Anemonia sulcata	sessile					X	X			X		NA	NA		NA
	Condylactis aurantiaca	sessile				X	X				X		10	LC		NO
	Cotylorhiza tuberculata	vagile			X								23	NA		NO
Crustacea	*Maja crispata*	vagile	X		X							X	NA	NA		NA

	Nephrops norvegicus	vagile				X	X		X				14	LC		NO
	Parapenaeus longirostris	vagile	X		X							X	10	NA		NO
	Penaeus kerathurus	vagile	X		X	X							17	NA		NO
	Carcinus maenas	vagile									X		10	NA		NO
	Carcinus mediterraneus	vagile	X		X	X							NA	NA		NA
	Pagurus bernhardus	vagile	X										NA	NA		NA
	Ceratothoa oestroides	vagile										X	NA	NA		NA
Echinodermata	*Paracentrotus lividus*	vagile	X					X				X	79	NA	Barcelona & Bern Conventions (Annex III)	YES
	Marthasterias glacialis	vagile	X			X		X					NA	NA		NA
	Holothuria tubulosa	vagile				X		X					20	LC		NO
	Holothuria polii	vagile								X			10	NA		NO
	Stichopus regalis	vagile	X			X				X			25	LC		NO
	Hippasteria phrygiana	vagile				X							NA	NA		NA
	Strongylocentrotus droebachiensis	vagile	X										NA	NA		NA
Gastropoda	*Euthria cornea*	vagile		X									10	NA		NO
	Buccinum undatum	vagile	X	X									NA	NA		NA
	Crepidula fornicata	vagile	X	X									NA	NA		NA
	Bolinus brandaris	vagile									X		10	NA		NO
	Neptunea antiqua	vagile									X	X	NA	NA		NA
	Stramonita haemastoma	vagile									X	X	NA	NA		NA
	Hexaplex trunculus	vagile	X				X				X		NA	NA		NA
Opisthobranchia	*Dendrodoris grandiflora*	vagile									X		NA	NA		NA
	Dendrodoris limbata	vagile									X		NA	NA		NA
	Doris verrucosa	vagile	X	X						X			NA	NA		NA
	Janolus cristatus	vagile									X		NA	NA		NA

	Spurilla neapolitana	vagile									X		10	NA		NO
	Aplysia fasciata	vagile					X	X					NA	NA		NA
	Aplysia depilans	vagile					X	X					NA	NA		NA
	Aplysia punctata	vagile				X	X						NA	NA		NA
Osteichthyes	*Trachinotus ovatus*	vagile	X										41	LC		NO
	Trachurus mediterraneus	vagile			X								47	LC		NO
	Sardina pilchardus	vagile			X			X					27	LC		NO
	Sardinella aurita	vagile	X		X								37	LC		NO
	Coris julis	vagile			X								39	LC		NO
	Umbrina cirrosa	vagile			X								60	NA	Barcelona & Bern Conventions (Annex III)	YES
	Sarda sarda	vagile			X							X	33	LC		NO
	Scomber japonicus	vagile			X	X							31	LC		NO
	Scomber scombrus	vagile	X		X								44	LC		NO
	Scophthalmus maximus	vagile	X										43	NA		NO
	Scorpaena notata	vagile			X							X	27	LC		NO
	Dicentrarchus labrax	vagile	X		X							X	60	NT		YES
	Dentex dentex	vagile	X										62	VU		YES
	Diplodus sargus	vagile										X	63	LC		YES
	Lithognathus mormyrus	vagile										X	40	LC		NO
	Hippocampus hippocampus	vagile			X			X					66	NT	CITES, Barcelona & Bern Conventions (annex II); List of species with special protection in Spain (Royal	YES

															Decree 139/2011)	
	Syngnathus acus	vagile				X							68	LC	Bern Convention (annex III)	YES
	Trachinus draco	vagile					X				X	X	42	LC		NO
	Sparus aurata	vagile	X		X							X	40	LC		NO
	Lophius piscatorius	vagile										X	72	LC		YES
	Epinephelus marginatus	vagile	X										72	VU	Barcelona & Bern Conventions (Annex III)	YES
	Solea senegalensis	vagile	X										49	DD		NO
	Mugil cephalus	vagile				X							42	LC		NO
	Thunnus alalunga	vagile						X					58	LC		NO
	Thunnus thynnus	vagile				X							74	EN	Barcelona Convention (Annex III)	YES
Porifera	*Geodia cydonium*	sessile				X		X					82	NA	Bern Convention (annex II); List of species with special protection in Spain (Royal decree 139/2011)	YES
	Spongia officinalis	sessile	X			X		X		X			NA	NA	Barcelona & Bern Conventions (annex III)	YES
	Axinella verrucosa	sessile										X	NA	NA	Barcelona & Bern Conventions (Annex III)	YES

