Universidad Católica de Valencia
San Vicente Mártir

TFG

TRABAJO FIN DE GRADO

GRADO EN
CIENCIAS DEL MAR

Review on the conservation status of autochthonous marine angiosperms in the Mediterranean Sea

Alumno: Alice Carrara

Tutor: Jorge Juan Vicedo

Curso académico: 2019/2020

Facultad de Veterinaria
y Ciencias Experimentales
Universidad Católica de Valencia
San Vicente Mártir

Alice Carrara

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Seagrasses are globally declining. They form important ecosystems, providing food and shelter for other marine organisms and services such as protection from coastal erosion and regulation of coastal water quality. For these reasons, their conservation should be of primary importance. A critical review was carried out determining whether the conservation efforts for autochthonous marine angiosperms in the Mediterranean Sea were consistent with their status based on their distribution, habitat, population dynamics, main threats, and the conservation strategies applied to each one of them. In general, seagrasses in the Mediterranean are declining and the conservation efforts applied to them vary substantially depending on the species. The species that have been studied the most between the years 2000 and 2020 were *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Asch. On the contrary, little information was available for *Ruppia maritima* L. The *in situ* conservation strategies implemented for these species are considerably more advanced than the *ex situ*. *P. oceanica* is the species that benefits from the highest level of legal protection, while the other species are mostly protected by actions directed to all seagrasses in general. Not many studies were developed for the *ex situ* conservation strategies and especially not for every species. Specimens in gene banks were only present for *Zostera marina* L., *Ruppia cirrhosa* (Petagna) Grande and *R. maritima.*

Keywords: *Seagrass, Mediterranean, conservation, in situ, ex situ, distribution.*

Resumen

Las angiospermas marinas están regresando globalmente. Forman ecosistemas de elevada importancia debido a que procuran comida y refugio para muchos organismos marinos, y también llevan a cabo servicios como la protección de la erosión costera y la regulación de la calidad del agua. Por estas razones, su conservación debería ser de importancia primaria. Para determinar el estado de conservación de las angiospermas marinas autóctonas del Mar Mediterráneo se ha llevado a cabo una revisión bibliográfica, en función de su distribución, dinámica poblacional, hábitat, amenazas y estrategias de conservación *in situ* y *ex situ* aplicadas a cada especie. Por lo general, las angiospermas marinas en el Mar Mediterráneo están en declive y las medidas de conservación varían considerablemente dependiendo de la especie. Las especies que más han sido estudiadas entre los años 2000 y 2020 han sido *Posidonia oceanica* (L.) Delile y *Cymodocea nodosa* (Ucria) Asch. Las estrategias de conservación *in situ* para estas especies están considerablemente más desarrolladas que las estrategias de conservación *ex situ. P. oceanica* es la especie que beneficia de la protección legal más elevada, mientras que otras especies están protegidas principalmente por legislaciones dirigidas a todas las angiospermas marinas en general. Los estudios que se han llevado a cabo para las estrategias de conservación *ex situ* son escasos y no existen para todas las especies. La presencia de material conservado en bancos genéticos sólo es disponible para *Zostera marina* L.*, Ruppia cirrhosa* (Petagna) Grande and *Ruppia maritima* L..

Palabras clave: *Angiospermas marinas, Mediterráneo, conservación, in situ, ex situ, distribución*.

1. INTRODUCTION

1.1 Seagrasses

1.1.1 Biology and diversity of seagrasses

Marine angiosperms, also known as seagrasses, are underwater marine flowering plants. Seagrasses are not true grasses, even though they are monocots, they are a polyphyletic group, meaning that they don't share the same evolutionary origins (Larkum *et al.*, 2006; Short *et al.*, 2016). In comparison to land plants, their biodiversity is quite low, there are only a few described species of seagrasses in the world. Currently there are 72 identified species according to Short *et al.* (2016), they are listed in Table I.1 of Annex I. These species are classified under 6 families (Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, Ruppiaceae, Zannichelliaceae, Zosteraceae) and 14 genera (Short *et al.,* 2016).

Seagrasses tend to form extensive meadows on sandy or muddy substrates that resemble land grass fields, also known as seagrass beds (Green *et al.*, 2003). These seagrass meadows form very complex ecosystems which are extremely important to the coastal marine environment, both locally and globally (Laffoley and Grimsditch, 2009). Seagrass beds are found in shallow marine and estuarine environments throughout coastal areas of the world, they can be found in all the continents except Antarctica (Short *et al.*, 2007). Seagrasses can also form mixed meadows with a combination of different species, or grow in isolated patches, they can even grow as part of a larger ecosystem associated with corals, mangroves, bivalve reefs, rocky benthos or salt marshes (Phillips and Meñez, 1988; Green *et al.*, 2003; Ruíz *et al.*, 2009; Pérez-Lloréns *et al.*, 2014; Hogarth, 2015).

According to Arber (1920), there are four properties that a marine water plant must possess in order to exist:

1. The plant must be adapted to life in a saline medium
2. The plant must be able to grow when fully submerged
3. The plant must have a secure anchoring system, able to withstand wave action and tidal currents
4. The plant must possess a hydrophilous pollination mechanism

All seagrasses fulfill these requirements, they live underwater in a marine environment, a saline medium. They present rhizomes as an anchoring system and, finally, they all have the capacity for hydrophilous pollination (Kuo and Den Hartog, 2007). A fifth essential property for seagrasses was added by Den Hartog (1970): the plant must have the ability to compete successfully with other organisms in the marine environment (Den Hartog, 1970; Green *et al.*, 2003).

These five properties described have led to different characteristics that are common to the majority of seagrass species. Firstly, marine angiosperms possess flattened leaves (with the exception of *Syringodium* Kützing and some *Phyllospadix* spp. W.J. Hooker), their leaves are also elongated or strap-like (with the exception of species in the genus *Halophila* Du Petit-Thouars) and finally, seagrasses have an extensive system of roots and rhizomes (Green *et al.*, 2003). The rhizomes are well-developed and buried in the substrate, while the leaves are usually green, and they are the most visible part of the plant (Bujang, 2012). In many seagrass genera it is very hard to observe flowers (Kuo and Den Hartog, 2007), this could be due to different reasons, in some cases they simply don't produce flowers, and in others they don't produce enough to be located. For example, *Halodule* Endlicher produces flowers under the sediment and thus they can only be seen in certain patches. *Thalassia testudinum* Banks and Sol. Ex J.Koenig grows into very dense vegetation and the flowers are hidden by it (Phillips and Meñez, 1988). The general structure of seagrasses can be observed in Figure 1. Examples of different seagrass species can be observed in Figure 2.

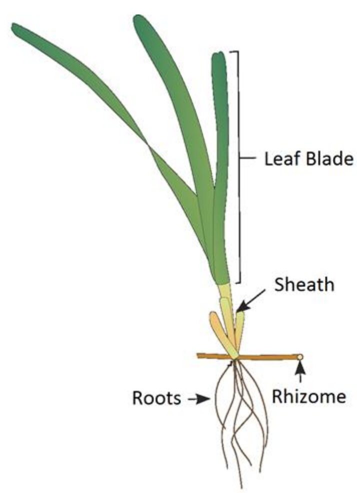

Figure 1. General structure of seagrasses. Source: McCormick (2018).

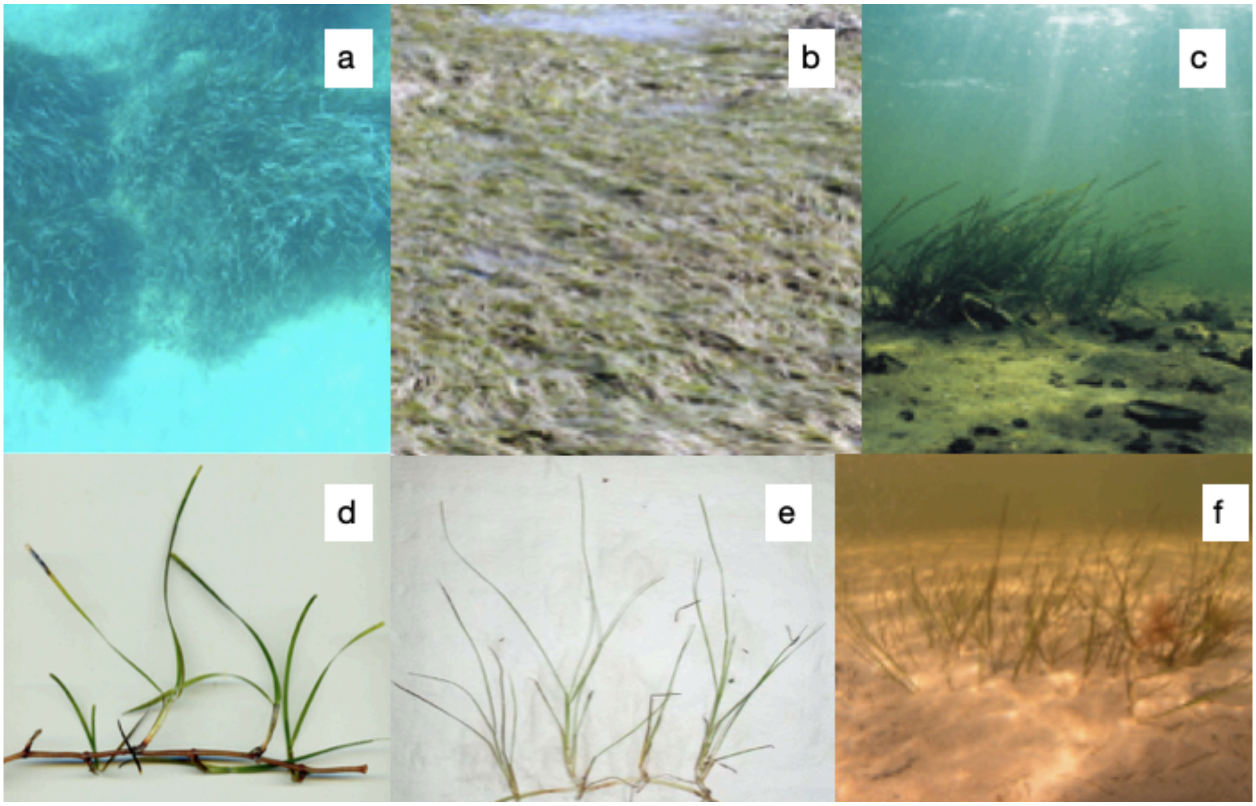

Figure 2. a) *Posidonia oceanica* (L.) Delile. b) *Zostera noltii* Hornem. c) *Zostera marina* L.. d) *Ruppia maritima* L. e) *Ruppia cirrhosa* (Petagna) Grande. f) *Cymodocea nodosa* (Ucria) Asch. Sources: Borum and Greve (2004), Short et al. (2010), FloraCatalana (n.d.), Radloff et al. (2010).

Marine angiosperms have developed certain adaptations that were essential for the colonization of the marine environment. Firstly, they possess subulate leaves with sheaths (see Figure 1): this allows them to live in high-energy environments, such as marine coastal habitats. Secondly, they carry out hydrophilous pollination which allows them to be pollinated under water, as a prerequisite for the adaptation to marine environments according to Arber (1920) (Phillips and Meñez, 1988; Kuo and Den Hartog, 2007). Lastly, they have a very extensive lacunar system, this allows the internal gas flow needed to maintain the correct oxygen supply that the structures require in the anoxic environment they live in (underground) (Hemminga and Duarte, 2000; Hogarth, 2015). The lacunae, also provide buoyancy, allowing the leaves to maintain themselves erect in the water (Phillips and Meñez, 1988; Larkum *et al.*, 2017). It is important that seagrasses receive high levels of light as a consequence of having structures that are belowground and therefore, these cannot carry out photosynthesis. Seagrasses, in fact, are the plant group that require one of the highest levels of light on the planet (Orth *et al.*, 2006). This restricts seagrasses to live in shallow waters as light penetration decreases rapidly with depth (Green *et al.*, 2003;

Hogarth, 2015), and it also causes seagrasses to be very susceptible to changes in their environment, especially those that cause water turbidity (Orth *et al.*, 2006).

As mentioned above, the majority of seagrass species possess relatively thin and flattened leaves (Kuo and Den Hartog, 2007). This characteristic allows marine angiosperms to reach a maximal diffusion of gases and nutrients between the leaves and the water, it also provides a wide surface to carry out photosynthesis and a maximal exposure of the chloroplasts to radiation (Phillips and Meñez, 1988). As the leaf blades lack mechanical support and thus, are very flexible, they follow the water movements exerting friction on the waves and dissipating their energy. By reducing wave energy, seagrasses also attenuate flooding and coastal erosion processes (Phillips and Meñez, 1988; Ondiviela *et al.*, 2014).

The rhizome and roots allow the anchorage to the substrate and the uptake of nutrients. All seagrasses have root hairs, their abundance depends on the species. The lacunae are located in the rhizome as well and they are continuous with those in the leaves (Larkum *et al*., 2006). The leaves have the ability to transport the oxygen down to the roots, allowing the plant to live with the rhizome in an anoxic environment (Phillips and Meñez, 1988; Hogarth, 2015).

In general, all seagrasses prefer sand-muddy substrates, but there are some exceptions (Larkum *et al.*, 2006; Ruiz *et al.*, 2015). Some species can be found on sand (sometimes *Halodule)* or rock (*Phyllospadix* and *Amphibolis* C. Agardh*)* (Phillips and Meñez, 1988). Most seagrasses, though, are most commonly found in waters where they are sheltered from wave action and where tidal currents are moderate (Phillips and Meñez, 1988; Ondiviela *et al.*, 2014; Hogarth, 2015).

Seagrasses' habitats are extremely important and they play a crucial role in many ecosystems, there are many marine organisms, plants and animals that are associated to them (Ruíz *et al.*, 2009; Hogarth, 2015). Seagrass beds provide habitat for fish, shellfish and can be a nursery area for larger species of the ocean (Serrano *et al.*, 2017). Seagrasses are the primary food source of dugongs, manatees and green sea turtles, which are all threatened species (Hogarth, 2015). Although there are few seagrass species, they are able to support great volumes of biomass and diversity of other organisms that are associated to them due to their very high productivity (Green *et al.*, 2003; Ondiviela *et al.*, 2014; Hogarth, 2015). They are considered to be one of the most important shallow water marine ecosystems to humans due to their considerable contribution in fisheries production; filtering coastal waters; dissipating wave energy and, as a consequence, reducing coastal erosion; and they also act as a great sediment anchoring system (Green *et al.*, 2003; Hogarth, 2015).

1.1.2 GLOBAL DISTRIBUTION

Although seagrasses are globally distributed, they are mainly concentrated along the temperate and tropical coastlines (Figure 3) (Short *et al.*, 2016; Jayathilake and Costello, 2018). It is worth mentioning that *Ruppia maritima* L. has only recently been classified as a marine angiosperm as it was previously considered a freshwater species. This species is one of the most distributed around the globe and it can be found in both tropical and temperate regions, as well as a variety of different habitats (Short *et al.*, 2016).

It is very difficult to determine the area of the ocean that is occupied by seagrass meadows, only less than a quarter of the world's seagrasses have been documented and mapped. Most studies only describe observations for seagrasses in specific locations which is unlikely to reflect the full geographic area occupied by them (Green *et al.*, 2003; Jayathilake and Costello, 2018). The total seagrass area across the world is estimated to occupy 1,646,789 km^2, this estimation is based on current mapped areas and point records (Figure 3) (Jayathilake and Costello, 2018).

Figure 3. Global seagrass distribution. Source: Jayathilake and Costello (2018).

Marine angiosperms have been divided into six bioregions, there are two tropical and four temperate. The temperate bioregions comprise the Temperate North Atlantic, the Temperate North Pacific, the Mediterranean, and the Temperate Southern Oceans. The two tropical bioregions consist of the Tropical Atlantic and the Tropical Indo-Pacific (Figure 4). (Short *et al.*, 2007). All of the temperate bioregions have in common species of the genera *Zostera* L. The tropical bioregions are habitat to mega-herbivore grazers, such as sea turtles, dugongs and manatees. The Tropical Atlantic bioregion has a high diversity of seagrasses on reefs and shallow banks, where the most abundant species is *T. testudinum.* The bioregion with the highest seagrass biodiversity in the world is the Tropical Indo-Pacific, containing 14 different seagrass species (Short *et al.*, 2016).

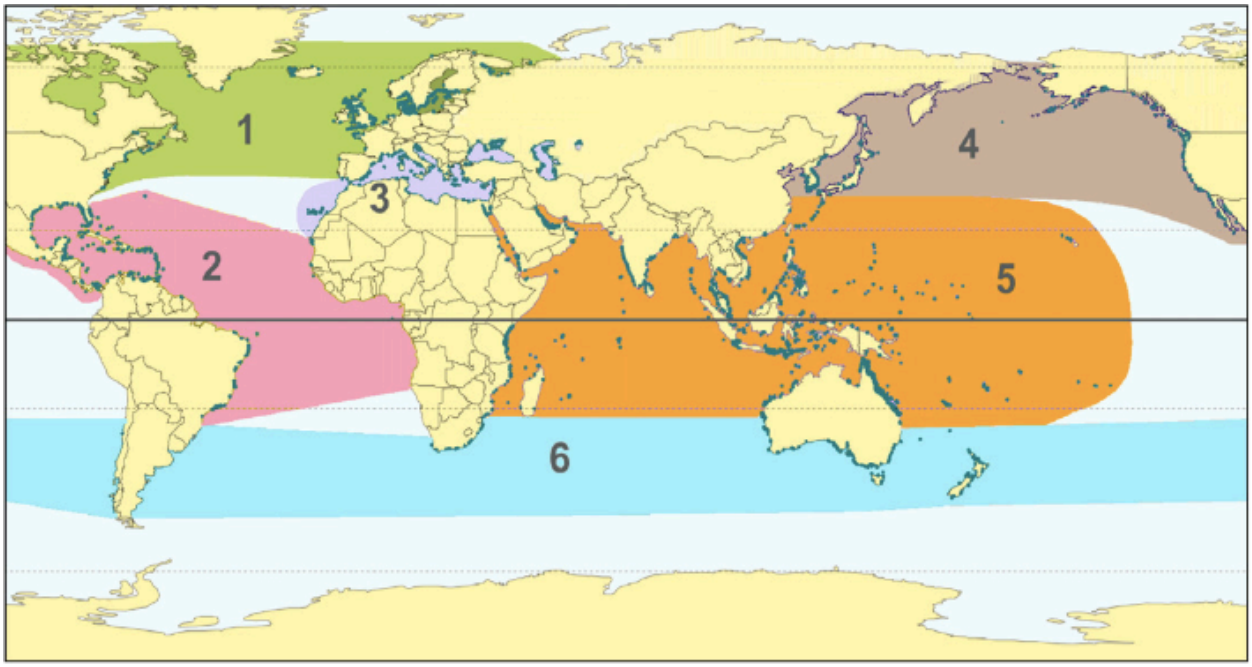

Figure 4. Geographic seagrass bioregions: 1. Temperate North Atlantic, 2. Tropical Atlantic, 3. Mediterranean, 4. Temperate North Pacific, 5. Tropical Indo-Pacific, 6. Temperate Southern Oceans. Source: Short et al. (2007).

The Mediterranean bioregion is characterized by presenting clear water and a moderate biodiversity which includes species of both the temperate and tropical seagrasses. This bioregion is dominated by vast meadows of the endemic species *Posidonia oceanica* L. (Delile) (Ruíz *et al.*, 2009; Short *et al.*, 2016). In the Mediterranean bioregion nine species are described: *Cymodocea nodosa* (Ucria) Asch., *Posidonia oceanica* L. (Delile), *Ruppia cirrhosa* (Petagna) Grande, *Ruppia maritima* L., *Zostera marina* L., *Zostera noltii* Hornem, *Halodule wrightii* Asch., *Halophila decipiens* Ostenf., *Halophila stipulacea* (Forssk.) Asch, but only seven of these can be found in the Mediterranean Sea (Short *et al.*, 2007). This difference is owed to the boundaries of the Mediterranean bioregion that extend outside of the Mediterranean Sea. Also, *H. stipulacea* is not an endemic species of the Mediterranean Sea, it migrated from the Red Sea through the Suez Canal and it was first observed in the Mediterranean Sea at the end of the 19th century (Sghaier *et al.*, 2011).

Therefore, the six autochthonous species of the Mediterranean Sea that will be discussed in this study are: *C. nodosa, P. oceanica, R. cirrhosa, R. maritima, Z. marina and Z. noltii* (Short *et al.*, 2007).

1.1.3 Main threats

Seagrass meadows are considered to be one of the most threatened marine ecosystems (Waycott *et al.*, 2009). They are declining at a global scale due to a different combination of anthropogenic and natural stressors (Green *et al.*, 2003; Orth *et al.*, 2006; Waycott *et al.*, 2009). Although water quality is one of the main concerns regarding the health of marine angiosperms due to their requirements of high levels of light penetration (Orth *et al.*, 2006; Waycott *et al.*, 2009), the impact that different factors combined together have on this ecosystem is cumulative and synergistic (Unsworth *et al.*, 2015; Cullen-Unsworth and Unsworth, 2016). The main threats can be divided into two large groups depending on their nature, natural or anthropogenic:

1. Natural threats

   1.1 Geological threats

   - Coastal uplift or subsidence (Green *et al.*, 2003)

   1.2 Meteorological threats

   - Major storm events, such as cyclones and tsunamis, which can lead to the erosion of wide areas in shallow waters (Green *et al.*, 2003; Waycott *et al.*, 2009; Grech *et al.*, 2012; Unsworth *et al.*, 2018)

   1.3 Biological threats (lesser impact except diseases)

   - Grazing by herbivores (Green *et al.*, 2003; Garrido *et al.*, 2013)
   - Burrowing and foraging animals that disrupt the sediment (Green *et al.*, 2003)
   - Diseases (Green *et al.*, 2003; Orth *et al.*, 2006; Waycott *et al.*, 2009)

2. Anthropogenic threats

   2.1 Direct

   - Destruction of the habitats by:
     - Coastal development (Green *et al.*, 2003; Orth *et al.*, 2006; Waycott *et al.*, 2009; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016; de los Santos *et al.*, 2019)

- Fishery practices such as benthic trawling (Green *et al.*, 2003; Orth *et al.*, 2006; Waycott *et al.*, 2009; Kocak *et al.*, 2011; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016)
- Boating damage (Green *et al.*, 2003; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016)
- Direct removal of seagrasses to “clean” beaches for tourists or to maintain navigation channels (Green *et al.*, 2003; Cullen-Unsworth and Unsworth, 2016)

2.2 Indirect

- Sediment deposition. It causes the turbidity of the water to increase and thus reduce light penetration. It can also smother seagrass meadows (Green *et al.*, 2003; Orth *et al.*, 2006; Waycott *et al.*, 2009; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016)
- High levels of nutrients that can come from sewage disposal, overland runoff and enriched groundwater discharge. Seagrasses can assimilate certain levels of nutrient and toxic pollutants, however, if these levels are above the accepted amount, it can lead to an excess of epiphytic overgrowth, planktonic blooms or competition from macroalgae and thus, reduce the ability of photosynthesis for seagrasses (Green *et al.*, 2003; Waycott *et al.*, 2009; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016)
- Introduction of alien or exotic species (Green *et al.*, 2003; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016)
- Overfishing of certain species causes the decline of predators and, as a consequence, of herbivores down the food chain. These are necessary to maintain seagrass beds free from fouling algae (Waycott *et al.*, 2009; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016)
- Climate change: rising sea levels, changing tidal regimes, localized decreases in salinity, damage from ultraviolet radiation, and changes in the distribution and intensity of extreme natural events (Brouns, 1994; Green *et al.*, 2003; Waycott *et al.*, 2009; Grech *et al.*, 2012; Cullen-Unsworth and Unsworth, 2016; Duarte *et al.*, 2018; Unsworth *et al.*, 2018)

1.2 Plant conservation strategies

Biodiversity is of extreme importance as it allows ecosystem stability and it ensures their proper functioning (Hooper *et al.*, 2005). For this reason, different conventions were formed throughout the years in order to promote and ensure the conservation of biodiversity.

The current development of biodiversity conservation strategies is the result of important international movements with the main objective of protecting the environment.

In the first World Conservation Strategy (1980), conservation was defined as: “The management of human use of the biosphere so that it may yield the greatest sustainable benefit to present generations, while maintaining its potential to meet the needs and aspirations of future generations. Thus, conservation is positive, embracing preservation, maintenance, sustainable utilization, restoration, and enhancement of the natural environment.”. The World Conservation Strategy is the first international document that reflects on the conservation of the world’s resources and it explains the need for a sustainable use of these (IUCN–UNEP–WWF, 1980).

The first international conference on the environment at a global scale is the United Nations Conference for Environment and Development (UNCED), more commonly known as the Earth Summit held in Rio de Janeiro in 1992. This conference set a milestone for the conservation of biodiversity as most of the world’s nations committed to a sustainable economic growth by protecting the environment. One of the main documents discussed and created during this conference is the Convention of Biological Diversity which came into force in 1993. This document required nations to create an inventory of their flora and fauna and to protect the endangered species (UN, 1992).

The Bern Convention (Convention on the conservation of European wildlife and natural habitats), signed in 1979 under the auspices of the European Council by several Mediterranean countries is one of the first international conventions that took into account seagrass meadows (Bern Convention, 1979). Three Magnoliophyte species of the Mediterranean Sea were classified as in need of protection: *C. nodosa, P. oceanica* and *Z. marina* (Appendix I Bern Convention, 1979).

The Barcelona Convention in 1975, was and important convention for the protection of species and areas of the Mediterranean. Until 1982 the convention was focused on marine pollution, but from that year on the focus shifted to the protection of marine habitats (Boudouresque *et al.*, 2012).

In 1995, on the 20th anniversary of the Marine Action Plan (MAP), a new convention was created, the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean. A new MAP was adopted with a new Protocol on Specially Protected Areas and Biological Diversity in the Mediterranean. In the second Annex of the Protocol, which was a list of endangered or threatened species, *P. oceanica, Z. noltii* and *Z. marina* were specifically mentioned (SPA/BD Protocol, 1999).

Additionally, a European Community Directive concerning the conservation of natural habitats of wild flora and fauna and the maintenance of biodiversity in the European Union was established, the Habitats Directive of 21 May 1992. In the first Annex of this Directive, where different types of natural habitat of community interest whose conservation requires the designation of Special Areas of Conservation are identified, *P. oceanica* is classified as a priority habitat (Council Directive 92/43/EEC, 1992).

Basically, strategies for plant conservation need to establish integrated and coordinated activities in the following areas (Laguna, 2012):

- Scientific research: The constant knowledge development about species and other aspects related and necessary to implement conservation strategies. (Taxonomy, ecology, reproductive biology, etc.).
- Techniques and methodologies: Directed to actions and management of species in their natural habitat (*in situ*) and outside (*ex situ*).
- Legal framework: To allow the establishment of a passive framework of preservation and protection of species and habitats.
- Educational areas: Directed towards the spread of knowledge from the specialized institutions (such as the scientific community) or from a reduced number of non-specialized groups (hiking societies, sailors, fishermen, tourists, etc.) to the general public.
- Supporting actions: Derived from other policies and actions that may have a synergistic effect with the conservation strategies.

Overall, all biodiversity conservation activities above mentioned can be grouped into two types of strategies based on their general aim, the *in situ* conservation strategies, applied in the natural habitat and *ex situ* conservation, outside of it (Laguna *et al*., 1998).

1.2.1 *In situ* conservation

In situ conservation strategies aim at the conservation of biodiversity in their natural habitats (Rajpurohit and Jhang, 2015). This consists in the maintenance and recovery of populations in their natural habitat in order to protect, manage and monitor population dynamics and adaptation to constant changes. To be able to carry out *in situ* conservation strategies, the habitats in which these species occur have to be identified, it is then important to protect both the habitat and the species. In the particular case of threatened species, the efforts should also be directed towards the containment or removal of the threats affecting them (Rajpurohit and Jhang, 2015).

In situ conservation includes both the preservation of species themselves but also of the environment they live in. Some of the activities for *in situ* conservation include (Heywood, 2014):

- Identification and protection of the most threatened flora (e.g. The Red List of Threatened Species regularly assessed by the IUCN)
- Single-species conservation and management plans
- For critically endangered species: preparation and implementation of recovery plans (e.g. reinforcement or reintroduction of populations)
- Multi-species management and recovery plans
- Habitat monitoring
- Habitat protection (e.g. Habitats Directive)
- Declaration of Protected Areas (e.g. Marine Reserves)
- Monitoring of outcomes of management interventions for endemic, rare, and/or threatened species

Before all of these conservation activities can be carried out, it is essential to obtain information on the taxonomy, distribution, ecology, genetic diversity, demography, variation, ethnobotany and conservation status of the populations of the species in interest, also known as ecogeographic surveying (Hunter and Heywood, 2011).

1.2.2 *Ex situ* conservation

Ex situ conservation is the preservation of components of biological diversity outside their natural habitats, commonly in those facilities named gene banks (UN, 1992).

Gene banks are extremely important as they can ensure at all times the continued availability of plant genetic diversity. In general, *ex situ* conservation is mostly used for species with one or more of the following characteristics (Kasso and Balakrishnan, 2013):

- Endangered species
- Species with a past, present or future local importance (e.g. agricultural varieties and crop relatives)
- Species of ethnobotanical interest
- Species of interest for restoration of local ecosystems
- Symbolic local species

- Taxonomically isolated species
- Monotypic or oligotypic genera

Ex situ conservation is a very valuable tool, it allows the conservation of biological resources, but it also allows for these resources to be studied and to gain a better understanding of them, this will allow the creation of a better and suitable conservation plan for each species. Some plant *ex situ* conservation practices include seed/plant/tissue and pollen collections, field gene banks, DNA banks and botanical gardens (Rajpurohit and Jhang, 2015). Due to the current rapid loss of biodiversity that the planet is experiencing, the *ex situ* conservation is becoming more and more valuable and necessary (Kasso and Balakrishnan, 2013). *Ex situ* conservation is also often used as a complementary measure to the *in situ* conservation. Through different techniques such as micropropagation, *in vitro* germination and cultures, new specimens can be grown in the laboratory to be later reintroduced in their natural habitat for restoration efforts (Bacchetta *et al.*, 2008).

1.3 Previous works

As a result of the increasing concern in marine biodiversity conservation, some works on seagrass biology, distribution and conservation have been published during the first decade of the 21st Century (Zharova *et al.*, 2001; Menéndez, 2002; Menéndez *et al.*, 2002; Agostini *et al.*, 2003; Borum and Greve, 2004; Malea *et al.*, 2004; González-Correa *et al.*, 2005; González-Correa *et al.*, 2005; Fernández-Torquemada and Sánchez-Lizaso, 2006; Bernard *et al.,* 2007; González-Correa *et al.*, 2007; Montefalcone *et al.*, 2009; Palomar and Losada, 2010; Calvo *et al.*, 2010; Ignacio *et al.*, 2010). This has led to the elaboration of the assessments on their conservation status by the International Union for Conservation of Nature (IUCN) between 2010 and 2016 (*Posidonia oceanica* (Pergent *et al*., 2016), *Cymodocea nodosa* (Pergent-Martini *et al.*, 2015), *Zostera marina* (Buia and Pergent-Martini, 2015); *Zostera noltii* (Pergent-Martini *et al.,* 2015); *Ruppia maritima* (Ali, 2010); *Ruppia cirrhosa* (Short *et al.,* 2010; Lansdown, 2011)). All of these species were classified as “Least Concern”, but different population trends were observed (See table 1).

Table 1. IUCN Red List of Endangered Species assessments. Source: IUCN Red List of Threatened Species.

Species	Scope and year of assessment	Population trend	Reference
Posidonia oceanica	Mediterranean (2013)	Decreasing	Pergent *et al.* (2016)

Cymodocea nodosa	Mediterranean (2013)	Stable	Pergent-Martini *et al.* (2015)
Zostera marina	Mediterranean (2013)	Decreasing	Buia and Pergent-Martini (2015)
Zostera noltii	Mediterranean (2013)	Decreasing	Pergent-Martini *et al.* (2015)
Ruppia cirrhosa	European (2010) Global (2007)	European: Stable Global: Stable	Global: Short *et al.* (2010) European: Lansdown (2011)
Ruppia maritima	Mediterranean (2007)	Decreasing	Ali (2010)

The interest and concern on coastal and marine conservation is currently gaining more attention, these habitats are highly exploited for their natural resources, tourism and navigation. Due to these uses, several environmental problems have recently been documented to have an impact on the habitat quality and on the survival of the biodiversity living in these areas (Boudouresque *et al.*, 2009; Kocak *et al.*, 2011; Boscutti *et al.*, 2015; Holon *et al.*, 2015; Brodersen *et al.*, 2018; de los Santos *et al.*, 2019). Therefore, careful management and proper conservation efforts are very much needed. In this regard, some institutions have implemented different programs for a better scientific understanding of the marine environment, the threats affecting it and possible measures of conservation. One example of these could be the assessments made by the International Union for Nature Conservation on the conservation status made on vascular plant species (including seagrasses) at the beginning of the last decade.

In order to achieve a better understanding of the problem, the following questions have been addressed: How has the scientific information on seagrasses' biology and conservation status increased during the last decade?, how has the knowledge of scientific information on *in situ* and *ex situ* conservation increased in this period of time?, what role do seagrasses have in different *ex situ* collections such as gene banks or DNA banks?. Lastly, based on the current knowledge of these species, what further steps can be taken in order to optimize the conservation status of seagrasses? In order to answer these questions, a set of aims were defined for this study.

2. AIMS OF THE STUDY

This review aims to analyze the conservation status and actions applied to each species in the Mediterranean Sea. To achieve the main goal, a set of specific objectives were defined as follows:

- To review the most important general information of each autochthonous Mediterranean seagrass species, including the distribution range, population dynamics, habitat and ecology and identified threats.
- To compile the different conservation strategies directed towards their preservation *in situ* based on the international legal framework, presence in natural protected areas and restoration actions.
- To check the available information on the different *ex situ* conservation strategies performed on these species, including the conservation in gene banks.
- To analyze all this information in order to identify possible gaps of knowledge.

3. MATERIAL AND METHODS

3.1 Research strategy

The methodology used to carry out the data analysis in this study was the classic method of bibliographic research. The sources of information were analyzed in order to obtain a general idea on the state of the art.

3.1.1 Databases

Two different kinds of databases were employed to obtain the information used throughout this review:

- Scientific Databases: used to review published scientific papers in peer-reviewed international Journals. These tools included Web of Science (WOS) and Science Direct.
- Other scientific databases with reference information for the topic: World Flora Online, the IUCN Red List of Threatened Species, Global Biodiversity Information Facility (GBIF), EUR-Lex and PlantSearch.

Web of Science (WOS)

Web of Science is a publisher-independent global citation database. It is a research engine that provides access to scientific citations and publications. It accounts for over than 1.7 billion cited references from more than 159 million records.

Science Direct

Science Direct is a platform for peer-reviewed literature. It allows for the access to more than 2500 scholarly journals and 39000 reference books, over 1.2 million articles are of open access on the platform.

World Flora Online (WFO)

The World Flora Online is an expanded and more recent version of The Plant List. The Plant List was created in response to Target 1 of the Global Strategy for Plant Conservation (GSPC) of 2002-2010 but it has not been updated since 2013. The World Flora Online was created for the 2011-2020 GSPC's updated Target 1.

This database was used to determine the taxonomy of the species and the species' official nomenclature.

Global Biodiversity Information Facility (GBIF)

The Global Biodiversity Information Facility is an international network and research database that allows users to access online information about all types of life on Earth.

This database was used to obtain the distribution map of those species where it was not available in the articles obtained through the bibliographic search.

The IUCN Red List of Threatened Species

The IUCN Red List of Threatened Species is the database developed by the International Union for Conservation of Nature devoted to providing information on the global conservation status of animal, fungus and plant species at different geographic levels.

This database was used to determine the information and knowledge available for each species related to the topic of the review and to compare the results obtained through the bibliographic search with this database.

EUR-Lex

EUR-Lex is an online database providing access to the European Law. It contains all EU legislation and legal documents, available in the 24 official European languages. It includes treaties, legal acts, preparatory documents, EU case law, international agreements, EFTA documents. The database is run by the Publications Office of the European Union.

EUR-Lex was used to find the legislation protecting the six species taken into account in this review at a European and Mediterranean level. This information was considered as part of the *in situ* conservation strategies.

PlantSearch

PlantSearch is the only online global database providing information about the living plants, gene and seed banks, cryopreserved and tissue culture collections of the world. It includes more than 1,478,703 collection records.

This database was used to determine whether *ex situ* conservation measures were carried out for each species. Unfortunately, the database can only provide information as to whether there are institutions conserving it *ex situ* and how many, but no information is given regarding the type of collection that is stored (seed, living plant, tissue) and its location.

3.1.2 Criteria of inclusion for the data analysis

Different criteria were used on each article to determine whether it could be included in the study or not. The criteria of inclusion were:

- Articles and papers published in scientific journals, in web portals, books or conferences.
- Articles written in one of the following three languages: English, Italian and Spanish.
- Articles regarding one or more of the six species studied: *P. oceanica, C. nodosa, Z. marina, Z. noltii, R. cirrhosa* and *R. maritima.*
- Articles related to the topic of this review.
- For the general equation regarding the distribution range, population dynamics, habitat and ecology and threats, the articles had to be published between the years 2000 and 2020 and restricted to the Mediterranean Sea.
- For the specific equations regarding the conservation strategies, *in situ* and *ex situ*, no publication date and Mediterranean Sea restriction was applied. This is because many protocols explaining *in vitro* germination, micropropagation or transplantation methods might only be written once and in dates preceding 2000 or based on areas outside of the Mediterranean Sea, as they explain methodologies that might remain unchanged.

3.1.3. Criteria of exclusion for the data analysis

- Articles and papers not published in scientific journals, in web portals, books or conferences.
- Articles not written in one of the following three languages: English, Italian and Spanish.
- Articles not regarding one of the six species studied: *P. oceanica, C. nodosa, Z. marina, Z. noltii, R. cirrhosa* and *R. maritima.*
- Articles not related to the topic of this review.
- For the general equation (distribution range, population dynamics, habitat and ecology and threats) the articles published in years preceding the year 2000 or studies carried out outside of the Mediterranean Sea were excluded.
- Articles already obtained through other databases.

3.1.4 Search equations

Keywords were selected and used to carry out the search for scientific papers, these had to be related and relevant to the topic and aim of the study. Different Boolean operators were used to connect keywords with one another, these are: “AND”, “OR” and “NOT”. The operator “*” was also used.

The operator "AND" was used to find records containing all the terms separated by the operator, "OR" was used to find records containing any of the terms separated by the operator (this operator is useful when used with synonyms, this enables a broader range of results). The operator "NOT" was used to exclude records containing certain words from the search, this was mostly used to exclude words that might give unwanted or irrelevant results. The operator "*" was used to find all the words related to the word written with the *. For example, if "conserv*" is used, the database will find all the words that begin with it, such as conservation, conserving, conserve, conserved etc.

Table 2 shows all the keywords and their synonyms, or words that are related to them, used to search for articles during the review.

Table 2. Keywords and synonyms used for the search equations.

Keyword	Synonyms/Related words
Conservation	Protection Restoration Preservation
Strategy	Method
In situ	Transplantation Restoration
Ex situ	Micropropagation *In vitro* Culture Gene bank Seed bank Seed storage Pollen storage DNA storage Seed germination Cryopreservation
Distribution	Range Presence
Habitat	
Population dynamics	Regression Decline Loss Increase Status
Threat	
Cymodocea nodosa	
Posidonia oceanica	
Ruppia cirrhosa	
Ruppia maritima	

Zostera marina	
Zostera noltii	*Zostera noltei*

To carry out the search for articles, these keywords were mainly implemented in the "topic" section as a broader range of results were obtained using this filter.

Three different search equations were created in order to obtain the desired information (Table 3). EQ1 was used as a general equation and it was used to find information related to distribution range, population dynamics, habitat and ecology and threats of each species. The specific equations used were SEQ1 and SEQ2, these were used to find scientific reports that could be used to develop *in situ* and *ex situ* conservation strategies respectively.

Table 3. Search equations used for the general (EQ1) and the specific equations (SEQ1 and SEQ2).

	Search equation
EQ1 (2000-2020)	("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* (distribution OR "population dynamic*" OR regres* OR declin* OR loss OR increase* OR gain OR status OR habitat OR threat*)
SEQ1 (All years)	("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* ("in situ" OR Conserv* OR protect* OR preserv* OR restor* OR transplant*)
SEQ2 (All years)	("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* ("in vitro" OR "gene bank" OR "seed bank" OR "seed* storage" OR culture OR "micropropagation" OR "seed germination" OR "ex situ conservation" OR "cryopreservation" OR "pollen storage" OR "DNA storage")

Furthermore, to determine the distribution of each species in the Mediterranean, all articles mentioning the species in a certain area of the Mediterranean were included, even though the aim of their study did not include the determination of the distribution of the species. Also, to give a better and more visual understanding of the distribution of each species, distribution maps from the articles obtained through the bibliographic search were employed. For the species for which a distribution map was not available in the articles, the distribution map provided by the GBIF was used.

3.2 Representation of results

Different tables and figures were used to represent the bibliographical results, these included the number of articles obtained with each database and the productivity per year for each search equation and also, a comparison of articles used between the two specific equations. The statistical treatment of the data obtained was carried out with the use of Microsoft Excel.

4. RESULTS AN DISCUSSION

4.1 Bibliometric results

A total number of 161 articles were reviewed in this study. In the following tables (Table 4, 5 and 6) the results obtained from each search equation and each database are shown. The tables also show the final number of articles that were used from each database.

In Table 4 the results obtained with EQ1 can be observed. The total number of articles obtained with EQ1 was 4555, from these, a total of 107 articles was used for this study based on the criteria of inclusion and exclusion. The number of articles found from this equation was quite high, this is due to the fact that this equation gave information about all six marine angiosperm species and many different aspects of each were included: distribution, population dynamics, habitat and threats. The database that gave result to the highest number of articles was Science Direct, the Web of Science seemed to be more specific with its results. Also, with Science Direct, each species had to be searched individually since the database only allows 8 Boolean operators to be used at once, thus there might have been many articles that were repeated. The percentage of the articles found with each database is represented in Figure 5. The database where most of the articles were used from was the Web of Science, this is because, as already stated, it gave more specific results and because all of the results obtained from the Science Direct that were already used from the Web of Science, were not considered in this table. The number 6, indicates that 6 articles were used from the Science Direct and were not found in the Web of Science.

Table 4. Results obtained with EQ1.

EQ1	**("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* (distribution OR "population dynamic*" OR declin* OR loss OR increase* OR gain OR recovery OR status OR habitat OR threat*) AND Mediterranean**	
	Articles obtained	**Articles used**
Web of Science	1524	101
Science Direct	3031	6
Total	4555	**107**

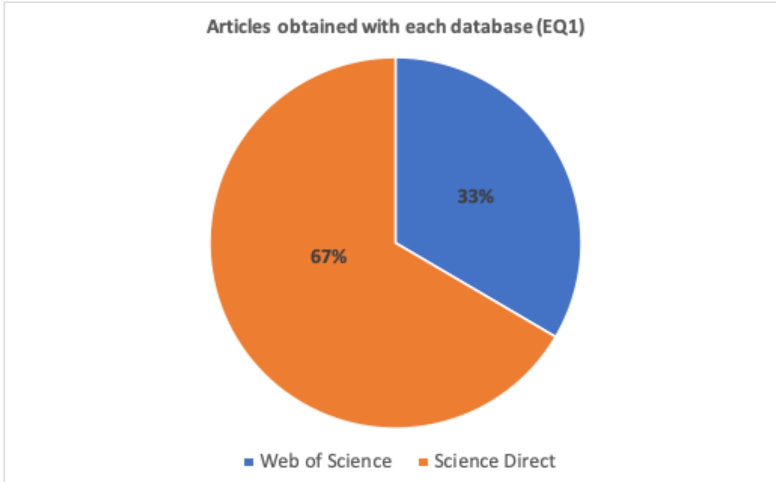

Figure 5. Percentage of articles found with each database for EQ1.

In Figure 6 the number of articles published per year based on the articles used for EQ1 between 2000 and 2020 can be observed. A general trend cannot be observed, but the majority of the articles were published after the year 2010, perhaps showing a slight increase in interest on the topic. It can be observed though, that until the year 2006 very few articles per year were published.

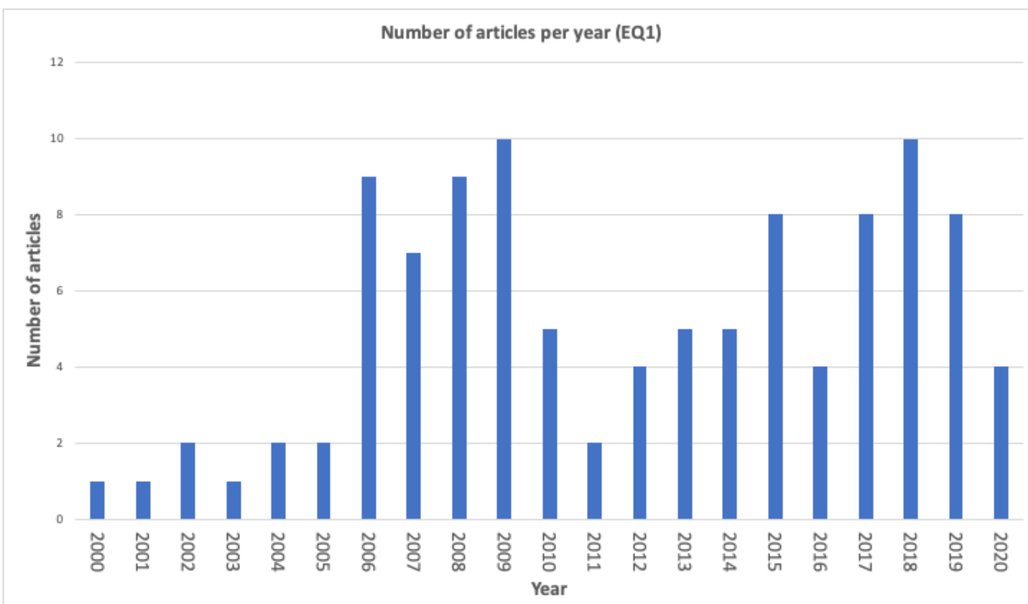

Figure 6. Number of articles published each year for the articles used in EQ1.

In Table 5 the results obtained for SEQ1 are represented. The total number of articles found with this equation was 6568 and after a revision of the articles based on the criteria of inclusion and exclusion, 45 articles were finally used in the study. The number of articles found with SEQ1 is higher than those found with EQ1, this is because no restrictions for the date of publication was applied and it also did not specify the location (Mediterranean). The database that provided the highest number of articles was Science Direct and the database from which the majority of articles were used from was Web of Science. Figure 7 represents the percentage of articles found with each database. In the articles used from Science Direct, the zero does not indicate that no used articles were found with this database, it indicates that all the articles found with it had already been found with the Web of Science.

Table 5. Results obtained with SEQ1.

SEQ1	**("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* ("in situ" OR Conserv* OR protect* OR preserv* OR restor* OR transplant*)**	
	Articles obtained	**Articles used**
Web of Science	2351	45
Science Direct	4217	0
Total	6568	45

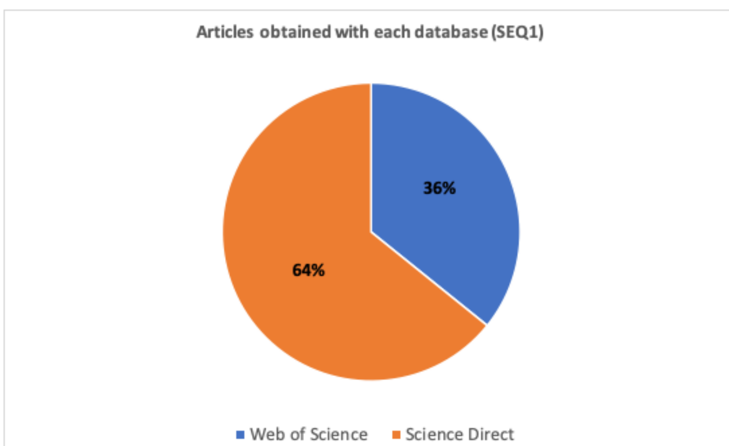

Figure 7. Percentage of articles found with each database for SEQ1.