	Axinella damicornis	sessile	X			X				X		X	10	NA		NO
	Axinella polypoides	sessile	X							X			NA	NA	Barcelona convention III	YES
	Aplysina aerophoba	sessile	X			X							NA	NA	Barcelona Convention (annex II)	YES
	Aplysina Cavernicola	sessile	X										NA	NA	Barcelona & Bern conventions (annex II)	YES
	Spongia agaricina	sessile					X						NA	NA	Barcelona & Bern Conventions (annex III)	YES
	Haliclona viscosa	sessile	X							X			NA	NA		NA
	Petrosia ficiformis	sessile	X				X						NA	NA		NA
	Hamacantha johnsoni	sessile								X			NA	NA		NA
	Hyrtios erecta	sessile				X	X						NA	NA		NA
	Jaspis johnstoni	sessile								X		X	NA	NA		NA
	Acanthella acuta	sessile	X			X				X			NA	NA		NA
	Scopalina lophyropoda	sessile	X							X			NA	NA		NA
	Chondrosia reniformis	sessile	X							X			NA	Least Concern		NO
	Agelas oroides	sessile	X			X				X			NA	NA		NA
	Dictyonella incisa	sessile	X							X			NA	NA		NA
	Haliclona mediterranea	sessile	X							X			NA	NA		NA
	Haliclona fulva	sessile	X							X			NA	NA		NA
	Haliclona mucosa	sessile	X			X	X			X			NA	NA		NA
	Phorbas fictitius	sessile	X				X			X			NA	NA		NA
	Phorbas tenacior	sessile	X							X			NA	NA		NA
	Crambe crambe	sessile	X	X			X			X			NA	NA		NA

	Crambe tailliezi	sessile	X							X			NA	NA		NA
	Hemimycalle columella	sessile	X										NA	NA		NA
	Scalarispongia scalaris	sessile	X				X			X			NA	NA		NA
	Hippospongia communis	sessile	X			X				X			NA	NA	Barcelona convention (Annex III)	YES
	Sarcotragus foetidus	sessile	X				X			X			NA	NA	Barcelona convention (Annex II)	YES
	Ircinia oros	sessile	X				X			X			NA	NA		NA
	Ircinia variabilis	sessile	X				X			X			NA	NA		NA
	Spongia nitens	sessile	X							X			10	NA		NO
	Raspaciona aculeata	sessile				X	X						NA	NA		NA
	Cliona carteri	sessile	X										NA	NA		NA
	Cliona celata	sessile	X										NA	NA		NA
	Cliona viridis	sessile	X		X					X			NA	NA		NA
	Crella mollior	sessile					X						NA	NA		NA
	Ciocalypta penicillus	sessile	X										NA	NA		NA
	Dysidea avara	sessile	X			X	X			X		X	NA	NA		NA
	Fasciospongia cavernosa	sessile	X			X		X					NA	NA		NA
	Halisarca dujardinii	sessile	X										NA	NA		NA
	Hamigera hamigera	sessile	X										NA	NA		NA
	Hymeniacidon perlevis	sessile	X							X			NA	NA		NA
	Ircinia dendroides	sessile	X				X						NA	NA		NA
	Sarcotragus spinosulus	sessile				X							NA	NA		NA
	Oscarella lobularis	sessile				X							NA	NA		NA
	Axinella rugosa	sessile	X										NA	NA		NA
	Plakortis simplex	sessile				X	X			X			NA	NA		NA
	Haliclona cratera	sessile					X						NA	NA		NA

Tunicata	*Halocynthia papillosa*	sessile	X				X						NA	NA		NA
	Ecteinascidia turbinata	sessile	X			X	X						NA	NA		NA
	Clavelina phlegraea	sessile					X						NA	NA		NA
	Clavelina oblonga	sessile								X			10	NA		NO
	Aplidium elegans	sessile				X	X						NA	NA		NA
	Aplidium tabarquensis	sessile					X					X	NA	NA		NA
	Pseudodistoma crucigaster	sessile	X							X			NA	NA		NA
	Clavelina lepadiformis	sessile										X	NA	NA		NA
	Aplidium conicum	sessile				X	X						NA	NA		NA
	Ascidia mentula	sessile	X				X						NA	NA		NA
	Cystodytes dellechiajei	sessile				X	X						NA	NA		NA
	Aplidium albicans	sessile				X	X						NA	NA		NA
	Didemnum coriaceum	sessile				X	X						NA	NA		NA
	Lissoclinum perforatum	sessile	X										NA	NA		NA
	Microcosmus polymorphus	sessile	X			X							10	NA		NO
	Phallusia fumigata	sessile					X						NA	NA		NA
	Trididemnum inarmatum	sessile				X							NA	NA		NA