In Figure 8 the articles published each year for the articles that were used for SEQ1 can be observed. The majority of the articles were published starting from 2010 to 2020. Until 1993 almost no articles were published, just one, and from this year on one or two articles per year were published, with a small gap between 2000 and 2002 where no results were obtained. Based on these results, a general increase in interest on this topic can be observed.

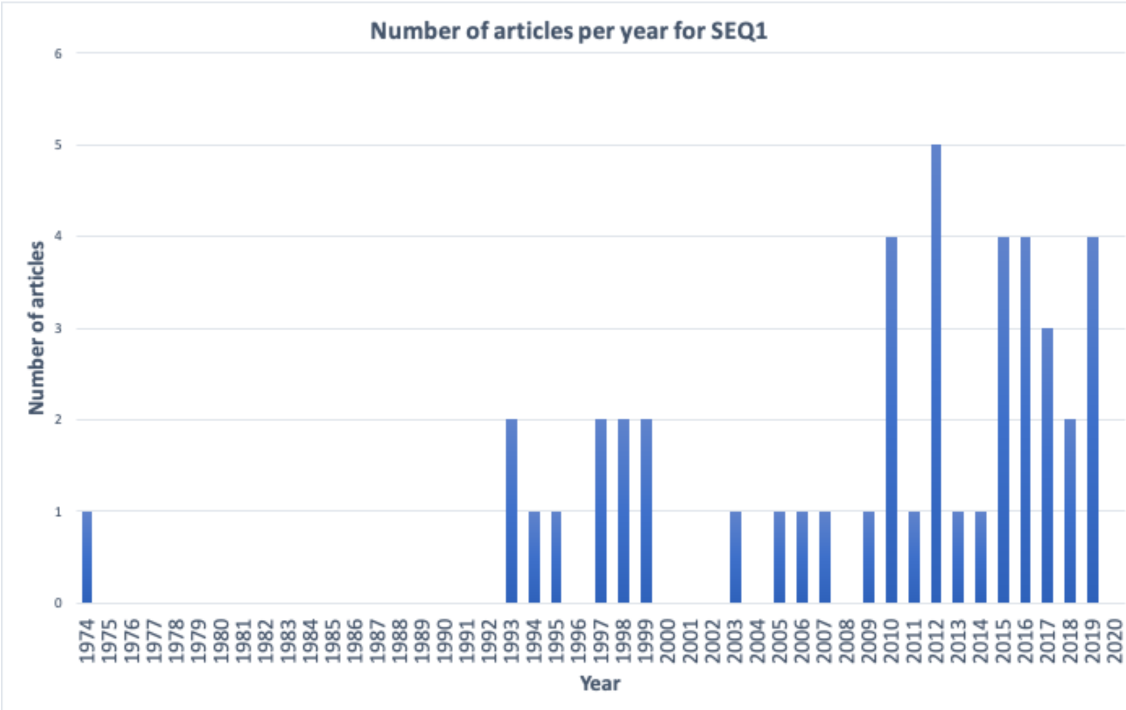

Figure 8. Number of articles published each year for the articles used in SEQ1.

Table 6 shows the number of articles found and used from Science Direct and Web of Science for the second specific equation, SEQ2. The total number of articles obtained with SEQ2 was 1907 and the number of articles that were used for this study were 9, based on the criteria of inclusion and exclusion. The number of articles found with SEQ2 is the lowest out of all the equations used. This is probably because it is a very specific research field and probably not many studies have been conducted on it yet. The database that provided the highest number of articles was Science Direct, in the same way as with the other two equations and the majority of articles used came from the Web of Science. Figure 9 shows the percentage of articles found with each database. As mentioned for SEQ1, the zero articles used for Science Direct indicates that they had already been found in the Web of Science and thus, they haven't been counted again.

Table 6. Results obtained with SEQ2.

SEQ2	**("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* ("in vitro" OR "gene bank" OR "seed bank" OR "seed* storage" OR culture OR "micropropagation" OR "seed germination" OR "ex situ conservation" OR "cryopreservation" OR "pollen storage" OR "DNA storage")**

	Articles obtained	Articles used
Web of Science	587	9
Science Direct	1320	0
Total	1907	9

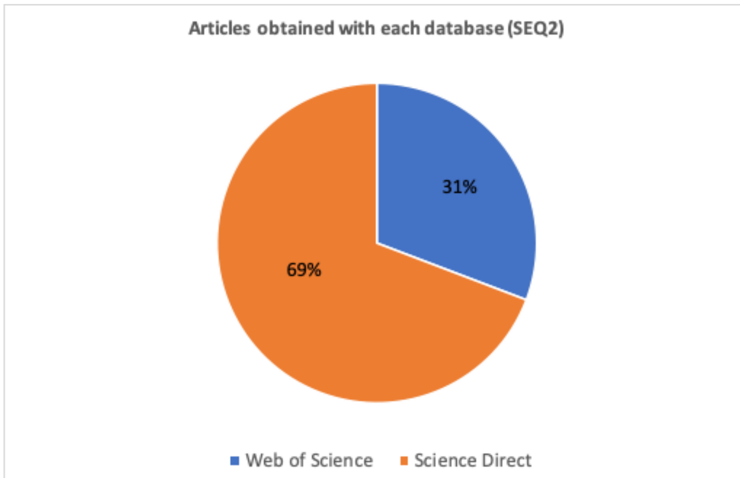

Figure 9. Percentage of articles found with each database for SEQ1.

In Figure 10 the number of articles published per year for SEQ2 can be observed. Very few articles have been published for the *ex situ* conservation strategies and they were all published before the year 1999, showing very low efforts dedicated to developing *ex situ* conservations for the Mediterranean marine angiosperms, especially in the most recent years.

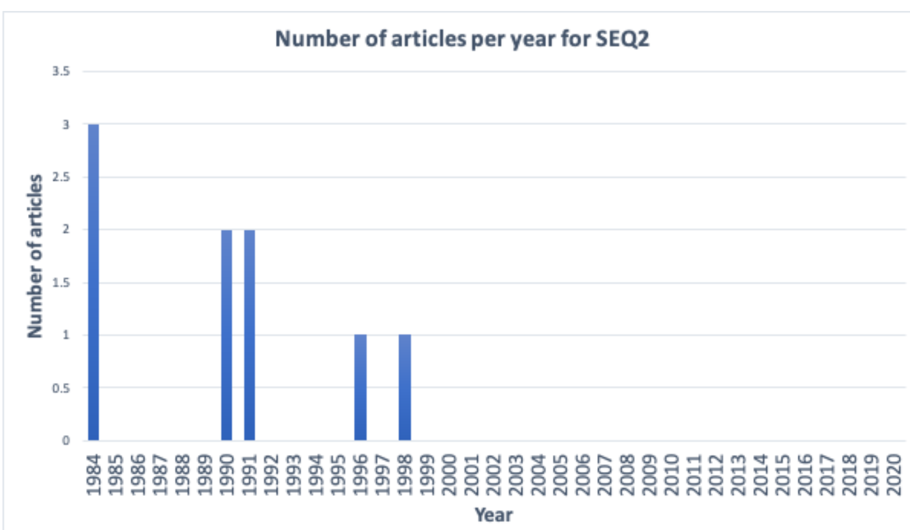

Figure 10. Number of articles published each year for the articles used in SEQ2.

The following table (Table 7) shows the number of articles used for each species and for each topic related to the species (e.g. from EQ1, the number of articles used of *P. oceanica* for its distribution, population dynamics, habitat and threats). The species for which the most articles were used was *C. nodosa* with 60 articles, followed by *P. oceanica* with 48 articles. The smallest number of articles used was for *R. maritima,* with only 15. The total number of articles that results from summing all the articles used for each species (224) is much greater than the total number of articles used for EQ1 (107, see Table 4). The reason for this is that many articles were used for more than one species or for more than one topic, resulting in a higher number of total articles used. The topic for which the most information was found was the distribution range, while the one with the least information was population dynamics.

Table 7. Articles used for each species and topic for EQ1.

EQ1	**("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR “Zostera noltei”) *AND* (distribution OR "population dynamic*" OR declin* OR loss OR increase* OR status OR habitat OR threat*)**				
Species	**Distribution range**	**Population dynamics**	**Habitat and ecology**	**Threats**	**Total**
Cymodocea nodosa	23	8	15	14	60
Posidonia oceanica	16	10	9	13	48
Zostera marina	13	6	10	8	37
Zostera noltii	9	6	6	10	31
Ruppia cirrhosa	18	4	8	3	33
Ruppia maritima	8	0	7	0	15
Total	87	34	55	48	

The next table, Table 8, represents the number of articles that were used for each species for SEQ1. The total number of articles sums up to 50 because some articles were used for more than one species, thus giving a higher number than the real number of articles used for SEQ1 which was 45 (see Table 5). The species for which the most articles were used was *Z. marina* (20 articles), followed by *P. oceanica* (14 articles) and the least articles were used for *R. cirrhosa* (1 article).

Table 8. Articles used for each species for SEQ1.

SEQ1	**("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* ("*in situ*" OR Conserv* OR protect* OR preserv* OR restor* OR transplant*)**
Species	**Articles used**
Cymodocea nodosa	5
Posidonia oceanica	14
Zostera marina	20
Zostera noltii	4
Ruppia cirrhosa	1
Ruppia maritima	6
Total	**50**

Table 9 shows the number of articles used for each species for SEQ2. The total number of articles that results from this table was 10 and the real number of articles used for SEQ2 was 9, this is because 1 article was repeated for two different species. For *C. nodosa* and *Z. marina* no results were found using SEQ2, the species with the most articles was *R. maritima.*

Table 9. Articles used for each species for SEQ2.

SEQ2	**("Posidonia oceanica" OR "Cymodocea nodosa" OR "Ruppia cirrhosa" OR "Ruppia maritima" OR "Zostera marina" OR "Zostera noltii" OR "Zostera noltei") *AND* ("in vitro" OR "gene bank" OR "seed bank" OR "seed* storage" OR culture OR "micropropagation" OR "seed germination" OR "ex situ conservation" OR "cryopreservation" OR "pollen storage" OR "DNA storage")**
Species	**Articles used**
Cymodocea nodosa	0
Posidonia oceanica	3
Zostera marina	0
Zostera noltii	1

Ruppia cirrhosa	1
Ruppia maritima	5
Total	**10**

Figure 11 represents the percentage of articles used for the two specific equations SEQ1 and SEQ2. 83% of the results obtained for the conservation strategies were of SEQ1, which refers to the *in situ* conservation strategies. A big difference in results obtained between SEQ1 and SEQ2 can be observed, showing the higher interest in the *in situ* conservation strategies rather than in the *ex situ.*

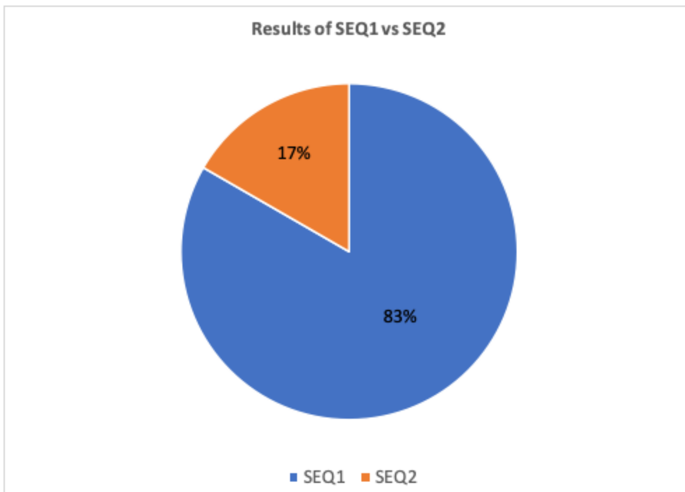

Figure 11. Percentage of articles used for SEQ1 and SEQ2.

4.2 Analytical results

4. 2. 1 *Cymodocea nodosa* (Ucria) Asch.

4.2.1.1 Taxonomy

The taxonomic information of *C. nodosa* is represented in the following figure (Figure 12).

Kingdom	Phylum	Class	Order	Family
Plantae	Tracheophyta	Liliopsida	Alismatales	Cymodoceaceae

Taxon name: *Cymodocea nodosa* (Ucria) Asch.

Synonyms:

- *Cymodocea aequorea* K.D. Koenig
- *Cymodocea major* (Willd.) Grande
- *Cymodocea preauxiana* Webb & Berthel.
- *Cymodocea webbiana* A. Juss.
- *Kernera nodosa* (Ucria) Schult. & Schult.f.
- *Phucagrostis major* Theophr. ex Cavolini
- *Phucagrostis major* Willd.
- *Phucagrostis nodosa* (Ucria) Kuntze
- *Zostera mediterranea* DC.
- *Zostera nodosa* Ucria

Figure 12. Taxonomic information of *Cymodocea nodosa* (Ucria) Asch. Source: World Flora Online (2020).

4.2.1.2 Distribution range

C. nodosa was found to be the second most distributed marine angiosperm of the Mediterranean, after *P. oceanica.* It extended throughout the Mediterranean Sea, part of the adjacent Atlantic coastal regions and the Black Sea (Chefaoui *et al.*, 2016). In the Atlantic it was found from the middle of Portugal down to Senegal and it was also present in the Canary Islands and Madeira (Alberto *et al.*, 2008; Duarte *et al.*, 2008; Cunha and Araújo, 2009).

In the Mediterranean Sea the species could be found along the coasts of different countries.

Chefaoui and her team (2018) mapped the presence of *C. nodosa* in the Mediterranean (Figure 13), this map was based on the probability of presence based on the suitable habitat for the species. The red parts though, were areas where the presence of *C. nodosa* was confirmed by other studies.

C. nodosa was reported in Spain including the Balearic Islands (Rueda *et al.*, 2008; Palomar and Losada, 2010; Pérez-Ruzafa *et al.*, 2012; Sánchez-Carnero *et al.*, 2012; Garrote-Moreno *et al.*, 2014; Canals *et al.*, 2020), France (Holon *et al.*, 2015), including Corsica (Garrido *et al.*, 2013), Italy (Boscutti *et al.*, 2015), and it was very abundant along the Sicilian coast (Calvo *et al.*, 2010). Its presence was also recorded in Croatia (Chefaoui *et al.*, 2018), Greece (Brodersen *et al.*, 2018; Traganos and Reinartz, 2018a, 2018b), Cyprus (Chefaoui *et al.,* 2018), Turkey (Duman *et al.*, 2019), Tunisia (Pérez-Lloréns *et al.*, 2014; Githaiga *et al.*, 2016), Egypt (Githaiga *et al.*, 2016; Chefaoui *et al.*, 2018), Libya (Pergent *et al.*, 2002) and Syria (Chefaoui *et al.*, 2018). *C. nodosa* is the most common seagrass species in the Eastern Mediterranean (Boudouresque *et al.*, 2009).

Figure 13. Distribution map of *Cymodocea nodosa* (Ucria) Asch..The scale shows the probability of presence. Source: Chefaoui et al. (2018).

4.2.1.3 Population dynamics

C. nodosa's populations have been reported to be in decline along the coasts of European countries (de los Santos *et al.*, 2019). Between 1869 and 2016, 17 *C. nodosa* sites in the European countries of the Mediterranean have demonstrated a decreasing trend in area (46%) and 4 sites showed a small increase (15.6%), resulting in an overall loss of 710 ha of 2,320 ha (de los Santos *et al.*, 2019). Nonetheless, another study conducted showed that *C. nodosa* is actually increasing in the Mediterranean, and at a rapid pace (Boudouresque *et al.*, 2009).

In Gran Canaria an important decline in the abundance of *C. nodosa* between 1990s and 2011 has been reported (Tuya *et al.*, 2013). These results were consistent with another study carried out in the Canary Islands, between 1991 and 2013, where a prevalence of decreasing trends of *C. nodosa* abundance had been observed (Manent *et al.*, 2020). In the Mar Menor lagoon (Spain), *C. nodosa* showed a gradual decrease in mean biomass for the period comprised between 1982 and 2008 (Pérez-Ruzafa *et al.*, 2012).

Although a general decline of *C. nodosa* has been observed in the Mediterranean, some studies showed an increase locally. In the eastern Thermaikos Gulf, Greece, between 2011 and 2016, *C. nodosa* beds have increased in area by 17.7% with a gain trend of 18 ha/yr (Traganos and Reinartz, 2018a). In Corsica, in the Urbino lagoon a 42% increase in the seagrass meadow studied has been observed from the early 1990s until 2011 (Garrido *et al.*, 2013).

A study conducted in the Alicante Bay (Spain) of a meadow close to two desalination plants, showed a constant decrease in area, mainly due to the high salinity and its fluctuations (Garrote-Moreno *et al.*, 2014).

4.2.1.4 Habitat and ecology

C. nodosa has been considered to be a warm water seagrass species (Borum and Greve, 2004; Pérez-Lloréns *et al.*, 2014; Pergent *et al.*, 2014) and its distribution was found to be mainly determined by sea surface temperature (SST) and salinity (Chefaoui *et al.*, 2016). Chefaoui and her team determined that the suitable temperatures for *C. nodosa* in the Mediterranean Sea ranged from 5.8ºC to 26.4ºC and the optimal temperature ranged from 20.7 ºC to 21.77 ºC in summer and 13.2 ºC to 15.44 ºC during winter (Chefaoui *et al.*, 2016), temperatures higher than 29-30 ºC were unsuitable for *C. nodosa* (Chefaoui *et al.*, 2016).

Water salinity was also an important factor delimiting the specie's distribution, optimal values ranged from 35.26 psu to 39.9 psu, but salinities between 17.5 psu to 39.3 psu were considered to be suitable as well. Hypersalinity did not seem to be a delimiting factor for *C. nodosa*, but low salinity might have explained the lack of presence of the species in most areas of the Black Sea (Chefaoui *et al.*, 2016). An experimental study conducted on *C. nodosa*'s tolerance to high salinities showed that the species was very sensitive to salinities higher than 41 psu (Fernández-Torquemada and Sánchez-Lizaso, 2006), whereas another experimental study showed that temperatures at 30ºC helped with the growth and overall performance of *C. nodosa* (Ontoria *et al.*, 2019).

The effect of the height of the waves was also studied, and waves higher than 2.5 m were a limiting factor for the presence of *C. nodosa* (Chefaoui *et al.*, 2016). Boscutti *et al.* (2015) also found that *C. nodosa* preferred a medium depth and a substrate fairly rich in nutrients.

A research conducted by Borum and Greve (2004) determined that this species mainly formed dense meadows in shallow subtidal areas. However, based on the results of other studies (for instance Pérez-Llorén and his team and Chefaoui and her team), *C. nodosa* could also reach lower depths (30-40 m) depending on water transparency (Pérez-Lloréns *et al.*, 2014; Chefaoui *et al.*, 2016). The depth range has been established to be between 0 m and 35 m by Chefaoui *et* *al.* (2016), but a study in Spain showed the presence of *C. nodosa* at up to 40 m deep (Sánchez-Carnero *et al.*, 2012).

C. nodosa was considered to be mostly an open sea species but it could also be encountered in bays or lagoons (Boudouresque *et al.*, 2009; Pérez-Lloréns *et al.*, 2014). This species was commonly found in sandy or sand-muddy substrates (Rueda *et al.*, 2008; Pérez-Lloréns *et al.*, 2014; Ruiz *et al.*, 2015). It has been observed as monospecific or mixed meadows with other marine angiosperms such as *Z. noltii, Z. marina, P. oceanica* and with the alga *Caulerpa prolifera* (Forsskål) J.V.Lamouroux 1809 (Pergent *et al.*, 2002). Another important finding was that *C. nodosa* is a fast growing species and it can colonize *P. oceanica*'s dead mattes (Pérez-Lloréns *et al.*, 2014; Unsworth *et al.*, 2015; Burgos *et al.*, 2017).

In a study of *C. nodosa* along the Sicilian coast, some meadows between 7-15 m of depth reached edaphic climax mostly due to low water transparency, muddy sediments and the sea floor morphology (Calvo *et al.*, 2010).

4.2.1.5 Threats

Two studies were conducted on the main pressures affecting *C. nodosa* in the Mediterranean and in European seas. The main factors observed to be threatening *C. nodosa* meadows in the Mediterranean Sea were water quality degradation, coastal modification (de los Santos *et al.*, 2019) and pollution (Brodersen *et al.*, 2018).

Global warming was also another factor that showed to play an important role in the regression of *C. nodosa* (Chefaoui *et al.*, 2018; Ontoria *et al.*, 2019) as it can lead to population declines and genetic loss due to the loss of suitable habitat (Chefaoui *et al.,* 2018). An experimental study showed that the growth of the plants exposed to 35ºC was clearly negatively affected (Ontoria *et al.*, 2019). Chefaoui *et al.* (2018) expected that the main decline of *C. nodosa* meadows would be concentrated in the southern Mediterranean region due to loss of suitable habitat, this would lead to the loss of unique gene pools that are present in this area. They also came to conclusion that by 2050 around 20.8% of suitable habitat would have been lost and by 2100 46.5%, the species might also decrease by 68.2% in the Eastern Mediterranean and by 60% in the Western Mediterranean by 2100. Furthermore, the study conducted by Ontoria *et al.* (2019) showed the effects of both eutrophication and increase in temperature on *C. nodosa*, the authors concluded that the joint effects of these factors would further threaten the species.

In a coastal lagoon in the Mar Menor (Spain), *C. prolifera* colonized the area in 1970 and as its biomass was increasing, the biomass of *C. nodosa* was constantly decreasing until 2008 leaving very few monospecific meadows of *C. nodosa* in the lagoon (Pérez-Ruzafa *et al.*, 2012).

In the Saronikos Gulf (Aegean Sea, Greece) the main threats observed were caused by the presence of fisheries, land-based activities, aquaculture activities and coastal defense infrastructure. These activities caused physical loss and disturbances due to littering, smothering due to biodeposition, abrasion and anchoring on the meadows. The introduction of non-indigenous species and the use of fertilizers and other nitrogen compounds were also observed to be an important threat to this species (Brodersen *et al.*, 2018).

In the Urbino lagoon, in Corsica, one of the main causes for the decline of *C. nodosa* in the 1990s was an increase in turbidity which was induced by different factors, natural and anthropogenic, being these: rainfall events, phytoplankton growth and dredging. Herbivores grazing was also a possible cause (Garrido *et al.*, 2013). A study showed an increase in consumption of *C. nodosa* by herbivores as a consequence of eutrophication, the added nutrients caused the leaves to be more palatable and this led to a local decline of *C. nodosa* (Jiménez-Ramos *et al.*, 2017).

The main threats to *C. nodosa* along the French Mediterranean coast were: urbanization, fishing, industrial effluents, coastal erosion, boat anchoring, man-made coastline, urban effluents, agriculture and aquaculture (Holon *et al.*, 2015).

From a bibliographic review of Boudouresque *et al.* (2009), the main threats to *C. nodosa* in the Mediterranean were: global warming, human activity, exceptionally high rainfall, pollution and brine discharges.

Other threats to *C. nodosa* in the Mediterranean have been observed to be discharges of sea water desalination plants (Fernández-Torquemada and Sánchez-Lizaso, 2006; Palomar and Losada, 2010; Garrote-Moreno *et al.*, 2014; Ruiz *et al.*, 2015), these installations increase the water salinity and showed to cause a decrease in growth and higher mortality rates of *C. nodosa* living in the surrounding areas (Garrote-Moreno *et al.*, 2014). Finally, other human activities developed along the coastal line such as destruction by land reclamation, dredging and coastal urbanization and degradation by organic sewage were also found to be important threats to *C. nodosa* populations (Ramos-Esplá *et al.*, 2007).

4.2.1.6 Conservation strategies

The following tables summarize the results obtained for the *in situ* and *ex situ* conservation strategies applied to *C. nodosa* in the Mediterranean Sea. Table 10 shows the legal protection that applies to *C. nodosa* as a species, as a seagrass, and for its presence in a protected habitat.

Table 10. *In situ* conservation strategies for *Cymodocea nodosa* (Ucria) Asch.: summary of the legal framework.

In situ	Description	Reference
IUCN Red List of Endangered Species.	Scope of assessment: Mediterranean. Least Concern. 2013.	Pergent-Martini *et al.* (2015)
Bern Convention on the Conservation of European Wildlife and Natural Habitats	Appendix 1: Strictly protected flora species.	Council Decision 82/72/EEC (1981)
Habitats Directive	Annex 1: Requires designation of special areas of conservation. Sandbanks which are slightly covered by sea water all the time (1110) (includes *C. nodosa*).	Council Directive 92/43/EEC (1992)
Conservation status of habitat types and species	Habitat 1110: Unfavorable-Inadequate (Between 2013-2018)	Article 17, Council Directive 92/43/EEC (1992)
Natura 2000 network	Sites with *C. nodosa* in the 1110 habitat sites.	Natura 2000
Council Regulation concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea	Protected habitats listed: Seagrass beds (including *C. nodosa*).	Article 4, Council Regulation (EC) No 1967/2006 (2006)
Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (2012-2017).	Priority at a species level and all seagrasses.	UNEP-RAC/SPA (2000)

Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD), from the Barcelona Convention	Annex II: List of endangered or threatened species.	UNEP/MAP-SPA/RAC (2018)
European Biodiversity Strategy for 2030	Protection of all seagrasses.	EU Biodiversity Strategy for 2030

C. nodosa in the Mediterranean is directly protected by three legislations, one that derives from the Bern Convention, listed in Appendix I as a strictly protected flora species (Council Decision 82/72/EEC, 1981), in the Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (UNEP-RAC/SPA, 2000) and also in the SPA/BD Protocol (UNEP/MAP-SPA/RAC, 2018).

The Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea was created by the SPA Protocol of the 1995 Barcelona Convention and it recommends different protection measures: to create an inventory of species and to map their distribution, to identify threats and elaborate and implement legislation for the protection of species and to establish protected areas. *C. nodosa* is listed as a priority species that requires particular attention (UNEP-RAC/SPA, 2000).

There are also general laws that indirectly protect *C. nodosa* as they protect all Mediterranean seagrass species. The Council Regulation (EC) N 1967/2006 in Article 4 concerning protected habitats lists seagrass beds as one of them. This article prohibits “Fishing with trawl nets, dredges, purse seines, boat seines, shore seines or similar nets above seagrass bed of, in particular, *Posidonia oceanica* or other marine phanerogams.” (Article 4, Council Regulation (EC) No 1967/2006: p. 18). The Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea also protects all seagrasses in general (UNEP-RAC/SPA, 2000). Furthermore, the new EU Biodiversity Strategy for 2030 states that it will be crucial to define, map, monitor and strictly protect all the EU’s significant carbon-rich ecosystems, where seagrasses are included.

Also, the Natura 2000 network indirectly affects *C. nodosa* as the species is not specifically mentioned, but it is included in the habitat “Sandbanks which are slightly covered by sea water all the time (1110)” (Council Directive 92/43/EEC, 1992). In the Natura 2000 Network habitats and species are not protected directly, but they must be maintained or restored to a favorable conservation status. Article 17 of the Habitats Directive (1992) requires all member states to monitor all the habitat types and all the species that are considered to be of community interest, which include all Natura 2000 habitats and species. The status of the habitat "sandbanks which are slightly covered by sea water all the time" in the Mediterranean has been assessed as Unfavorable-Inadequate, meaning that Article 17 is not being met (Art. 17 Council Directive 92/43/EEC, 1992).

Table 11 shows the presence of *C. nodosa* in Specially Protected Areas of Mediterranean Importance (SPAMI) sites. The SPAMI list derives from the SPA/BD Protocol, where the Contracting Parties of the Barcelona Convention have to establish a list of Specially Protected Areas of Mediterranean Importance with the aim of promoting management and conservation of these natural areas, including threatened habitats and species present in them. The complete list of SPAMI sites and the marine angiosperms present in each can be consulted in Table II.1 of Annex II.

Table 11. *In situ* conservation strategies for *Cymodocea nodosa* (Ucria) Asch.: Presence in SPAMI sites.

Country	SPAMI
Cyprus	1. Lara-Toxeftra Turtle Reserve
France	1. The Blue Coast Marine Park 2. Natural Reserve of Bouches de Bonifacio 3. The Embiez Archipelago (Six Fours) 4. Calanques National Park 5. Port Cros National Park
Lebanon	1. Palm Islands Natural Park
Slovenia	1. Strunjan Landscape Park
Italy	1. Plemmirio Marine Protected Area 2. Capo carbonara Marine Protected Area 3. Portofino Marine Protected Area 4. Egadi Islands Marine Protected Area 5. Miramare Marine Protected Area 6. Penisola del Sinis – Isola di Mal di Ventre Marine Protected Area 7. Porto Cesareo Marine Protected Area

	8. Torre Guaceto Marine Protected Area and Natural Reserve
Spain	1. Cabo de Gata-Nijar Natural Park 2. Cap de Creus Natural Park 3. Mar Menor and Oriental zone of the Region of Murcia coast 4. Archipelago of Cabrera National Park 5. Maro-Cerro Gordo Cliffs
Tunisia	1. La Galite Archipelago 2. Kneirr Islands

In the following tables, Table 12 and Table 13, the *in situ* and *ex situ* protocols developed for the conservation of the species are listed.

Table 12. *In situ* conservation strategies for *Cymodocea nodosa* (Ucria) Asch.: protocols

Description and location	Reference
Restoration through sods and rhizomes. Sods showed a higher percentage of success. Location: Italy	Sfriso *et al.* (2019)
Restoration through staples, mesh frames and sods. Only sods gave successful results. Assessment of donor seagrass species, transplant season and source location. Location: Portugal	Paulo *et al.* (2019)
Protocol for the production of transplants with reduced mortality rates from seeds cultured in a nursery. Location: Italy	Balestri and Lardicci (2012)
In vitro germinated seedlings transplanted in field with artificial seagrass leaves, which improved the survivorship of these. Location: Gran Canaria Island	Tuya *et al.* (2017)
Protocol for germination *in vitro*, seedling culture and field transplant of *Cymodocea nodosa* from seeds collected. Location: Gran Canaria Island	Zarranz *et al.* (2010)

Table 13. *Ex situ* conservation strategies for *Cymodocea nodosa* (Ucria) Asch. ("x" indicates absence of data).

Protocols	
Description and location	**Reference**
x	-
Gene banks	
x	

A remarkable amount of recently published scientific information on *C. nodosa* in the Mediterranean Sea was found. For instance, it is worth mentioning the work of Chefaoui *et al.* (2018) carried out along the coasts of Mediterranean countries. They mapped a large part of these coasts and determined the specie's presence in order to construct an updated distribution map of the species.

The last assessment made by the IUCN on 2013 (published in 2015) stated that *C. nodosa* showed a stable population trend in the Mediterranean (Pergent-Martini *et al*., 2015). However, based on the results obtained in this review, the population trend seems to be declining along the coast of the Mediterranean-European countries (Chefaoui *et al*., 2016; de los Santos *et al*., 2019). The majority of the other works found during this research explained the population trend of a specific time and location and both declines (Pérez-Ruzafa *et al*., 2012; Tuya *et al*., 2013; Garrote-Moreno *et al*., 2014; Manent *et al*., 2020) and progressions (Garrido *et al*., 2013; Traganos and Reinartz, 2018a) were observed, therefore, it is not possible to make a general assessment. More studies should be carried out at a larger scale to truly understand the population dynamics of the species in the Mediterranean. This knowledge would help understanding whether sufficient conservation strategies are being adopted or whether there is a need for better effort.

Chefaoui *et al.* (2018) determined the characteristics and values for a suitable habitat of *C. nodosa* and predicted a future decline of the species in the Mediterranean Sea due to the loss of suitable habitat as a result of global warming. This study is very important as it gives the possibility to plan strategies for possible conservation actions and prevent the loss of *C. nodosa* in many areas of the Mediterranean. In this regard, it could be very useful to adopt *ex situ* conservation strategies with the aim of not losing genetic variability of the species as it plays a very important role in the success of restoration actions (Reynolds *et al.,* 2012).

As already mentioned above, *C. nodosa* is protected at different levels, as a species in the Bern Convention Appendix I (Council Decision 82/72/EEC,1981), in the Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (UNEP-RAC/SPA, 2000) and in the SPA/BD Protocol. These give the species a solid base of protection, but further research to determine whether these legal protection systems are actually being implemented and applied at a practical level should be carried out. *C. nodosa* is also present in a great number of SPAMIs (24 sites) of 7 different countries. Italy is the country with the most SPAMIs that include *C. nodosa*. The meadows present in these sites benefit from a higher level of protection than the ones that are not included, so this gives an optimistic perception on the conservation of this species *in situ.*

Regarding the protocols found through the bibliographic search that can be applied on *in situ* conservation strategies, the information is quite abundant. Two articles were based in the Mediterranean (Italy) (Balestri and Lardicci, 2012; Sfriso *et al.*, 2019) and three in the Atlantic Ocean (one in Portugal and two in Gran Canaria Island) (Zarranz *et al.*, 2010; Tuya *et al.*, 2017; Paulo *et al.*, 2019). The protocol created by Zarranz *et al.* (2010) has set a milestone for the restoration of *C. nodosa* through plant production using *in vitro* culture techniques, where other authors have used it as a method to carry out their study (Tuya *et al.*, 2017). These articles show the possibility of restoration of *C. nodosa* meadows, even at a large scale. More studies could be carried out on the development of restoration methods with seedlings or plantlets, as the majority of the results showed a higher mortality rate in these and an easier and more successful method with sods (Paulo *et al.*, 2019; Sfriso *et al.*, 2019). In addition, the information found in these studies could also be applied to perform different *ex situ* conservation strategies (for instance, *in vitro* germplasm banks) that are less developed for *C. nodosa* in comparison to other topics on conservation biology. In this regard, it is worth mentioning that no *ex situ* conservation strategies were found for this species. This is probably not a very surprising result due to the wide distribution of the species in the Mediterranean and its abundance, as well as the conclusion of the last IUCN assessment on the conservation status of *C. nodosa* that defined the population trend of the species as stable (Pergent-Martini *et al.*, 2015). Nonetheless, applying different *ex situ* conservation strategies might be a very good way to store and maintain genetic diversity of the species as global warming might lead to its habitat and genetic loss (Chefaoui *et al.,* 2018). For this reason, more research efforts should be directed towards the research on methods for seed storage, cell and tissue preservation (including cryopreservation) as well as pollen and DNA collection.

4. 2. 2 *Posidonia oceanica* (L.) Delile

4.2.2.1 Taxonomy

The taxonomic information of *P. oceanica* is represented in Figure 14.

Kingdom	Phylum	Class	Order	Family
Plantae	Tracheophyta	Liliopsida	Alismatales	Posidoniaceae

Taxon name: *Posidonia oceanica* (L.) Delile

Synonyms:

- *Aegle fragilis* Dulac
- *Alga oceanica* (L.) Kuntze
- *Caulinia oceanica* (L.) DC.
- *Kernera oceanica* (L.) Willd.
- *Posidonia caulini* K. D. Koenig
- *Taenidium acuminatum* Targ. Tozz.
- *Taenidium oceanicum* (L.) Targ. Tozz.
- *Zostera oceanica* L.

Figure 14. Taxonomic information of *Posidonia oceanica* (L.) Delile. Source: World Flora Online (2020).

4.2.2.2 Distribution range

In the study of Boudouresque *et al.* (2012) *P. oceanica* was considered an endemic species of the Mediterranean Sea (native and restricted to this sea). It could be found in both the western and eastern basin of the Mediterranean.

Figure 15 shows the current distribution of *P. oceanica* in the Mediterranean Sea, as well as the areas where no data was available or existent and areas where it was known that *P. oceanica* was absent (Stramska and Aniskiewicz, 2019).

Studies on *P. oceanica* meadows have been recorded along the coasts of the following Mediterranean countries, Spain (Guillén *et al.*, 2013; Marba *et al.*, 2014; Telesca *et al.*, 2015), France (González-Correa *et al.*, 2007; Marba *et al.*, 2014; Balestri *et al.*, 2017; Abadie *et al.*, 2018), Italy (Badalamenti *et al.*, 2006; González-Correa *et al.*, 2007; Montefalcone *et al.*, 2009;

Calvo *et al.*, 2010; Marba *et al.*, 2014; Burgos *et al.*, 2017), Algeria (Githaiga *et al.*, 2016), Morocco (Rova *et al.*, 2018; Stramska and Aniskiewicz, 2019), Malta (Borg *et al.*, 2009) and Tunisia (Duman *et al.*, 2019).

Telesca *et al.* (2015) reviewed the area occupied by *P. oceanica* meadows in each Mediterranean country. Tunisia was the country where the species had extended the most, followed by Italy, Spain and then France. The other countries included in this review were: Slovenia, Croatia, Albania, Malta, Greece, Turkey, Cyprus, Libya and Algeria.

It was confirmed that the species was absent in Syria, Lebanon and Israel (Telesca *et al.*, 2015). In Spain and France (including Corsica) the meadows were quite consistent along all of the coastline (Telesca *et al.*, 2015) with the exception of the Gulf of Lion, probably due to the presence of strong wave action (Stramska and Aniskiewicz, 2019). *P. oceanica* was rather continuous along the coastline of Italy in the Tyrrhenian, Ionian and South-Western Adriatic Sea with interruptions where the main river mouths were situated. In the Northern and Central-Western Adriatic Sea no meadows of *P. oceanica* were reported and on the Eastern Adriatic coasts some small meadows in Slovenia and in some parts of Croatia were observed. The meadows were abundant along the coasts of Greece and Albania. In Morocco *P. oceanica* was absent except for the Chafarinas Islands. Along the Southern Mediterranean coast the information available on the distribution of *P. oceanica* was quite scarce, but they were mostly present along the coastlines of Tunisia, Libya and Egypt (Telesca *et al.*, 2015).

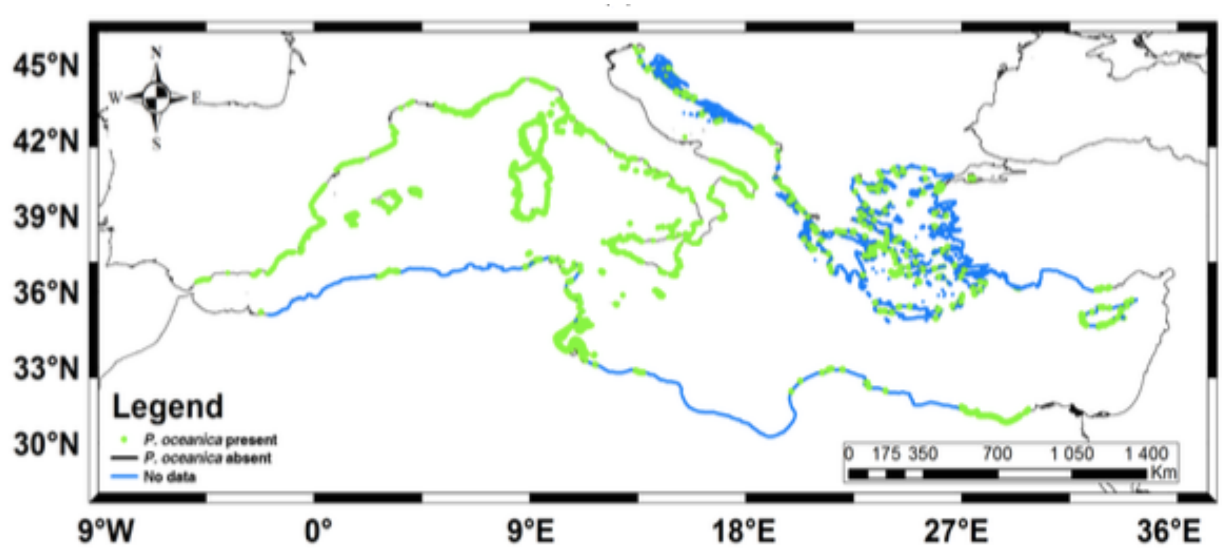

Figure 15. *Posidonia oceanica* (L.) Delile current distribution. Green = *P. oceanica* presence. Blue = No data available. Black = *P. oceanica* absence. Source: Stramska and Aniskiewicz (2019).

4.2.2.3 Population dynamics

A trajectory of decline for *P. oceanica* meadows in the Mediterranean has been recorded by different authors (Boudouresque *et al.*, 2009; Marba *et al.,* 2014; Telesca *et al.*, 2015; Balestri *et al.,* 2017; de los Santos *et al.*, 2019), especially since the second half of the 20th Century (Marba *et al.*, 2014). However, the current total percentage of *P. oceanica* area lost in the Mediterranean is still unclear due to a lack of detailed information. A review on the loss of *P. oceanica* meadows between 1842 and 2009 showed an increasing regression through the years, with a possible loss of 13% to 38% of mapped aerial extent, where the majority of losses were recorded during the second half of the 20th century (Marba *et al.*, 2014). Also, de los Santos *et al.* (2019) estimated a loss of 21% for the period comprised between 1869 and 2016. In the work of Telesca *et al.* (2015) a regression of about 34% in the extent area has been documented since the 1960s, and Marba *et al.* (2014) documented a loss between 13% and 38% in the years 1842 and 2009.

In Spain, data collected from 1993 to 2011 showed a regression of *P. oceanica* meadows of 29%; France, from 1980 to 2011, showed a regression of 9%; Italy showed a 25% loss from 1990 to 2005 and Tunisia a 2% from 1972 to 2010 (Telesca *et al.*, 2015). The largest net loss of the seagrass meadow area for a single location recorded from 1860 to 2016 was in Cape Circeo and Sperlonga in Italy, showing a loss of 4364 ha (de los Santos *et al.*, 2019).

Two studies on the status of *P. oceanica* meadows along the Ligurian coast, in Italy, showed that all the meadows in this region were regressing, even the ones included in marine protected areas (Montefalcone *et al.*, 2009; Burgos *et al.*, 2017). Another study based along the Italian coast of the Tyrrhenian Sea showed a regression of 60% between 1959 and 2005 (Ardizzone *et al.*, 2006). In the Thermaikos Gulf (Greece) *P. oceanica* declined by 4.1% in 5 years, between 2011 and 2016 (Traganos and Reinartz, 2018a)

It is worth mentioning that not all the information found reported a decreasing trend, based on the data collected in the years between 1869 to 2016, 128 sites showed a loss in area, 158 sites showed no change over the years and 72 sites showed an increase in area occupied by *P. oceanica* meadows (de los Santos *et al.*, 2019). A study conducted between 2002 and 2011 monitoring *P. oceanica* meadows in the Valencia Region (Spain), showed that most of the species' meadows were increasing in density and covering or remaining the same (Guillén *et al.*, 2013). This is one example of exception to the overall decreasing trend observed for *P. oceanica* meadows in the Mediterranean.

4.2.2.4 HABITAT AND ECOLOGY

Fernández-Torquemada and Sánchez-Lizaso (2013) determined that *P. oceanica* is very sensitive to high and low salinities. The mean salinity for the Mediterranean is around 37-38 psu and *P. oceanica* could only tolerate 1 or 2 psu higher than the mean value (Sánchez-Lizaso *et al.*, 2008).

P. oceanica has been encountered forming extensive meadows that spread from the sea level and can reach up to 25 to 40 m of depth depending on the transparency of the water (Boudouresque *et al.*, 2006). In fact, light, was found to be one of the main factors that influence its distribution along the depth gradient (Boudouresque *et al.*, 2012; Vacchi *et al.*, 2017). Another important factor that was found to affect its distribution was the influence of the nearshore hydrodynamics: *P. oceanica* did not occur where there was strong wave breaking action near the shore (Vacchi *et al.*, 2017).

Also, Vacchi *et al.* (2017) determined different parameters that affect the meadow's limits, light attenuation created a shaded limit of the meadow, while a change in the substrate created a sharp limit and an eroded limit was caused by the presence of bottom currents.

P. oceanica meadows can form organic formations known as "mattes" (Abadie *et al.*, 2018). The vertical rhizomes grow in height and sedimentation processes elevate the seafloor. Dead roots, rhizomes and leaves accumulate and are buried under, creating a large organic deposit (Hemminga and Duarte, 2000).

A very important feature observed of *P. oceanica* was its very slow growth (Unsworth *et al.*, 2015; Chefaoui *et al.*, 2018), this aspect is very important in terms of conservation of the species and it will be discussed further down in the document.

4.2.2.5 Threats

Different factors have been identified as a cause for *P. oceanica* meadows regression in the Mediterranean. According to Marba *et al.* (2014) the main threats were human induced, directly or indirectly, and they often worked together creating a synergistic negative effect on the meadows (Boudouresque *et al.*, 2009; 2012).

These threats included coastal development and modification of sedimentary flow, industrial and urban pollution and presence of excessive concentrations of nutrients and chemical contaminants (Bull *et al.*, 2010), water quality degradation (Pérez-Lloréns *et al.*, 2014), trawling, coastal aquaculture (Kocak *et al.*, 2011), anchoring (Montefalcone *et al.*, 2006), dredging, sand extraction and dumping (Boudouresque *et al.*, 2009), laying of underwater cables and pipes, brine discharges, competition with introduced species (Marba *et al.*, 2014), overgrazing (Ruiz-Fernández *et al.*, 2009) and climate change (Boudouresque *et al.*, 2009, 2012; Marba *et al.*, 2014; Telesca *et al.*, 2015; de los Santos *et al.*, 2019) .

Based on a bibliographic review carried out by de los Santos *et al.* (2019), the main factor observed to be threatening *P. oceanica* meadows was water quality degradation, although coastal modification and mechanical damage also had a large impact on the species.

A recent model developed by Chefaoui *et al.* (2018), showed that the increase in temperatures of the Mediterranean as a result of climate change would cause a serious population regression and genetic loss of *P. oceanica.* It was estimated that *P. oceanica* would lose 70%-75% of suitable habitat by 2050, meaning that there was a high probability of the disappearance of the species by that year (Chefaoui *et al.*, 2018). The loss of genetic diversity would lead to a lower tolerance to environmental changes, and therefore make *P. oceanica* more vulnerable to any changes in its environment (Jump *et al.*, 2009). High temperatures due to global warming have also shown to affect recently germinated seedlings of *P. oceanica*, increasing their mortality rates and altering their development (Guerrero-Meseguer *et al.*, 2017).

4.2.2.6 Conservation strategies

The following tables show the results obtained for the conservation strategies of *P. oceanica* in the Mediterranean. Table 14 shows the legal framework, Table 16 the presence of the species is SPAMI sites, Table 17 the *in situ* conservation protocols and Table 18 the *ex situ* conservation protocols and the presence or absence of gene banks storing this species.

Table 14. *In situ* conservation strategies for *Posidonia oceanica* (L.) Delile: summary of the legal framework.

In situ	**Description**	**Reference**
IUCN Red List of Endangered Species	Scope of assessment: Mediterranean. Least Concern. 2013.	Pergent *et al.* (2016)
Bern Convention on the Conservation of European Wildlife and Natural Habitats	Appendix 1: Strictly protected flora species.	Council Decision 82/72/EEC (1981)
Habitats Directive on the conservation of wild fauna and flora	Annex 1: Requires designation of special areas of conservation. *Posidonia* beds (1120, priority habitat) and Sandbanks which are slightly covered by seawater all the time (1110).	Council Directive 92/43/EEC (1992)
Conservation status of habitat types and species	Habitat 1120: Unfavorable-inadequate. Habitat 1110: Unfavorable-inadequate. (Between 2013-2018)	Article 17, Council Directive 92/43/EEC (1992)
Natura 2000 network	Habitat 1120 sites present in Cyprus, Spain, France, Italy, Malta, Greece, Croatia and Slovenia (See table 14)	EEA (2019)
Council Regulation concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea	Protected habitats listed: Seagrass beds (including *P. marina*).	Article 4, Council Regulation (EC) No 1967/2006 (2006)
Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (2012-2017).	Priority at a species level and all seagrasses.	UNEP-RAC/SPA (2000)

Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD), from the Barcelona Convention	Annex II: List of endangered or threatened species.	UNEP/MAP-SPA/RAC (2018)
European Biodiversity Strategy for 2030	Protection of all seagrasses.	EU Biodiversity Strategy for 2030

There are 488 Natura 2000 sites designated to the habitat *Posidonia oceanica* beds (1120), these are shown in Table 15.

Table 15. Natura 2000 sites for *Posidonia oceanica* (L.) Delile in each country. Source: EEA (2019).

Country	Number of sites
Cyprus	7
Spain	84
France	30
Greece	53
Croatia	104
Italy	204
Malta	5
Slovenia	1

Table 16 shows the SPAMI sites that include *P. oceanica.*

Table 16. *In situ* conservation strategies for *Posidonia oceanica* (L.) Delile: Presence in SPAMI sites.

Country	SPAMI
Albania	1. Karaburun Sazan National Marine Park
Cyprus	1. Lara-Toxeftra Turtle Reserve
France	1. The Blue Coast Marine Park 2. Natural Reserve of Bouches de Bonifacio 3. Cerbère-Banyuls Marine Nature Reserve 4. The embiez Archipelago (Six Fours) 5. Calanques National Park

	6. Port Cros National Park
Lebanon	1. Palm Islands Natural Park
Monaco, Italy, France	1. Pelagos Sanctuary
Italy	1. Capo Caccia- Isola Piana Marine Protected Area 2. Plemmirio Marine Protected Area 3. Capo carbonara Marine Protected Area 4. Portofino Marine Protected Area 5. Egadi Islands Marine Protected Area 6. Miramare Marine Protected Area 7. Penisola del Sinis – Isola di Mal di Ventre Marine Protected Area 8. Porto Cesareo Marine Protected Area 9. Punta Campanella Marine Protected Area 10. Torre Guaceto Marine Protected Area and Natural Reserve 11. Tavolara Punta Coda Cavallo Marine Protected Area
Spain	1. Cabo de gata- Nijar Natural Park 2. Cap de Creus Natural Park 3. Sea Bottom of the Levante of Almeria 4. Mar Menor and Oriental zone of the Region of Murcia coast 5. Medes Islands 6. Archipelago of Cabrera National Park 7. Maro-Cerro Gordo Cliffs
Tunisia	1. Zembra and Zembretta National Park 2. La Galite Archipelago 3. Kneirr Islands

Table 17. *In situ* conservation strategies for *Posidonia oceanica* (L.) Delile: protocols.

Description and location	Reference
Use of plant growth stimulants on seed germination and use of three different growth regulators on root initiation and development of seedlings to obtain more vigorous seedlings for reseeding projects. Auxins could give higher success rates. Location: Italy.	Balestri and Bertini (2003)
Implementation of anti-trawling reefs as a method of *P. oceanica* protection. Results show that by eliminating the cause of stress on the meadow, recovery is possible. However, full recovery could take up to 100 years. Prevention through better management should be the main objective. Location: Spain.	González-Correa *et al.* (2005)
Use of beach-cast fruits as a source for *P. oceanica* seeds for restoration projects. Culture of seeds in aquaria until seedlings development. Studies on the effects of substratum type, and planting level on survivorship. Planting seedlings in dead matte gives better results. Location: Spain.	Terrados *et al.* (2013)
Cuttings and seedlings originating from 12 different populations of different Mediterranean countries used for transplantation project in National Park of Port-Cros. Location: France.	Meinesz *et al.* (1993)
Transplantation of 500 m^2 of *P. oceanica* clods using SafeBent protocol. Explanation of SafeBent method. Short term results seem to be a success, monitoring will continue until 2021. Location: France.	Descamp *et al.* (2017)
P. oceanica sods were transplantation from an area affected by the enlargement of a marina to a non-affected area. The aim of the study was to determine the feasibility of this type of technique. Resulted in high mortality and degradation of the shoots. Transplant techniques on a large scale do not work for *P. oceanica.* Location: Spain.	Sanchez-Lizaso *et al.* (2009)

Dead matte colonized by *Caulerpa cylindracea* Sonder as a substratum for *P. oceanica* seedlings transplantation. Seedlings obtained in laboratory from seeds of fruits collected on the beach. Seedlings survival in the field were positively affected by the presence of *C. cylindracea* by enhanced production of roots. Location: Spain.	Pereda-Briones *et al.* (2018)
Pilot transplantation experiment to determine the feasibility of restoration projects in disturbed areas. Transplants were able to survive even though high turbidity and exposure affected the site. Location: Italy.	Piazzi *et al.* (1998) Balestri *et al.* (1998)
Cuttings of *P. oceanica* transplanted in three different substrates and in different arrangements and spacings to determine the more successful method. Most successful results obtained with cuttings transplanted closer together and in mattes. Location: France.	Molenaar and Meinesz (1995)
Use of auxins to stimulate root formation due to the current (2006) low success rate of rooting by rhizome cuttings in transplantation projects. Results show that root formation is enhanced with auxins. Possible pre-planting practice for more successful transplantation results. Location: Italy.	Balestri and Lardicci (2006)
Vegetative fragments of *P. oceanica* collected from the beach after storms as possible material for restoration. Fragments are regenerated in aquaculture systems and survived up to 3 years in tanks. The use of fragments of *P. oceanica* presents major advantages for restoration techniques, including no impact on donor populations. Location: Italy.	Balestri *et al.* (2011)
Restoration of *P. oceanica* meadows impacted by a fish-farm by planting seedlings inside of mesh-pots in different areas: in dead matte and in a *P. oceanica* meadow. Seedlings grown in awuaria from seeds collected at the beach. Seedlings planted in the dead	Domínguez *et al.* (2012)

matte showed a high rate of survival, while the opposite occurred for those planted in the meadow. Location: Spain.	
Transplantation of 40 m^2 of a *P. oceanica* meadow. Transplant shoot density increased by 16%. Location: Italy.	Pirrotta *et al.* (2015)

Table 18. *Ex situ* conservation strategies for *Posidonia oceanica* (L.) Delile. ("x" indicates absence of data).

Protocols	
Description and location	**Reference**
Study on the effects of culture media and fruit storage on the germination, development and survival of *P. oceanica* seedlings. Development of a protocol for *in vitro* culture of *P. oceanica.* Location: Italy.	Balestri *et al.* (1998)
Preliminary results for a future *ex situ* conservation *in vitro*. Axenic culture from tissue material of *P. oceanica* achieved. Disinfection protocol was developed for each tissue material. Location: France.	Loques *et al.* (1990)
Orthotropic rhizomes were collected from *P. oceanica* meadow and cultivated for two years in laboratory. High death rate for the first few months, and then decreased. Location: France.	Meinesz *et al.* (1991)
Gene banks	
x	

The distribution of *P. oceanica* in the Mediterranean is well documented globally and especially in the Northern Mediterranean countries. Less data is available on the distribution in the Southern and North-Eastern Mediterranean countries (Stramska and Aniskiewicz, 2019). Spain, France, Italy, Slovenia, Montenegro, Albania, Malta, Syria, Lebanon, Israel and Morocco have all surveyed their coastlines completely, giving very specific data on where the species is found or not in these areas (Telesca *et al.*, 2015). This is a very important step forward since knowing the distribution of the species is essential and the starting point to allow the institution of proper management and conservation. For this reason, it would be a good prospect for future projects to complete the mapping of *P. oceanica* along all of the coasts of the Mediterranean.

Based on the results, it is quite clear that *P. oceanica* is regressing at a global scale, this result is consistent with the las IUCN Red List's assessment of 2013 of the specie's population trend, where it was classified as decreasing (Pergent *et al.,* 2016). This decreasing trend could be related to the Conservation status of habitat types and species (Article 17 of the Habitats Directive) where the Natura 2000 habitat *Posidonia oceanica* beds (1120) was assessed as unfavorable-inadequate (Art. 17 Council Directive 92/43/EEC, 1992). Furthermore, *P. oceanica* is present in 31 SPAMI sites, this elevated number of sites is probably attributable to the wide distribution of *P. oceanica* in the Mediterranean Sea. The fact that *P. oceanica* is present in such a great number of SPAMIs is important because in these areas the species is protected even further.

This species benefits from having an important level of protection in the Mediterranean since it is identified as a habitat itself in Natura 2000, *"Posidonia oceanica* beds". This is the only marine phanerogam to which such importance has been attributed. This is probably mainly because *P. oceanica* is the only seagrass species that is strictly restricted to the Mediterranean and it is the most abundant (Boudouresque *et al.*, 2012). Nonetheless, the species is still regressing (Telesca *et al.*, 2015; de los Santos *et al.*, 2019). The main causes attributed to this decline were human-induced and among these trawling played a big part (Figure 16) (Marba *et al.*, 2014), which is very disappointing considering that this activity is prohibited on all seagrass beds, with special attention to *P. oceanica*, based on Article 4 of the Council Regulation 1967/2006 (Art. 4, Council Regulation (EC) No 1967/2006).

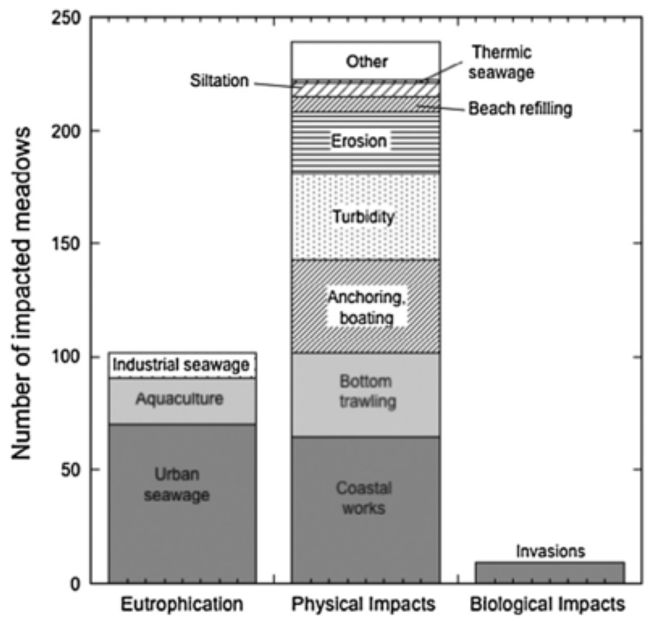

Figure 16. Main pressures identified as causes of *Posidonia oceanica* (L.) Delile decline. Number of *P. oceanica* meadows impacted by each pressure. Source: Marba et al. (2014).

Based on the results obtained, the information available on the *in situ* conservation of the species is quite abundant. In addition, specific methods that could be implemented in different *in situ* conservation strategies have been developed. For example, restoration by using alternatives to donor beds for transplantations are very well developed, such as fruits and vegetative fragments found on the shore (Balestri *et al*., 2011; Terrados *et al.,* 2013) and this allows for more sustainable conservation actions. An interesting observation can be made on the countries that are mostly studying these techniques, Spain, Italy and France, which goes in accordance with the area where this species habitat, distribution and threats are better understood. No protocols created by other countries were found. Regarding the year of publication, there is quite a constant trend, where almost 1 article per year is published, since 1993 to 2018.

On the contrary to what was stated for the *in situ* conservation strategies, the information for the *ex situ* conservation is very reduced. Only three articles were found and all with preliminary results or basic information that need to be further developed in order to be implemented as *ex situ* conservation actions. In fact, no facilities storing collections of *P. oceanica*'s seeds, living specimens, tissues, DNA, pollen etc. were found. Only very few species of marine phanerogams are stored in such manner, so it is not a surprising result. Nevertheless, it would be a very useful conservation strategy to avoid the genetic diversity loss that has been predicted to occur due to global warming.

Overall, *P. oceanica* is protected by a high level of legal actions, but this does not seem to be enough to ensure its long-term survival. *P. oceanica* is still decreasing even in marine protected areas (MPAs) (Montefalcone *et al.*, 2009; Burgos *et al.*, 2017), and mostly due to the anthropogenic pressures. Since *P. oceanica* presents such a slow growth, restoration actions are not the best strategy as it would take too much time to even observe a slight change. For this reason, more and better managing and monitoring need to be emplaced in order to really protect this species.

4. 2. 3 *ZOSTERA MARINA* L.

4.2.3.1 TAXONOMY

Figure 17 shows the taxonomic information related to *Z. marina.*

Kingdom	Phylum	Class	Order	Family
Plantae	Tracheophyta	Liliopsida	Alismatales	Zosteraceae

Taxon name: *Zostera marina* L.

Synonyms:

- *Alga marina* (L.) Lam.
- *Zostera latifolia* (Morong) Morong
- *Zostera marina f. latifolia* (Morong) Setch.
- *Zostera marina f. sulcatifolia* Setch.
- *Zostera marina var. atam* T.W.H. Backman
- *Zostera marina var. izembekensis* T.W.H. Backman
- *Zostera marina var. latifolia* Morong
- *Zostera marina var. marina*
- *Zostera marina var. phillipsii* T.W.H. Backman
- *Zostera marina var. stenophylla* (Raf.) Asch. & Graebn.
- *Zostera maritima* Gaertn.
- *Zostera oregana* S. Watson
- *Zostera pacifica* S. Watson
- *Zostera stenophylla* Raf.

Figure 17. Taxonomic information of *Zostera marina* L. Source: World Flora Online.

4.2.3.2 Distribution range

Z. marina was considered to have a very wide distribution range by the authors Borum and Greve (2004), according to these researchers it was found in arctic waters and in abundance in Northern Europe, especially the Baltic Sea.

The presence of *Z. marina* in the Mediterranean has been reported for different countries, but with scarce abundance and distribution (Bull *et al.*, 2010); it can be observed in Figure 18. The common occurrence in Northern Europe could be related to its affinity to colder waters (Pergent *et al.*, 2014).

It was also cited in the Black Sea (along the coast of Turkey) (Bilgin *et al.*, 2007) and more recently in the Marmara Sea (Artüz, 2017). In Italy, there are reports of presence in the Northern Adriatic Sea (Bergmann *et al.*, 2010; Boscutti *et al.*, 2015; de los Santos *et al.*, 2019), in the Venice Lagoon (Zharova *et al.*, 2001) and in only one location of the Sicilian coast (Calvo *et al.*, 2010). It was also observed in Southern Spain (Rueda *et al.*, 2008; Rueda *et al.*, 2009; Bull *et al.*, 2010; de los Santos *et al.*, 2019) and in France (Bernard *et al.*, 2007; de los Santos *et al.*, 2019).

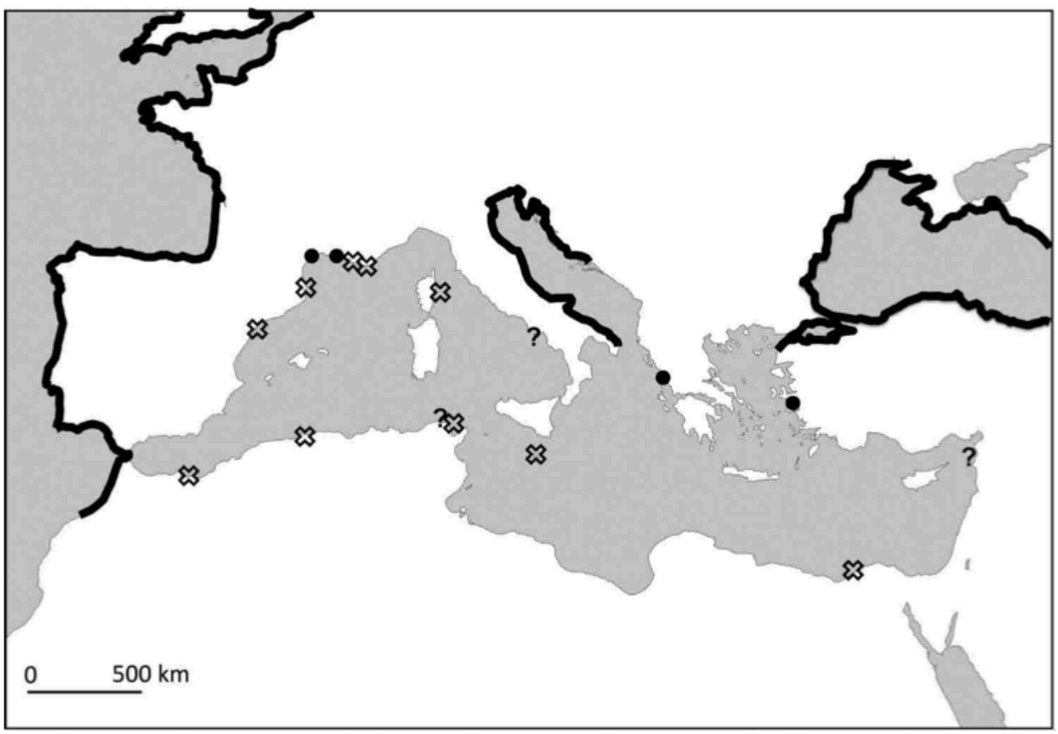

Figure 18. *Zostera marina* L. distribution map in the Mediterranean Sea. (black outline: area where the species is frequently found; black cross: localities where the species has disappeared; black dots: isolated locations; question marks: presence to be confirmed.). Source: Pergent *et al.* (2014).

4.3.2.3 Population dynamics

Based on the study carried out by Boudouresque *et al.* (2009), *Z. marina* populations have regressed in the Mediterranean region. More recently, recordings on *Z. marina*'s disappearance have been also been reported in many sites of the Mediterranean Sea according to Pergent *et al.* (2014) as it is shown in Figure 18.

In addition, different local losses of *Z. marina* meadows have been reported in certain areas of the Mediterranean Sea. For instance, in the Straits of Gibraltar, the complete disappearance of the species was observed leaving behind empty sandy substratum. No single factor was attributed to this loss, but it was considered to be mainly human-induced (Bull *et al.*, 2010). Another example of local regression was observed in the Berre Lagoon (France). *Z. marina* meadows were completely substituted by *Z. noltii* beds in the 1990s due to different stressors (Bernard *et al.,* 2007). However, the species was observed again in 2002, after almost three decades of its disappearance. The authors attributed the recolonization to an improvement of environmental conditions of the lagoon (Bernard *et al.*, 2005).

A thorough study conducted by de los Santos *et al.* (2019) demonstrated a 57% of net loss of *Z. marina* beds in Europe between 1869 and 2016. The study was based on the European distribution of *Z. marina* making it hard to determine whether the regression occurred in the Mediterranean or mostly in the Atlantic. The main cause observed for this regression by the authors was the wasting disease in the Atlantic Ocean, indicating that the Mediterranean meadows probably were not affected by it, or at least not to such a great extent.

4.3.2.4 Habitat and ecology

Z. marina in the Mediterranean Sea was most commonly recorded in brackish waters, usually in sheltered areas like lagoons or close to estuaries (Boudouresque *et al.*, 2009; Rueda *et al.*, 2009; Calvo *et al.*, 2010), but where the influence of seawater is prevalent (Boudouresque *et al.*, 2009). They were usually found as isolated patches, but also as extensive meadows in some areas (Borum and Greve, 2004; Bilgin *et al.*, 2007).

Populations of *Z. marina* have been observed living on soft muddy or firm sandy bottoms (Rueda *et al.*, 2008; Calvo *et al.*, 2010), in intertidal zones (Boscutti *et al.*, 2015; de los Santos *et al.*, 2019) at 1.5 to 3 m depth (Bergmann *et al.*, 2010) or subtidal zones, between 5 to 16-17 m depth (Rueda *et al.*, 2008; 2009).

Different studies have reported a high tolerance to salinity of *Z. marina* (Bernard *et al.*, 2005; Boscutti *et al.*, 2015). The species was found in both fully marine waters and brackish waters with salinities as low as 5 psu (Bernard *et al.*, 2005). The results of an experimental study conducted by Bernard and his research team in 2005 showed that the vegetative growth significantly thrived in temperatures between 10ºC and 15ºC. Also, these authors discovered that the production of generative shoots increased in temperatures slightly higher, between 15ºC to 20ºC (Bernard *et al.*, 2005).

Other important limiting factors for *Z. marina*'s distribution were light availability, turbidity, sediment dynamics and nutrient load (Bernard *et al.*, 2005).

4.3.2.5 Threats

According to Bull *et al.*, (2010) the main stressors found to be affecting *Z. marina* in the Strait of Gibraltar were pollution, high temperatures, different anthropogenic activities and disturbances such as dredging and coastal works, the latter being the one that seemed to have the highest impact. Whereas in the Berre Lagoon, where *Z. marina* beds had completely disappeared, the main problem seemed to be the increase of freshwater input and urban and industrial pollution (Bernard *et al.,* 2007).

Coastal erosion, man-made coastlines (like harbors and artificial beaches), industrial effluents, anchoring, aquaculture were considered to be other important threats to *Z. marina* in the Mediterranean (Holon *et al.*, 2015). Eutrophication was also observed to be a cause of regression in the Grado Marano lagoon (Italy) (Boscutti *et al.*, 2015).

Pergent *et al.* (2014) postulated that if global warming was going to persist and intensify, *Z. marina* would most likely decrease, or even disappear from the Mediterranean Sea. According to these authors the temperature increase of the Mediterranean Sea would cause the replacement of *Z. marina* by species that are more adapted to warmer temperatures or invasive species such as the green algae belonging to *Caulerpa* sp.

Boudouresque *et al.* (2009) found that turbidity, pollution, coastal developments, mooring and urban waste were the main factors affecting *Z. marina* populations. In addition, Holon *et al.* (2015) reported that industrial effluents, coastal erosion and man-made coastlines were also negatively affecting this species. Finally, de los Santos *et al.* (2019) indicated that the main stressors for *Z. marina* were water quality degradation, mechanical damage, coastal modification and extreme events.

4.2.3.6 Conservation strategies

Table 19 shows the legal framework that applies to *Z. marina* in the Mediterranean Sea at an international level.

Table 19. *In situ* conservation strategies for *Zostera marina* L.: summary of the legal framework.

In situ	Description	Reference
IUCN Red List of Endangered Species.	Scope of assessment: Mediterranean. Least Concern. 2013.	Buia and Pergent-Martini (2015)
Bern Convention on the Conservation of European Wildlife and Natural Habitats	Appendix 1: Strictly protected flora species.	Council Decision 82/72/EEC (1981)
Habitats Directive on the conservation of wild fauna and flora	Annex 1: Requires designation of special areas of conservation. Estuaries (1130) and Large shallow inlets and bays (1160). (Both include *Zostera spp.* beds).	Council Directive 92/43/EEC (1992)
Conservation status of habitat types and species	Habitat 1130: Unfavorable-Inadequate Habitat 1160: Unfavorable-Bad (Between 2013-2018)	Article 17, Council Directive 92/43/EEC (1992)
Council Regulation concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea	Protected habitats listed: Seagrass beds (including *Z. marina*).	Article 4, Council Regulation (EC) No 1967/2006 (2006)
Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (2012-2017).	Priority at a species level and all seagrasses.	UNEP-RAC/SPA (2000)

Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD), from the Barcelona Convention	Annex II: List of endangered or threatened species.	UNEP/MAP-SPA/RAC (2018)
Natura 2000 Network	Sites with *Z. marina* in the 1130 and 1160 habitat sites.	Natura 2000
European Biodiversity Strategy for 2030	Protection of all seagrasses.	EU Biodiversity Strategy for 2030

Table 20 shows the SPAMI sites where *Z. marina* is present.

Table 20. *In situ* conservation strategies for *Zostera marina* L.: Presence in SPAMI sites.

Country	SPAMI
Lebanon	1. Palm Islands Natural Park 2. Tyre Coast Nature Reserve
Slovenia	1. Strunjan Landscape Park
Spain	1. Maro-Cerro Gordo Cliffs

The following table (Table 21) shows the *in situ* conservation protocols developed for *Z. marina.*

Table 21. *In situ* conservation strategies for *Zostera marina* L.: protocols.

Description and location	Reference
Restoration through sods and rhizomes. Sods showed a higher percentage of success. A large-scale restoration was achieved. Location: Italy	Sfriso *et al.* (2019)
Restoration through staples, mesh frames and sods. Only sods gave successful results. Assessment of donor seagrass species, transplant season and source location. Location: Portugal	Paulo *et al.* (2019)

Successful restoration of *Z. marina* and development of a protocol to identify a suitable restoration site. Location: USA	Thom *et al.* (2018)
Determination on the influence of burial depth and sediment type on seed germination and seedling establishment. Location: China	Wang *et al.* (2016)
Laboratory and field experiments to determine the effect of protecting seeds on their survival or germination rate for a large-scale restoration project. Location: USA	Harwell and Orth (1999)
Experiment to determine the effect of Loess coating on the success of seed germination. Resulted in higher germination rate and thus an effective technique for large-scale restoration. Location: Korea	Park and Lee (2007)
Series of experiments to determine the most suitable conditions for large-scale restoration of *Z. marina:* mechanical seed planting and harvesting techniques were a useful tool. Location: USA	Marion and Orth (2010)
New method for sowing seeds with hessian bags as protection, minimizing seed mortality. Location: China	Zhang *et al.* (2015)
Successful restoration of *Z. marina* meadows showed that seagrass restoration is a viable option and they can recover and expand once established. Location: USA	Bologna and Sinnema (2012)
Seed planting for large-scale restoration. Assessment of major causes of seed loss and best planting method. They suggest more research be put into how to avoid high seed mortality during restoration projects. Location: Sweden	Infantes *et al.* (2016)
Growth of seeds in aquaculture system for two years for restoration project. Growing *Z. marina* from seeds could be an alternative to donor beds.	Yang *et al.* (2016)

Location: China	
Development of a method for deploying seeds using hessian bags for successful large-scale restoration. Location: UK	Unsworth *et al.* (2019)
Use of the "horizontal rhizome method" for successful large-scale restoration with minimum impact on the area. Location: USA	Davis and Short (1997)
Assessment of the effects of source population identity and diversity on the success of the transplant. Planting shoots from more source sites increased success. Location: USA	Novak *et al.* (2017)
Development of a transplantation protocol of unanchored shoots with rhizomes planted by hand in the sediment. It showed to be very successful. Location: USA	Orth *et al.* (1999)
Method of seed-based restoration to conserve genetic diversity by using a buoy-deployed seeding technique. Location: USA	Orth *et al.* (2014)
Aquaculture system to grow *Z. marina* seeds into shoots and use them for restoration projects. Alternative to harvesting shoots from donor beds. Location: USA	Tanner and Parham (2010)
Development of a technique of *Z. marina* transplantation by using long shoots. Results indicated a higher success of survival when trimmed before transplanting. Location: China	Liu *et al.* (2019)
Transplantation of *Z. marina* with 75% of silt and clay content of sediments had a higher success of transplants survival. Location: China	Zhang *et al.* (2015)
Determination of the more successful source for *Z. marina* transplantation between seeds and sods (sods) and the implications for using each. For a successful restoration, large quantities of sods	Phillips (1974)

were needed, and seeds were easily lost in the field and showed difficulties to establish. Location: USA	

Finally, Table 22 shows the *ex situ* conservation strategies applied to *Z. marina.*

Table 22. *Ex situ* conservation strategies for *Zostera marina* L. ("x" indicates absence of data).

Protocols	
Description and location	**Reference**
x	-
Gene banks	
1	

Based on the results obtained, only one case of progression of *Z. marina* meadows (Bernard *et al.*, 2005) has been recorded in the Mediterranean since the year 2000. The rest of the articles found observed either local regressions (Boudouresque *et al.*, 2007; Bull *et al.*, 2010) or at a European (de los Santos *et al.*, 2019) and Mediterranean scale (Boudouresque *et al.*, 2009; Pergent *et al.*, 2014). The overall trend that can be observed from these results is a remarkable regression of *Z. marina* in the Mediterranean Sea. Nevertheless, the study conducted in Europe includes the meadows of the Atlantic coasts that were largely affected by the wasting disease (de los Santos *et al.*, 2019) and the meadows in Mediterranean did not seem to be impacted by it, thus such a trend might not be present in the Mediterranean area. Also, it is important to mention that only two articles conducted a study on the population trend of the species in the Mediterranean, one of which was published 11 years ago (Boudouresque *et al.*, 2009) and thus, it would be desirable to have more updated information on this topic in order to assess the current situation of the species.

The population trend observed from the results of this study seems to be consistent with the last assessment made by the IUCN Red List in the year 2013 (published in 2015) (Buia and Pergent-Martini, 2015).

The information available on *Z. marina*'s distribution in the Mediterranean Sea is not scarce considering that it is not very abundant in this area, but most of the studies were focused on a specific area and not at a global scale. Further research should be directed towards mapping its presence along the Mediterranean coasts in order to get a more complete view on its distribution since the distribution map found could present some important limitations (Pergent *et al.*, 2014). Also, distribution data for *Z. marina* are not available on the last assessment made by the IUCN (Buia and Pergent-Martini, 2015) indicating a large gap of knowledge on the species in this aspect. This could be an important issue to focus on since, based on this study, the population trend is declining. A better understanding of the distribution of the species will enable proper conservation and protection measures in the most suitable areas.

Z. marina is protected by different legal actions. The Bern Convention, Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea and the SPA/BD protocol protect it as a species itself. It is interesting to mention that *Z. marina* is listed in the Annex II of the SPA/BD 2018 protocol as an endangered or threatened species (Annex II UNEP/MAP-SPA/RAC, 2018), even though, based on the criteria of the IUCN, it was classified as Least Concern (Buia and Pergent-Martini, 2015). Also, *Z. marina* is not directly protected by the Habitats Directive (Council Directive 92/43/EEC, 1992), but *Zostera* beds are included in two habitat types: estuaries (1130) and large shallow inlets and bays (1160). Article 17 of the Habitat Directive demands a “favorable” conservation status of these habitats, but in the last assessment (2013-2018) the habitat 1130 was classified as “unfavorable-inadequate” and 1160 as “unfavorable-bad” (Article 17 Council Directive 92/43/EEC, 1992), showing that the criteria are not being achieved. Nonetheless, the species is protected in 5 SPAMIs of three different countries. Special attention and conservation measures are attributed to the meadows present in these sites.

Regarding the *in situ* conservation protocols, a remarkable amount of information is currently available. Many studies were conducted with the aim of expanding knowledge on, mainly, restoration actions of *Z. marina.* The unfortunate finding was that only one out of the 20 studies available was conducted in the Mediterranean region (Sfriso *et al.*, 2019). The great majority of these studies were based in the USA and China, probably where this species is more abundant. Different restoration protocols have been developed using sods, shoots, rhizomes and seeds (Davis and Short, 1997; Orth *et al.*, 1999; Marion and Orth, 2010; Bologna and Sinnema, 2012; Infantes *et al.*, 2016; Novak *et al.*, 2017; Sfriso *et al.*, 2019), being the latter the one that showed the most difficulties. Also, different techniques and methods were carried out to optimize these protocols or to try to obtain more successful results using seeds for restoration (Harwell and Orth, 1999; Park and Lee, 2007; Tanner and Parham, 2010; Zhang *et al.*, 2015; Wang *et al.*, 2016; Yang *et al.*, 2016). Most of these articles (11 out of 20) were published from the year 2015 and on, showing a large increase in interest towards the restoration of this species in the last 5 years.

As the last assessment made by the IUCN for the European and Mediterranean status of the species was made before this period (Buia and Pergent-Martini, 2015), it is necessary to include all this recent information in a new assessment in order to achieve a better understanding of the current conservation status of the species.

No results were found for the *ex situ* conservation strategies with the bibliographic research, but one *ex situ* conservation site worldwide was found on PlantSearch. Unfortunately, as already mentioned in the methods section, this database only provides the number of sites, but no information on the type of collection (living collection, seeds, DNA…) or the locality. Nonetheless, this is a very important finding as very little seagrass species are stored in gene banks of any type, this type of conservation is mostly directed to terrestrial plant species, with special emphasis to crops and crop relatives.

The overall observations that can be made based on the results obtained are that more conservation actions and protection should be dedicated to *Z. marina.* The population trend seems to be declining, the habitat types that include *Z. marina* are not properly conserved, a great amount of information is available for restoration practices but almost none of them have been applied in the Mediterranean area. Finally, no information for the *ex situ* conservation strategies was available.

4.2.4 *Zostera noltii* Hornem

4.2.4.1 Taxonomy

The following figure (Figure 19) represents the information on the taxonomy of *Z. noltii.*

Kingdom	**Phylum**	**Class**	**Order**	**Family**
Plantae	Tracheophyta	Liliopsida	Alismatales	Zosteraceae

Taxon name: *Zostera noltii* Hornem.

Synonyms:

- *Nanostera noltii* (Hornem.) Toml. & Posl.
- *Zostera emrginata* Ehrenb. & Hempr. ex Asch.
- *Zostera minor* Nolte ex Rchb.
- *Zostera nana var. Latifolia* Harmsen
- *Zostera pumila* Le Gall

Figure 19. Taxonomic information of *Zostera noltii* Hornem. Source: World Flora Online.

4.2.4.2 Distribution range

Z. noltii could be found in Europe and North Africa (Mosbahi *et al.,* 2018), it spread from Mauritania to the Baltic Sea (Pergent *et al.*, 2014). It was also present in the Black, Azov, Caspian Aral and Mediterranean Seas (Valle *et al.*, 2014) and in the Canary Islands (Elso *et al.*, 2018) Its distribution can be observed in Figure 20.

In the Mediterranean Sea, *Z. noltii*, was observed along the coast of different countries, Spain, along the Catalan coast (Canals *et al.*, 2020), in France (Holon *et al.*, 2015; Espel *et al.*, 2019) and in two lagoons of Corsica (Pergent-Martini *et al.*, 2005; Holon *et al.,* 2015), in a shallow lagoon of Camargue, the Vaccarès lagoon (Espel *et al.*, 2019; Charpentier *et al*, 2005) and in the Berre lagoon of Provence (Bernard *et al.,* 2007), in Italy, small patches have been observed along the Sicilian coast but it was not very common (Calvo *et al.,* 2010), in the Urbino lagoon (Pergent-Martini *et al.*, 2005) and in the Northern Adriatic Sea, specifically in the Grado-Marano lagoon (Boscutti *et al.,* 2015), in Tunisia (Githaiga *et al.*, 2016; Mosbahi *et al.*, 2018; Mosbahi *et al.*, 2020) and in Morocco (Githaiga *et al.*, 2016).

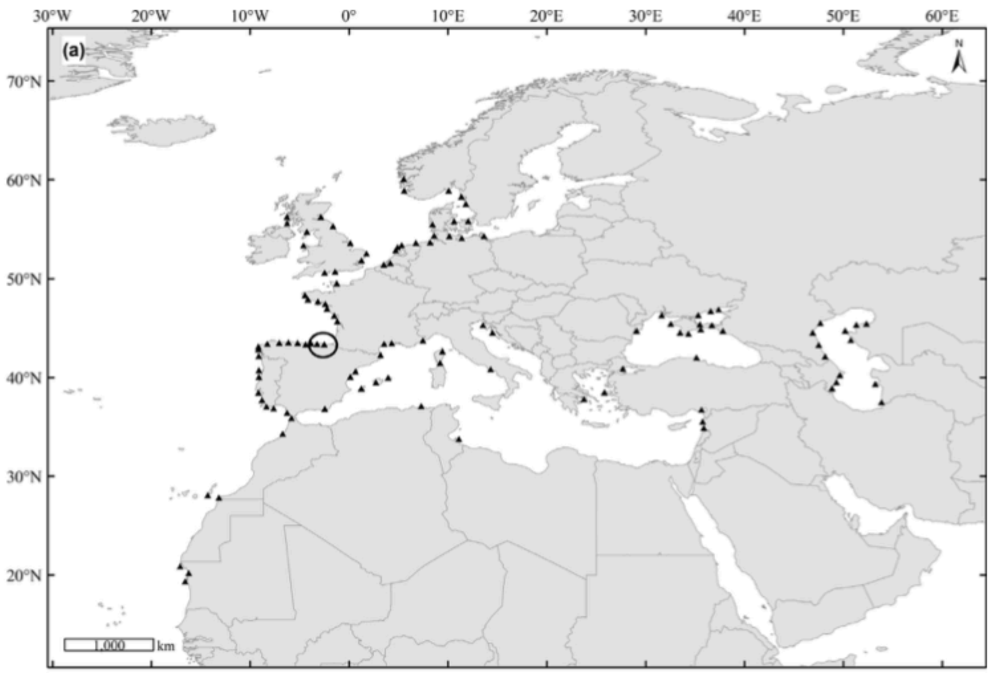

Figure 20. Distribution of *Zostera noltii* Hornem represented by black triangles. Source: Valle et al. (2014).

4.2.4.3 Population dynamics

The study conducted by Short *et al.* (2011) suggested that the global population trend of *Z. noltii* was declining.

Variations at a local scale have been specifically observed in the Vaccarès Lagoon (France), *Z. noltii* experienced two important regressions since 1996, leading it to the complete disappearance at one point, but in 2003 the species seemed to have recovered, occupying 20% of the original area (Charpentier *et al.*, 2005; Garrido *et al.*, 2013; Espel *et al.*, 2019). Another important regression in the Mediterranean has been reported in the Berre lagoon, also in France, where it experienced a great loss of *Z. noltii* in the last decades (Bernard *et al.*, 2007). In the late 19th century *Z. noltii* covered 6000 ha of the lagoon, in 2007 it decreased to less than 1.5 ha leaving the bed of the lagoon mostly occupied by bare silt. In 2009 though, a very small recovery was observed (Bernard *et al.*, 2007; Jahnke *et al.*, 2015). In the Ghar El Melh Lagoon (Tunisia), an increase of local salinity caused the *Z. noltii* meadow to decrease and be replaced by *Ruppia sp.* (Boudouresque *et al.*, 2009). However, the authors also stated that the regression of *Z. noltii* meadows in the Mediterranean was probably not an overall trend, but simply local fluctuations that do not affect the overall balance (Boudouresque *et al.*, 2009). They also found that *Z. noltii* was actually rapidly growing and recolonizing areas previously occupied by other species, as it happened in the Berre Lagoon (Bernard *et al.,* 2007). The same conclusion was deducted by Pergent and his team: the cases reported of regression were all mostly at a local scale, therefore not indicating a general declining trend (Pergent *et al.*, 2014).

The only study found on population dynamics of *Z. noltii* that was carried out on a larger scale, Europe, during the 2000s, showed a large gain in area, mainly attributed to the Atlantic coasts (79%) but also in the Mediterranean Sea (7.2%) in the Vaccarès Lagoon (de Los Santos *et al.,* 2019).

Few studies in general have been carried out on the dynamics of the species both at local and, especially, at Mediterranean levels. It is therefore not possible to make a clear statement on whether *Z. noltii* meadows are regressing or increasing. Also, usually, more research efforts are devoted to regression trends, and this probably leads to a bias towards studies dedicated to regressing populations instead of increasing ones. Overall, based on the results obtained and the conclusions made by Boudouresque *et al*. (2009), Bernard *et al.* (2007) and Pergent *et al.* (2014), the population trend of *Z. noltii* at a Mediterranean Sea scale seems to be quite stable with different episodes of local regression.

4.2.4.4 Habitat and ecology

Z. noltii was commonly found in the intertidal zone (Valle *et al.*, 2014; Mosbahi *et al.,* 2018) and in subtidal habitats, it usually lived in muddy sheltered and shallow areas, such as bays or brackish lagoons (Boudouresque *et al.*, 2009; Pérez-Lloréns *et al.,* 2014; Canals *et al.*, 2020) where the influence of seawater is greater (Boudouresque *et al.,* 2009). It has also been found on anaerobic sandy sediments (Boscutti *et al.,* 2015). *Z. noltii* was observed forming both monospecific and mixed meadows, and it has been frequently found forming mixed meadows with *C. nodosa* or *Z. marina* (*Boudouresque et al.*, 2009; Pérez-Lloréns *et al.,* 2014). These mixed meadows were mostly observed in areas where variations of light intensity and temperatures were high (Valle *et al.*, 2014).

Z. noltii showed a relatively high tolerance threshold to salinity and it was considered a euryhaline species (Cardoso *et al.*, 2008; Pérez-Lloréns *et al.*, 2014), it was observed that it was well adapted to open sea salinities even though it thrived more in brackish waters (Boudouresque *et al.*, 2009). It showed negative sensitivity to freshwater inputs (Boscutti *et al.*, 2015). It has been found living in an area where high salinities during summertime are common (60-70 g/L) (Calvo *et al.*, 2010). It has also been studied that the plant is sensitive to salinities higher than 41 psu, while salinities lower than 10 psu have shown to improve its germination and seedling development (Boudouresque *et al.*, 2009). The species was also quite tolerant to changes, such as pollution and coastal development, it demonstrated to have a high resilience level (Boudouresque *et al.*, 2009; Valle *et al.*, 2014).

4.2.4.5 Threats

Important factors that were observed to be threatening *Z. noltii* meadows in the Mediterranean region were coastal erosion, coastal modification, industrial effluents (Bernard *et al.,* 2007; Holon *et al.*, 2015), urban wastewater discharge (Bernard *et al.*, 2007; Cabaço *et al.*, 2008), eutrophication (Boudouresque *et al.*, 2009; Pérez-Lloréns *et al.*, 2014), mechanical habitat destruction, introduced species (Pérez-Lloréns *et al.*, 2014; de los Santos *et al.*, 2019) but the main threat seemed to be water quality degradation (de Los Santos *et al.,* 2019).

Also, the alteration of environmental factors played a big role in the regression of *Z. noltii*, salinity (Charpentier *et al.*, 2005; Pergent *et al.*, 2014) and turbidity variations (Pergent e*t al*., 2014) were, for example, the main cause for the first episode of regression of the species in Vaccarès lagoon and eutrophication, temperature increase and chemical contamination of trace elements and organic contaminants were the causes for the second main regression that *Z. noltii* suffered in the lagoon above mentioned (Espel *et al.*, 2019). In the Berre lagoon, the diversion of the Durance River caused a great impact on *Z. noltii*'s meadows due to a great increase in freshwater and silt inputs (Bernard *et al.,* 2007). Another case of low salinity impact on the species occurred in the Ghar El Melh Lagoon, where a reduction in the exchange of seawater with the lagoon caused *Z. noltii* to regress and be replaced by *Ruppia sp*. (Boudouresque *et al.*, 2009).

Another important threat to the *Z. noltii* meadows was sediment load and the presence of algae (Vieira *et al.*, 2020) as well as invasive species like *Caulerpa taxifolia* (M.Vahl) C.Agardh *and Caulerpa racemosa* (Forsskål) J.Agard (Pérez-Lloréns *et al.*, 2014; Pergent *et al.*, 2014) and shellfishing (Garmendia *et al.*, 2017).

Natural extreme events indirectly affected *Z. noltii*, droughts caused the increase in salinity values which negatively affected the species (Cardoso *et al.,* 2008; de Los Santos *et al.,* 2019).

Global warming was also observed to be a major threat to *Z. noltii* by Pérez-Lloréns *et al.* (2014). The study of Valle *et al.* (2014) showed that the increase of the sea surface temperature (SST) would probably cause the loss of 18.5% of the species' suitable habitat, change its distribution and abundance as temperature directly affected flowering and seed germination. Valle and her team also estimated that by the end of the 21st century, the suitable habitat of the species might suffer a poleward shift of almost 900 km due to its affinity to colder waters.

Nevertheless, natural and human induced impacts usually work synergistically, and it is difficult to separate them and to determine which one has the greatest impact on the species.

4.2.4.6 Conservation strategies

The results obtained through an extensive search for legal actions protecting *Z. noltii* in the Mediterranean at an international or European level, as part of the *in situ* conservation strategies, have been listed in Table 23. The presence of the species in SPAMI sites is presented in Table 24. The results obtained through the bibliographic research of *in situ* and *ex situ* conservation strategies are represented in Table 25 and 26 respectively.

Table 23. *In situ* conservation strategies for *Zostera noltii* Hornem.: summary of the legal framework at the International level.

In situ	**Description**	**Reference**
IUCN Red List of Endangered Species.	Scope of assessment: Mediterranean. Least Concern. 2013.	Pergent-Martini *et al.* (2015)
Habitats Directive on the conservation of wild fauna and flora	Annex 1: Requires designation of special areas of conservation. Estuaries (1130, *Z. noltii*) and Large shallow inlets and bays (1160, *Zostera spp.*).	Council Directive 92/43/EEC (1992)
Conservation status of habitat types and species	Habitat 1130: Unfavorable-Inadequate Habitat 1160: Unfavorable-Bad (Between 2013-2018)	Article 17, Council Directive 92/43/EEC (1992)

Council Regulation concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea	Protected habitats listed: Seagrass beds (including *Z. noltii*).	Article 4, Council Regulation (EC) No 1967/2006 (2006)
Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (2012-2017).	Priority at a species level and all seagrasses.	UNEP-RAC/SPA (2000)
Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD), from the Barcelona Convention	Annex II: List of endangered or threatened species.	Annex II, UNEP/MAP-SPA/RAC (2018)
Natura 2000	Sites with *Z. noltii* in the 1130 and 1160 habitat sites.	Natura 2000
European Biodiversity Strategy for 2030	Protection of all seagrasses.	EU Biodiversity Strategy for 2030

Table 24. *In situ* conservation strategies for *Zostera noltii* Hornem: Presence in SPAMI sites.

Country	SPAMI
France	1. The Embiez Archipelago (Six Fours)
Monaco, France, Italy	1. Pelagos Sanctuary
Lebanon	1. Palm Islands Natural Park 2. Tyre Coast Nature Reserve
Monaco, Italy, France	1. Pelagos Sanctuary
Slovenia	1. Strunjan Landscape Park
Italy	1. Miramare Marine Protected Area 2. Penisola del Sinis – Isola di Mal di Ventre Marine Protected Area 3. Torre Guaceto Marine Protected Area and Natural Reserve 4. Tavolara Punta Coda Cavallo Marine Protected Area
Spain	1. Sea Bottom of the Levante of Almeria 2. Archipelago of Cabrera National Park

The following table (Table 25) shows the *in situ* conservation protocols developed for *Z. noltii.*

Table 25. *In situ* conservation strategies for *Zostera noltii* Hornem: protocols.

Description and location	Reference
Protocol for large-scale restoration. No transplant survived on the long term. Location: Portugal	Paulo *et al.* (2019)
Transplantation project of *Z. noltii* using sods and single shoots. 43% of sod transplants survived at the long term, all single shoots transplants failed within the first 3 months. Location: The Netherlands	Suykerbuyk *et al.* (2016)
Restoration field experiments on the influence of the type of sediment, the selection of appropriate locations, the time required for the natural recovery of the donor beds. Guidelines to improve the success rate of field restorations. Location: Spain, Atlantic Ocean	Valle *et al.* (2015)
Restoration project. Guidelines for restoration. Determination of characteristics to improve success rate. Removing negative interactions with other species helped with the initial establishment phase. Location: The Netherlands	Suykerbuyk *et al.* (2012)

Finally, Table 26 shows the *ex situ* conservation strategies applied to *Z. noltii.*

Table 26. *Ex situ* conservation strategies for Zostera noltii Hornem. ("x" indicates absence of data).

Protocols	
Description and location	**Reference**
x	-
Gene banks	
x	

Based on the results obtained, the scientific information available for *Z. noltii* in the Mediterranean region is quite scarce. No studies have been carried out specifically on its complete distribution range along the coasts of the Mediterranean Sea and the information available for this region is only based on specific locations. Probably, this is the reason why a distribution map for this area was also not available in reputed databases such as the IUCN Red List of Threatened Species for the Mediterranean assessment, indicating a lack of information and mappings carried out in the Mediterranean Sea.

The last assessment on the general population trend was made by the IUCN Red List of Threatened Species in 2013 (Pergent-Martini *et al.,* 2015). The authors concluded that population trend was "decreasing". However, based on the results presented in this review this statement seems not to match the more recently described trends by Garrido *et al.* (2013); Pergent *et al*. (2014); de Los Santos *et al.* (2019) and Espel *et al.* (2019). This fact points out the need for more scientific research on population dynamics (especially at global scales) to make informed conclusions and to implement proper conservation and managing strategies.

The IUCN found that the conservation status for *Z. noltii* was "Least Concern" (Pergent-Martini *et al.,* 2015). Therefore, it seems not to be worthy of primary attention and conservation efforts. However, other normative included this species in higher levels of concern as Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (UNEP-RAC/SPA, 2000) and the SPA/BD protocol, where it is listed in Annex II (List of endangered or threatened species) (UNEP/MAP-SPA/RAC, 2018). Also, the habitats where this species lives in was taken into account in the Habitats Directive (Council Directive 92/43/EEC, 1992) and Natura 2000. Therefore, there is a wide array of different national and international normative aimed to protect legally both, species and habitats of *Z. noltii*.

Z. noltii is present in 12 Specially Protected Areas of Mediterranean Importance (SPAMI). Considering its narrow distribution in the Mediterranean Sea, the number of SPAMIs that include this species is actually quite numerous and it probably indicates that a large portion of *Z. noltii* meadows benefit from this level of protection.

Scientific information that could be applied in different *in situ* conservation strategies applied for *Z. noltii* was found in four articles. Unfortunately, none of these studies were based in the Mediterranean Sea. These articles explain different restoration projects by transplanting sods and rhizomes and give directions on how to achieve a higher percentage of success. These protocols are very useful and can be taken as example for future restoration projects in the Mediterranean Sea.

Regarding the *ex situ* conservation strategies, very little has been found in this bibliographic research. As far as germplasm conservation concerns, no record of the species was found in seedbanks, tissue culture, pollen or DNA banks. This indicates the little effort that has being made to conserve this species.

Basic research conducted on *ex situ* conservation of *Z. noltii* was found in one article published in the year 1990, the authors developed an *in vitro* seed germination protocol, but not with the aim of conserving the species *ex situ* (Loques *et al.,* 1990b). However, this is a very valuable information that could make it easier to manage seeds in seedbanks, thus helping with the application of *ex situ* conservation strategies that are currently lacking.

Overall, at a global scale the *in situ* conservation strategies applied to *Z. noltii* are much greater than those for the *ex situ* conservation. The data available for this species in the Mediterranean is rather scarce. This could be due to a scarce presence of meadows in the Mediterranean or to its classification as Least Concern by the IUCN Red List of Threatened Species. Furthermore, the little information available does not allow a clear understanding of the status of *Z. noltii* in the Mediterranean, and this could be a reason for the lack of conservation strategies.

4. 2. 5 *Ruppia cirrhosa* (Petagna) Grande

4.2.5.1 Taxonomy

The taxonomic information of *R. cirrhosa* is shown in Figure 21.

Kingdom	Phylum	Class	Order	Family
Plantae	Tracheophyta	Liliopsida	Alismatales	Ruppiaceae

Taxon name: *Ruppia cirrhosa* (Petagna) Grande

Synonyms:

- *Buccaferrea cirrhosa* Petagna
- *Dzieduszyckia limnobis* Rehmann
- *Ruppia cirrhosa subsp. occidentalis* (S. Watson) Á.Löve & D.Löve
- *Ruppia cirrhosa var. truncatifolia* (Miki) H.Hara
- *Ruppia lacustris* Macoun
- *Ruppia maritima subsp. spiralis* (Dumort.) Asch. & Graebn.
- *Ruppia maritima var. occidentalis* (S. Watson) Graebn.
- *Ruppia maritima var. pedunculata* Hartm. ex Ledeb.
- *Ruppia maritima var. spiralis* (Dumort.) Moris
- *Ruppia occidentalis* S. Watson
- *Ruppia spiralis* Dumort.
- *Ruppia truncatifolia* Miki

Figure 21. Taxonomic information of *Ruppia cirrhosa* (Petagna) Grande. Source: World Flora Online.

4.2.5.2 Distribution range

R. cirrhosa was defined as cosmopolitan, having been reported in the Mediterranean Sea, in European Seas of the Atlantic Ocean in Portugal, Germany and The Netherlands (Mannino *et al.*, 2015). There are also reports of its presence in South and Central America, as well as South Africa (Mannino *et al.*, 2015). In the Mediterranean Sea, *R. cirrhosa* was observed in Croatia, France, Greece, Israel, Egypt, Italy, Morocco, Slovenia, Spain and Tunisia (Orfanidis *et al.*, 2008; Mannino *et al.*, 2015; Triest *et al.*, 2018; Tsioli *et al.*, 2019).

Specifically, in the Western Mediterannean region Its presence was also recorded by different studies in Spain (Menéndez *et al.*, 2002; Obrador *et al.*, 2007; Triest and Sierens, 2009; Obrador and Pretus, 2010; Prado *et al.*, 2013), in France (Menéndez *et al.,* 2002; Agostini *et al.*, 2003; Pasqualini *et al.*, 2006, 2017; Pergent *et al.*, 2006), Tunisia (Shili *et al.*, 2007; Dhib *et al.*, 2013), Italy (Orfanidis *et al.*, 2008; Signorini *et al.*, 2008; Mannino and Geraci, 2016; Mannino and Graziano, 2016) and in Greece (Orfanidis *et al.*, 2008; Tsioli *et al.*, 2019)

No distribution map of *R. cirrhosa* in the Mediterranean was found with the bibliographic search. The following figure (Figure 22) shows a distribution map of this species taken from the GBIF. This distribution map should not be considered as a completely reliable source of distribution since it is based on observations made by both reliable occurrence records, but also by individuals with qualifications that are unknown. Therefore, it is very possible that this database contains misidentifications as they are not evaluated by a scientific community (Aubry *et al.*, 2017).

Figure 22. Distribution of Ruppia cirrhosa (Petagna) Grande in the Mediterranean. Source: Global Biodiversity Information Facility - GBIF (2019).

4.2.5.3 Population dynamics

The first dramatic population decline reported for *R. cirrhosa* was in a coastal lagoon of Menorca in 2007 where the species completely disappeared (Obrador and Pretus, 2010).

Pasqualini *et al.* (2017) also observed an evident decrease in coverage of *R. cirrhosa* in a coastal lagoon in Corsica during the years 1970 and 2007. The authors also reported that many other shallow coastal lagoons experienced the same decline during that period of time.

In contrast, a case of progression was also observed in a Spanish coastal lagoon concomitant with the regression of *Potamogeton pectinatus* L.: *R. cirrhosa* quickly took over and increased in coverage occupying *P. pectinatus*'s area (Menéndez *et al.*, 2002). A similar situation occurred in Western Greece where *R. cirrhosa* gradually replaced *Z. noltii* and increased in area coverage (Christia *et al.*, 2018).

4.2.5.4 Habitat and ecology

Populations of *R. cirrhosa* were observed to form dense and extensive meadows (Menéndez *et al.*, 2002; Obrador *et al.*, 2007; Mannino and Graziano, 2016), which may be monospecific (Mannino and Graziano, 2016) or hybrid meadows with the closely related species *R. maritima* (Mannino *et al.*, 2015), or even different seagrasses such as *Z. noltii* (Menéndez *et al.*, 2002). Due to this ability to resist and tolerate extreme variations and environmental stresses, *R. cirrhosa* was considered to be a very competitive seagrass species, more than other macrophytes (Mannino and Sara, 2006; Mannino and Graziano, 2016).

It was observed that *R. cirrhosa* could perform both annual and perennial life cycles (Mannino and Sara, 2006). This feature was reported from different coastal lagoons in the Mediterranean. In the North-Western Mediterranean Sea, *R. cirrhosa* was found to be living in both temporary and permanent coastal lagoons. Also, they occurred in areas with different ranges of temperature, salinity, light and depth. *R. cirrhosa* was able to show a perennial or annual life cycle depending on the environment it was living in. In the temporary lagoon the annual cycle was adopted and only the seeds remained viable during the dry period, while in the permanent lagoon, *R. cirrhosa* adopted the perennial strategy (Mannino *et al.*, 2015). This difference in life cycles was also observed in Italy, in some lagoons *R. cirrhosa* had developed a perennial life cycle, while in others an annual growth cycle (Mannino and Graziano, 2016).

R. cirrhosa may tolerate wide ranges of temperature and salinity, being 5ºC to 30ºC and 1.5 psu to 60-80 psu respectively (Mannino *et al.*, 2015), although values of 3 and 100 psu were also recorded (Orfanidis *et al.*, 2008). The optimal salinity for the growth of *R. cirrhosa* was that of seawater (between 37 and 40 psu) (Mannino and Sara, 2006). In accordance to this statement, *R. cirrhosa* was found to form denser meadows in areas where salinity was about 40 psu in a lagoon in North-eastern Tunisia (Dhib *et al.*, 2013). The same authors found that seagrass density was reduced greatly in areas close to the freshwater inputs.

Light availability was the main factor affecting the biomass production and distribution of the species according to Obrador and Pretus (2010) and Mannino *et al.* (2015).

4.2.5.5 Threats

The growth of seaweeds in the same environment as *R. cirrhosa* showed to have a negative effect, the higher the macroalgaes coverage the lower the area occupied by *R. cirrhosa* (Mannino and Sara, 2006).

An important factor that caused different declines of *R. cirrhosa* populatons in Corsica was the increase in nutrient inputs that led to the generation of large blooms of opportunistic seaweeds such as *Ulva* L. and *Gracilaria* Greville and, as a consequence, the water transparency decreased (Pasqualini *et al.*, 2017). Turbidity created by algal blooms was also observed to cause a negative impact on *R. cirrhosa* in Italy (Mannino and Graziano, 2016).

4.2.5.6 Conservation strategies

Table 27, 28 and 29 show the *in situ* conservation strategies, the legal protection (Table 27), the presence in SPAMI sites (Table 28) and the protocols developed (Table 29).

Table 27. *In situ* conservation strategies for *Ruppia cirrhosa* (Petagna) Grande.: Summary of the legal framework at the International level.

In situ	**Description**	**Reference**
IUCN Red List of Endangered Species.	Scope of assessment: Global (2007) and Europe (2010). Least Concern.	Global: Short *et al.* (2010) Europe: Lansdown (2011)
Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (2012-2017).	Protection of all seagrasses.	UNEP-RAC/SPA (2000)
European Biodiversity Strategy for 2030	Protection of all seagrasses.	EU Biodiversity Strategy for 2030

Table 28 shows the SPAMI sites that include *R. cirrhosa.* In the Italian SPAMI, the species included in this site is not specified, it simply describes the presence of the genus *Ruppia spp.*

Table 28. *In situ* conservation strategies for *Ruppia cirrhosa* (Petagna) Grande.: Presence in SPAMI sites.

Country	**SPAMI**
Slovenia	1. Strunjan Landscape Park
Cyprus	1. Lara-Toxeftra Turtle Reserve
Italy	1. Tavolara Punta Coda Cavallo Marine Protected Area **(*Ruppia spp.*)**

Table 29. *In situ* conservation strategies *Ruppia cirrhosa* (Petagna) Grande: protocols.

Description and location	Reference and location
Use of sods and rhizomes for transplantation of *R. cirrhosa* in restoration project. Sods showed a higher success for transplantation than rhizomes. The study showed the feasibility of large-scale restoration for *R. cirrhosa.* Location: Italy	Sfriso *et al.* (2019)

Table 30 shows the *ex situ* conservation strategies, the protocols developed and the presence in gene banks.

Table 30. *Ex situ* conservation strategies for *Ruppia cirrhosa* (Petagna) Grande. ("x" indicates absence of data).

Protocols	
Description and location	**Reference**
Study on the germination of *Ruppia* taxa testing the effect of chlorinity and temperature, determination of optimal values for both. Location: The Netherlands	Van Vierssen *et al.* (1984)
Gene banks	
1	

The distribution of *R. cirrhosa* in the Mediterranean region is not very clear, there are several reports of its presence in different coastal lagoons of various countries, but a general distribution study in the Mediterranean has not been carried out yet. Probably for this reason, an updated distribution map of the species in the Mediterranean was not found through the present bibliographic research. In addition, the distribution map was also not available in the IUCN Red List for both the global and European assessment (a Mediterranean assessment has not been carried out) (Short *et al.*, 2010; Lansdown, 2011).

Studies on local declines and progressions were both reported. However, these studies cannot give a general idea on whether the species is actually regressing, progressing or remaining stable. The IUCN Red List for both European and global assessments declare that the population trend is stable (Short *et al.*, 2010; Lansdown, 2011). However, they also report that there is no specific information about the population of this species and therefore, further taxonomical work would be desirable in order to obtain accurate information on their distribution (Short *et al.,* 2010).

Problems with taxonomic identifications of the species have been reported in different studies, as they describe their results simply by using the genus *Ruppia sp.* (Boudouresque *et al.*, 2009; Triest and Sierens, 2009; Mannino *et al.*, 2015). This explains the little amount of studies found for this species in the Mediterranean.

Some studies described the local habitat and ecology of the species (Menéndez *et al.*, 2002; Mannino and Sara, 2006; Obrador *et al.*, 2007; Orfanidis *et al.*, 2008; Obrador and Pretus, 2010; Dhib *et al.*, 2013; Mannino *et al.*, 2015; Mannino and Graziano, 2016) and only three articles (Mannino and Sara, 2006; Mannino and Graziano, 2016; Pasqualini *et al.*, 2017) described the possible stressors that could have caused the regressions as described in the Population dynamics section.

Unlike other marine angiosperms, there is no specific legal protection for *R. cirrhosa* in the Mediterranean area. It is only protected by the Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea and the European Biodiversity Strategy for 2030. In any case, this is a general framework for the protection of all seagrasses (UNEP-RAC/SPA, 2000). These results are consistent with the general information of the species found in this work. If a good base of knowledge is not available, it is not possible to create and implement proper conservation measures.

Furthermore, the species was included in 2 SPAMI sites, and it was also present at a genus (*Ruppia sp.*) level in one SPAMI site in Italy. Since it is unsure of what species is present in this area, it cannot be determined whether this species benefits from such degree of protection in Italy. Interestingly, the species is mostly distributed in France and in Spain, based on the distribution map (Figure 22), but the SPAMI sites that include it are in Cyprus and Slovenia.

Two studies were found dealing with scientific information that could be applied to different conservation strategies of the species. Regarding the *in situ* conservation strategies restoration efforts with *R. cirrhosa* can be achieved in the Mediterranean area by planting either rhizomes or sods, although sods would ensure a higher success rate (Sfriso *et al.*, 2019). The second work was carried out by Van Vierssen *et al.* (1984) and it is more suitable to be applied in *ex situ* preservation strategies as it offers a preliminary study of *R. cirrhosa* cultures in the laboratory. Although the latter was not conducted in the Mediterranean Sea, the results presented could serve as a scientific basis for the development of a strategy focused in the Mediterranean area.

There is the presence of *R. cirrhosa* in one gene bank, but unfortunately, as already explained, no information on the location or the type of specimen stored was available on PlantSearch.

This lack of knowledge on the species could be explained by the fact that the genus *Ruppia* has only recently been considered as marine (Short *et al*., 2016). Therefore, the interest on the species might grow in the near future in scientific works of Marine Sciences. The first step in order to take to gain a better understanding of the species and its conservation status would be to properly determine the taxonomic differences among *Ruppia* species, this is a starting point that needs to be achieved before anything else could be more accurately studied.

4. 2. 6 *Ruppia maritima* L.

4.2.6.1 Taxonomy

Figure 23 shows the taxonomic information of *R. maritima.*

Kingdom	Phylum	Class	Order	Family
Plantae	Tracheophyta	Liliopsida	Alismatales	Ruppiaceae

Taxon name: *Ruppia maritima* L.

Synonyms:

- *Buccaferrea maritima* (L.) Lunell
- *Ruppia andina* Phil.
- *Ruppia brachypus* J.Gay
- *Ruppia brevipes* Bertol. ex Griseb.
- *Ruppia cirrhosa subsp. longipes* (Hagstr.) Á.Löve
- *Ruppia curvicarpa* A.Nelson
- *Ruppia intermedia* C.G.H.Thed.
- *Ruppia marina* Fr.
- *Ruppia maritima f. aculeata* Hagstr.
- *Ruppia maritima f. curvirostris* H. St.John & Fosberg
- *Ruppia maritima f. pectinata* Hagstr.
- *Ruppia maritima subsp. brachypus* (J.Gay) Schlegel
- *Ruppia maritima subsp. brachypus* Á. Löve
- *Ruppia maritima subsp. brevirostris* C.Agardh
- *Ruppia maritima subsp. intermedia* (C.G.H.Thed.) Piper & Beattie
- *Ruppia maritima subsp. obliqua* (Griseb. & Schenk) Á.Löve & D.Löve
- *Ruppia maritima subsp. rostellata* (W.D.J.Koch ex Rchb.) Asch. & Graebn.
- *Ruppia maritima subsp. rostellata* Maire
- *Ruppia maritima subsp. rostrata* (C.Agardh) Piper & Beattie
- *Ruppia maritima var. brachypus* (Gay.) Garcke
- *Ruppia maritima var. brachypus* (J.Gay) K.Schum.
- *Ruppia maritima var. brevirostris* (C.Agardh) Asch. & Graebn.

- *Ruppia maritima var. brevirostris* C. Agardh
- *Ruppia maritima var. curvicarpa* (A.Nelson) Fernald & Wiegand
- *Ruppia maritima var. exigua* Fernald & Wiegand
- *Ruppia maritima var. floridana* Graebn.
- *Ruppia maritima var. intermedia* (C.G.H.Thed.) Asch. & Graebn.
- *Ruppia maritima var. japonica* H. Hara
- *Ruppia maritima var. longipes* Hagstr.
- *Ruppia maritima var. mari4tima*
- *Ruppia maritima var. minor* Mert. & W.D.J.Koch
- *Ruppia maritima var. obliqua* (Griseb. & Schenk) Asch. & Graebn.
- *Ruppia maritima var. onondagensis* Fernald & Wiegand
- *Ruppia maritima var. pacifica* H.St.John & Fosberg
- *Ruppia maritima var. recta* Moris
- *Ruppia maritima var. rostellata* Asch. & Graebn.
- *Ruppia maritima var. rostrata* C.Agardh
- *Ruppia maritima var. subcapitata* Fernald & Wiegand
- *Ruppia obliqua* Griseb. & Schenk
- *Ruppia pectinata* Rydb.
- *Ruppia rostellata* W.D.J.Koch ex Rchb.
- *Ruppia rostellata var. brachypus* (J.Gay) T.Marsson
- *Ruppia rostellata var. brachypus* Gay.
- *Ruppia salina* Schur
- *Ruppia spiralis subsp. longipes* (Hagstr.) Á.Löve & D.Löve
- *Ruppia spiralis subsp. transsilvanica* (Schur) Nyman
- *Ruppia subsessilis* Thwaites
- *Ruppia taquetii* H.Lév.
- *Ruppia transsilvanica* Schur
- *Ruppia trichodes* Durand
- *Ruppia zosteroides* Lojac.

Figure 23. Taxonomic information of *Ruppia maritima* L. Source: World Flora Online.

4.2.6.2 Distribution range

R. maritima was classified as a cosmopolitan species by different authors (Orfanidis *et al.*, 2008; Triest and Sierens, 2009; Mannino *et al.*, 2015). Its distribution though, even if found in all the continents, it was observed to be quite discontinuous (Triest and Sierens, 2009). Outside the Mediterranean region *R. maritima* has been observed in Portugal, North America, Central and South America, Canada, South Africa, India, Philippines, Japan and Australia (Mannino *et al.*, 2015).

Local observations have been recorded it in Spain (Triest and Sierens, 2009), specifically in Murcia (Gutiérrez-Cánovas *et al.*, 2009) and along the coast of Catalonia (Menéndez *et al.*, 2019). It was also reported to be present in Italy (Orfanidis *et al.*, 2008), Greece (Orfanidis *et al.*, 2008) and Algeria (Magni *et al.*, 2015) and Turkey (Malea *et al., 2004*; Malea *et al.*, 2008).

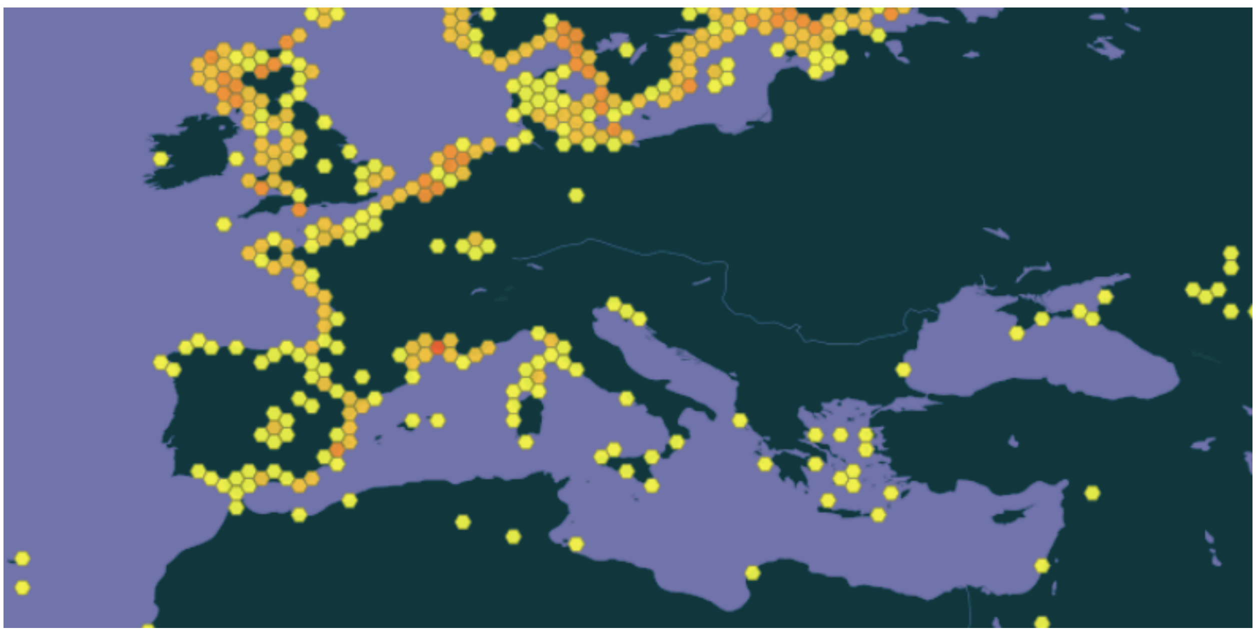

Figure 24. Mediterranean distribution of *Ruppia maritima* L. Source: Source: Global Biodiversity Information Facility - GBIF (2019).

As it occured for *R. cirrhosa* a distribution map of *R. maritima* in the Mediterranean was not available in the bibliographic research here presented. Figure 24 shows its distribution, this map though, should not be taken as a definitive distribution since these results have not been reviewed by the scientific community and since very often *R. maritima* is mistaken for other *Ruppia* species (Mannino *et al.*, 2015).

4.2.6.3 Population dynamics

As a result of the bibliographic research on this species, no information was obtained on the population dynamics of *R. maritima* for the Mediterranean area.

4.2.6.4 Habitat and ecology

It has been determined that *R. maritima* can tolerate high salinity fluctuations: Mannino *et al.* (2015) found that the range of salinity that the species could withstand from 0.3 to 15 psu, while Orfadinis *et al.* (2008) stated that the salinity range was from 0.6 to 27 psu.

It was more commonly found as monospecific meadows, but some were also found forming hybrid populations with *R. cirrhosa* (Mannino *et al.*, 2015).

R. maritima in the Monolimni and Drana Lagoon, (Northern Aegean Sea) showed a different type of life cycle in each lagoon. In the Monolimni Lagoon, permanently flooded, *R. maritima* showed a perennial life cycle. On the contrary, in the Drana Lagoon it showed an annual life cycle, and the vegetative organisms of the meadows disappeared when the lagoon dried out. The seeds remained viable and regenerated the meadow once the water was back (Malea *et al.*, 2004). The vegetative growth of *R. maritima* was determined to occur when the water temperature was higher than 10ºC for the minimum temperature and 15ºC for maximum. This study determined that growth and reproduction of *R. maritima* were mainly affected by water temperature and transparency (Malea *et al.*, 2004).

4.2.6.5 Threats

No results were obtained regarding the main threats or pressures that affected *R. maritima* in the Mediterranean Sea.

4.2.6.6 Conservation strategies

The following table (Table 31) shows the legal protection of *R. maritima* in the Mediterranean.

Table 31. *In situ* conservation strategies for *Ruppia maritima* L.: summary of the legal framework at the International level.

In situ	Description	Reference
IUCN Red List of Endangered Species.	Scope of assessment: Mediterranean. Least Concern. 2007.	Ali (2010)
Habitats Directive on the conservation of wild fauna and flora	Annex 1: Requires designation of special areas of conservation. Estuaries (1130), Large shallow inlets and bays (1160) and Coastal lagoons (1150, priority habitat). (All include *R. maritima*).	Council Directive 92/43/EEC (1992)
Conservation status of habitat types and species	Habitat 1130: Unfavorable-inadequate Habitat 1160: Unfavorable- bad Habitat 1150: Unfavorable-bad (Between 2013-2018)	Article 17, Council Directive 92/43/EEC (1992)
Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (2012-2017).	Protection of all seagrasses.	UNEP-RAC/SPA (2000)
Natura 2000 Network	Sites with *R. maritima* in the 1130, 1150 and 1160 habitat sites.	Natura 2000
European Biodiversity Strategy for 2030	Protection of all seagrasses.	EU Biodiversity Strategy for 2030

Table 32 shows the SPAMI sites that include *R. maritima*, although the site in Italy does not specify the species of *Ruppia* sp.

Table 32. *In situ* conservation strategies for *Ruppia maritima* L.: Presence in SPAMI sites.

Country	SPAMI
Slovenia	1. Strunjan Landscape Park
Italy	1. Tavolara Punta Coda Cavallo Marine Protected Area **(*Ruppia spp.*)**

Table 33 and table 34 show the *in situ* and *ex situ* conservation protocols developed for *R. maritima,* respectively.

Table 33. *In situ* conservation strategies for *Ruppia maritima* L.: protocols.

Description and location	Reference
Study on the effects of salinity on rhizome growth and rooting cultured *in vitro*. Determination of optimal salinity for *in vitro* culture of *R. maritima*. Plants were also rooted *ex vitro* with a success of 100%. Results showed the feasibility for a rapid *in vitro* culture of *R. maritima.* Location: USA	Bird *et al.* (1993)
Determination of feasibility of *R. maritima* meadow restoration through *in vitro* propagation. Two experiments carried out: 1. Comparation of different planting methods. 2. *In vitro* propagation, growth in flowing seawater system and planting. Successful results from both experiments showed that the species can be propagated i*n vitro* and used for meadows restoration. Location: USA	Bird *et al.* (1994)
Experiments to test techniques for culturing and planting *R. maritima.* Development of techniques to maximize the vigor of the plant material used in field plantings and the rate of tissue propagation. The suitability for field restoratition from cultures was demonstrated. Location: USA	De Leon *et al.* (1997)

Study on the effects of planting depth, sediment grain size and nutrients on the growth and emergence of seedlings. Determination of optimal parameters for more successful restoration projects. Location: USA	Ailstock *et al.* (2010)
Development of a protocol for the use of seeds in large-scale restorations. The effects of collection date, processing technique, aeration, storage and induction temperature and salinity and storage period on seed germination were all determined. Location: USA	Ailstock *et al.* (2010)
Restoration project by *R. maritima* transplantation. Evidence that restoration of *R. maritima* is possible. Location: USA	Bologna and Sinnema (2012)

Table 34. *Ex situ* conservation strategies for *Ruppia maritima* L.

Protocols	
Description	**Reference and location**
First successful protocol for algae-free culture of *R. maritima.* Determination of nutrient concentration for optimal growth in culture. Location: USA	Thursby (1984)
Study on the germination of *Ruppia taxa* testing the effect of chlorinity and temperature, determination of optimal values for both. Location: The Netherlands	Van Vierssen *et al.* (1984)
Protocol for the germination of *R. maritima* into algal-free culture. Description of basic culture conditions. Location: Brazil	Seeliger *et al.* (1984)
Effects of auxin and cytokinin on *in vitro* plant growth and development. Use of axenic tissue cultures of *R. maritima* and clonal propagation from terminal rhizome segments. Protocol for the collection, preparation, sterilization and culture of terminal rhizome explants. Location: USA	Koch and Durako (1991)

Comparison of culture media for *R. maritima.* Culture of plant segments. Results showed better results for an inorganic medium. Location: USA	Bird *et al.* (1996)
Gene banks	
4	

Very little scientific works on the general characteristics of *R. maritima* are available for the Mediterranean region. No records of reported possible threats or population trends were found and therefore, it is very difficult to discuss on its conservation needs. The information on the habitat and the distribution were mainly taken from local studies, thus not giving a general perspective of the species' distribution range in the Mediterranean area, or at a global level. The IUCN Red List classifies its population trend as decreasing (Ali, 2010), even though there were no real information on population dynamics to understand why and how this conclusion was made. The distribution map was not available both in the bibliographical results and in the IUCN Red List assessment, making their current population trend assessment quite difficult.

R. maritima is included in three different habitat types based on the Natura 2000 network. Estuaries (1130), Large shallow inlets and bays (1160) and Coastal lagoons (1150), all these habitats are under the protection within Habitats Directive (Council Directive 92/43/EEC, 1992), and an important degree of protection is attributed to Coastal lagoons as it is under the priority habitats. The conservation status of these habitats was determined based on Article 17 of the Habitats Directive and the results are not positive. 1130 was unfavorable-inadequate and 1150 and 1160 were unfavorable-bad (Article 17 Council Directive 92/43/EEC, 1992). This could show the potential of a low conservation status of *R. maritima* based on the conservation of the habitats that it lives in. Also, the species is present in only one SPAMI site, meaning that the protection of this species in this sense is very scarce. Nonetheless, it should be taken under consideration that another SPAMI site includes the genus *Ruppia* sp*.* without specifying the species. This does not allow a proper assessment of the conservation of *R. maritima* in protected marine areas.

The scientific studies that could be applied on different *in situ* conservation strategies were quite numerous. However, they were all carried out in the USA and none in the Mediterranean region. Different protocols for *in vitro* propagation of *R. maritima* were developed with the aim of restoring affected areas (Bird *et al.*, 1993; De Leon *et al.*, 1997) and the research team of Ailstock performed different works for the *in vitro* germination of seeds and the optimal techniques to be employed when doing restoration projects (Ailstock *et al.*, 2010a, 2010b).

A good base of information was also available for the *ex situ* conservation of *R. maritima.* Different preliminary works were carried out and determined protocols for *in vitro* cultures of *R. maritima* (Thursby, 1984; Koch and Durako, 1991; Bird *et al.*, 1996) and germination procedures (Seeliger *et al.*, 1984; Van Vierssen *et al.*, 1984). This information would be very valuable for the material stored in the 4 gene banks. It is the species with the most results in this area and it is quite surprising since no published articles explain strategies for the storage of this species.

Finally, there is not enough available information for this species in the Mediterranean region in order to make proper conclusions on its conservation status, due to this lack of information it is also difficult to properly manage and conserve the species. As mentioned for *R. cirrhosa*, the first efforts to be implemented for a better understanding of the species should be directed towards the taxonomic identification. Only after gaining this knowledge, the next steps can be taken in order to understand specifically the distribution of each taxon, the population dynamics and main threats which are essential to develop a good conservation strategy, if needed.

4.2.7 GENERAL DISCUSSION

In the following Table 35 a summary of the conservation strategies applied for each species can be observed. The *in situ* conservation strategies have been divided into three categories: the legal framework directed at the species specifically, the presence in SPAMI sites and conservation protocols developed. The legal framework that protects certain species in particular are the Bern Convention, Habitats Directive, Action Plan for the Conservation of Marine Vegetation in the Mediterranean Sea (APCMVMS), and the SPA/BD Protocol.

The *ex situ* conservation strategies have also been separated into two different categories, the protocols developed for each species and the presence of the species in gene banks.

Table 35. Summary of the results obtained regarding the conservation strategies applied to each species. ("*" indicates that although protocols for the species exist, none were carried out in the Mediterranean area).

	In situ						***Ex situ***	
Species	**Legal framework directed (species)**				**SPAMI sites**	**Protocols**	**Protocols**	**Gene bank**
	Bern Convention	**Habitats Directive**	**APCMVMS**	**SPA/BD Protocol**				
C. nodosa	√	-	√	√	√	√	-	-
P. oceanica	√	√	√	√	√	√	√	-
Z. marina	√	-	√	√	√	√	-	√
Z. noltii	-	-	√	√	√	√*	-	-
R. cirrhosa	-	-	-	-	√	√	√*	√
R. maritima	-	-	-	-	√	√*	√*	√

A few general observations can be drawn from these results. *P. oceanica* is the species that benefits from the highest number of conservation strategies. Results for *in situ* conservation were presented for each category, showing that an important interest and efforts are dedicated to the conservation of this species in its natural habitat. *P. oceanica* is also the only species that presents *ex situ* conservation protocols carried out in the Mediterranean Sea. These findings seem to be natural as *P. oceanica* is very well distributed in the Mediterranean Sea.

Results for *R. cirrhosa* and *R. maritima* were also obtained regarding the *ex situ* protocols, but none of these were conducted in the Mediterranean, probably showing a higher interest in these species outside of this region.

C. nodosa and *Z. marina* showed an equal level of protection *in situ.* These results are quite surprising if compared to the results obtained with the bibliographic research on their background information. On one hand, *C. nodosa* is very well studied, especially its distribution and main threats, which are important parameters to determine the level of protection that the species requires. On the other hand, *Z. marina* showed a much lower level of knowledge, especially considering its distribution, which would make assume that not as many conservation strategies would be applied to the species, but this is not the case.

Both *Ruppia* species are not legally protected at a species level, but they are both present in SPAMI sites. These results are consistent with the background information, as not much is known about these species in the Mediterranean Sea and especially because they have only recently been classified as marine angiosperms. Interestingly, they are the only species that present both *ex situ* conservation strategies, although none of the protocols have been carried out in the Mediterranean.

5. CONCLUSIONS

Based on the results obtained with the present study, the following conclusions can be made:

1. The results obtained on the available general information of each seagrass (distribution range, population dynamics, habitat and ecology and threats) varies greatly depending on the species. *Posidonia oceanica* and *Cymodocea nodosa* are the species for which the information is the most abundant and precise. It is safe to say that these two species were the most studied during the time period included between 2000 and 2020 in the Mediterranean Sea. On the contrary, not many studies are available for *Ruppia maritima* in the Mediterranean Sea and information is lacking, especially for its distribution, population dynamics and main threats. For the rest of the species (*Zostera marina, Zostera noltii* and *Ruppia cirrhosa*) the information available was also relatively scarce, but quite proportional to their distribution range in the Mediterranean.
2. Overall, *P. oceanica* is the species that benefits from the highest number of *in situ* conservation strategies, followed by *C. nodosa* and *Z. marina.* These three species are the most protected *in situ*, reflecting the knowledge available on their background information, except for *Z. marina*. The species in the genus *Ruppia* are the only ones that are simply protected by general legal actions and not as species. Restorations and transplantations are the main strategies observed in the protocols for the conservation of seagrasses in the Mediterranean. All seagrasses are present in at least one SPAMI site.
3. The *ex situ* conservation strategies applied to seagrasses in the Mediterranean are very scarce. The main strategies being implemented are *in vitro* cultures and *in vitro* germination of seeds, but not for all of the species. Gene banks are not a common conservation measure implemented for marine angiosperms, only *Z. marina, R. cirrhosa* and *R. maritima* benefit from this strategy. *P. oceanica* is the only species that presents a preliminary protocol for its conservation *ex situ* in the Mediterranean Sea.
4. The main gaps of knowledge observed with this review were on the distribution and population dynamics of these species at a level of the Mediterranean Sea, especially for *Z. noltii, R. cirrhosa* and *R. maritima.* The same can be said for the *ex situ* conservation actions, as the protocols are mainly preliminary studies that could be useful for the development of *ex situ* conservation strategies.

6. FUTURE RESEARCH DIRECTIONS

In the future, it would be interesting to further develop and investigate the following areas:

- To carry out a complete mapping of all the coasts of the Mediterranean Sea for the presence or absence of these species, in order to obtain a global perception on the distribution of each species, especially for *Z. marina, Z. noltii, R. cirrhosa* and *R. maritima*.
- To conduct studies assessing the population dynamics of *Z. marina, Z. noltii, R. cirrhosa* and *R. maritima* at a Mediterranean scale.
- To include marine angiosperms in *ex situ* conservation strategies carried out in genebanks. In order to properly achieve this goal, more scientific efforts directed towards a better understanding on the germplasm (seeds, vegetative propagules, cell and tissue cultures and so on) response to different storage conditions would be very useful.
- To study the effect that global warming could have on these species, in order to predict their future distribution and population trend, and plan conservation strategies ahead of time.

7. REFERENCES

Abadie, A., Pace, M., Gobert, S., Borg, J.A., (2018). Seascape ecology in Posidonia oceanica seagrass meadows: Linking structure and ecological processes for management, *Ecological Indicators* Elsevier 87, 1–13.

Agostini, S., Capiomont, A., Marchand, B., Pergent, G., (2003). Distribution and estimation of basal area coverage of subtidal seagrass meadows in a Mediterranean coastal lagoon, *Estuarine, Coastal and Shelf Science* Elsevier 56(5–6), 1021–1028.

Ailstock, M.S., Shafer, D.J., Magoun, A.D., (2010A). Effects of planting depth, sediment grain size, and nutrients on Ruppia maritima and Potamogeton perfoliatus seedling emergence and growth, *Restoration Ecology* Wiley Online Library 18(4), 574–583.

Ailstock, M. S., Shafer, D.J., Magoun, A.D., (2010B). Protocols for use of Potamogeton perfoliatus and Ruppia maritima seeds in large-scale restoration, *Restoration ecology* Wiley Online Library 18(4), 560–573.

Alberto, F., Massa, S., Manent, P., Diaz-Almela, E., Arnaud-Haond, S., Duarte, C.M., Serrao, E.A., (2008). Genetic differentiation and secondary contact zone in the seagrass Cymodocea nodosa across the Mediterranean–Atlantic transition region, *Journal of Biogeography* Wiley Online Library 35(7), 1279–1294.

Ali, M.M. (2010): *Ruppia maritima*. *The IUCN Red List of Threatened Species*. Available at: https://www.iucnredlist.org/species/164508/5897205 [Accessed on: 10-04-2020].

Ardizzone, G., Belluscio, A., Maiorano, L., (2006). Long-term change in the structure of a Posidonia oceanica landscape and its reference for a monitoring plan, *Marine Ecology* Wiley Online Library 27(4), 299–309.

Artüz, M.L., (2017). First record of Bopyrina ocellata (Isopoda, Bopyridae) from the Sea of Marmara, Turkey, *Crustaceana* Brill 90(6), 685–694.

Aubry, K.B., Raley, C.M., McKelvey, K.S., (2017). The importance of data quality for generating reliable distribution models for rare, elusive, and cryptic species, *PLoS One* Public Library of Science San Francisco, CA USA 12(6), e0179152.

Badalamenti, F., Di Carlo, G., D'Anna, G., Gristina, M., Toccaceli, M., (2006). Effects of dredging activities on population dynamics of Posidonia oceanica (L.) Delile in the Mediterranean sea: the case study of Capo Feto (SW Sicily, Italy), in *Marine Biodiversity* Springer , 253–261.

Balestri, E., Bertini, S., (2003). Growth and development of Posidonia oceanica seedlings treated with plant growth regulators: possible implications for meadow restoration, *Aquatic Botany* Elsevier 76(4), 291–297.

Balestri, E., Lardicci, C., (2006). Stimulation of root formation in Posidonia oceanica cuttings by application of auxins (NAA and IBA): a potential useful pre-planting practice for meadow restoration.

Balestri, E., Lardicci, C., (2012). Nursery-propagated plants from seed: a novel tool to improve the effectiveness and sustainability of seagrass restoration, *Journal of Applied Ecology* Wiley Online Library 49(6), 1426–1435.

Balestri, E., Piazzi, L., Cinelli, F., (1998). In vitro germination and seedling development of Posidonia oceanica, *Aquatic Botany* Elsevier 60(1), 83–93.

Balestri, E., Piazzi, L., Cinelli, F., (1998). Survival and growth of transplanted and natural seedlings of Posidonia oceanica (L.) Delile in a damaged coastal area, *Journal of Experimental Marine Biology and Ecology* Elsevier 228(2), 209–225.

Balestri, E., Vallerini, F., Lardicci, C., (2011). Storm-generated fragments of the seagrass Posidonia oceanica from beach wrack–A potential source of transplants for restoration, *Biological conservation* Elsevier 144(5), 1644–1654.

Balestri, E., Vallerini, F., Lardicci, C., (2017). Recruitment and patch establishment by seed in the seagrass Posidonia oceanica: importance and conservation implications, *Frontiers in plant science* Frontiers 8, 1067.

Bergmann, N., Winters, G., Rauch, G., Eizaguirre, C., Gu, J., Nelle, P., (2010). Population-specificity of heat stress gene induction in northern and southern eelgrass Zostera marina populations under simulated global warming, *Molecular Ecology* Wiley Online Library 19(14), 2870–2883.

Bernard, G., Bonhomme, P., Boudouresque, C.F., (2005). Recovery of the seagrass Zostera marina in a disturbed Mediterranean lagoon (Etang de Berre, Bouches-du-Rhône, Southern France), *Hydrobiologia* Springer 539(1), 157–161.

Bernard, G., Boudouresque, C.F., Picon, P., (2007). Long term changes in Zostera meadows in the Berre lagoon (Provence, Mediterranean Sea), *Estuarine, Coastal and Shelf Science*

Elsevier 73(3–4), 617–629.

Bilgin, S., Çelik, E.Ş., Ateş, A.S., (2007). The Brachyura (Decapoda) community of Zostera marina meadows in the coastal area of the Southern Black Sea (Sinop Peninsula, Turkey), *Crustaceana* Brill 80(6), 717–730.

Bird, K.T., Cody, B.R., Jewett-Smith, J., Kane, M.E., (1993). Salinity effects on Ruppia maritima L. cultured in vitro, *Botanica Marina* Walter de Gruyter, Berlin/New York 36(1), 23–28.

Bird, K.T., Brown, M.S., Henderson, T.T., O'Hara, C.E., Robbie, J.M., (1996). Culture studies of Ruppia maritima L. in bicarbonate-and sucrose-based media, *Journal of experimental marine biology and ecology* Elsevier 199(2), 153–164.

Bird, K.T., Jewett-Smith, J., Fonseca, M.S., (1994). Use of in vitro propagated Ruppia maritima for seagrass meadow restoration, *Journal of Coastal Research* JSTOR , 732–737.

Bologna, P.A.X., Sinnema, M.S., (2012). Restoration of Seagrass Habitat in New Jersey, United States, *Journal of Coastal Research* The Coastal Education and Research Foundation 1656 Cypress Row Drive, West … 28(1A), 99–104.

Borg, J.A., Rowden, A.A., Attrill, M.J., Schembri, P.J., Jones, M.B., (2009). Occurrence and distribution of different bed types of seagrass Posidonia oceanica around the Maltese Islands, *Mediterranean Marine Science* 10(2), 45–62.

Borum, J., Greve, T.M., Krause-Jensen, D., Duarte, C.M., (2004). *European seagrasses: an introduction to monitoring and management.* EU.The M&MS project.

Boscutti, F., Marcorin, I., Sigura, M., Bressan, E., Tamberlich, F., Vianello, A., Casolo, V., (2015). Distribution modeling of seagrasses in brackish waters of Grado-Marano lagoon (Northern Adriatic Sea), *Estuarine, Coastal and Shelf Science* Elsevier 164, 183–193.

Botanic Gardens Conservation International (n.d.): PlantSearch. Available at: https://tools.bgci.org/plant_search.php [Accessed on: 02-04-2020]

Boudouresque, C.F., Bernard, G., Bonhomme, P., Charbonnel, E., Diviacco, G., Meinesz, A., Pergent, G, Pergent-Martini, C., Ruitton, S., Tunesi, L., (2012). *Protection and conservation of Posidonia oceanica meadows,* RAMOGE and RAC/SPA publisher.

Boudouresque, C.F., Bernard, G., Bonhomme, P., Charbonnel, E., Le Diréach, L., Ruitton, S., (2007). Monitoring methods for Posidonia oceanica seagrass meadows in Provence and the

French Riviera, *Sci Rep Port-Cros Natl Park* 22, 17–38.

Boudouresque, C.F., Bernard, G., Pergent, G., Shili, A., Verlaque, M., (2009). Regression of Mediterranean seagrasses caused by natural processes and anthropogenic disturbances and stress: a critical review, *Botanica Marina* Walter de Gruyter 52(5), 395–418.

Boudouresque, C.F., Mayot, N., Pergent, G., (2006). The outstanding traits of the functioning of the Posidonia oceanica seagrass ecosystem, *Biol Mar Medit* 13(4), 109–113.

Brodersen, M.M., Pantazi, M., Kokkali, A., Panayotidis, P., Gerakaris, V., Maina, I., Kavadas, S., Kaberi, H., Vassilopoulou, V., (2018). Cumulative impacts from multiple human activities on seagrass meadows in eastern Mediterranean waters: the case of Saronikos Gulf (Aegean Sea, Greece), *Environmental Science and Pollution Research* Springer 25(27), 26809–26822.

Brouns, J.J., Pernetta, J.C., Leemans, R., Elder, D., Humphrey, S., (1994): *Impacts of Climate Change on Ecosystems and Species: Marine and Coastal ecosystems,* Gland, Switzerland. IUCN.

Buia, M. & Pergent-Martini, C. (2015): *Zostera marina*. *The IUCN Red List of Threatened Species*. Available at: https://www.iucnredlist.org/species/164508/5897205 [Accessed on: 10-04-2020].

Bujang, J.S., (2012). *The Marine Angiosperms, Seagrass*, Doctoral Thesis, University Putra Malaysia, Serdang.

Bull, J.C., Kenyon, E.J., Edmunds, D., Cook, K.J., (2010). Recent loss of Gibraltar seagrasses, *Botanica Marina* De Gruyter 53(1), 89–91.

Burgos, E., Montefalcone, M., Ferrari, M., Paoli, C., Vassallo, P., Morri, C., Bianchi, C., Nike (2017). Ecosystem functions and economic wealth: Trajectories of change in seagrass meadows, *Journal of Cleaner Production* Elsevier 168, 1108–1119.

Cabaço, S., Machás, R., Vieira, V., Santos, R., (2008). Impacts of urban wastewater discharge on seagrass meadows (Zostera noltii), *Estuarine, Coastal and Shelf Science* Elsevier 78(1), 1–13.

Calvo, S., Tomasello, A., Di Maida, G., Pirrotta, M., Cristina Buia, M., Cinelli, F., Cormaci, M., Furnari, G., Giaccone, G., Luzzu, F., (2010). Seagrasses along the Sicilian coasts, *Chemistry and Ecology* Taylor & Francis 26(S1), 249–266.

Canals, M., Amblas, D., Rayo, X., Romero, J., Ylla, J., (2020). Distribution of seagrass communities north of Barcelona, Northwestern Mediterranean Sea, in *Seafloor Geomorphology as Benthic Habitat* Elsevier , 385–401.

Cardoso, P.G., Raffaelli, D., Pardal, M.A., (2008). The impact of extreme weather events on the seagrass Zostera noltii and related Hydrobia ulvae population, *Marine Pollution Bulletin* Elsevier 56(3), 483–492.

Charpentier, A., Grillas, P., Lescuyer, F., Coulet, E., Auby, I., (2005). Spatio-temporal dynamics of a Zostera noltii dominated community over a period of fluctuating salinity in a shallow lagoon, Southern France, *Estuarine, Coastal and Shelf Science* Elsevier 64(2–3), 307–315.

Chefaoui, R.M., Assis, J., Duarte, C.M., Serrão, E.A., (2016). Large-scale prediction of seagrass distribution integrating landscape metrics and environmental factors: the case of Cymodocea nodosa (Mediterranean–Atlantic), *Estuaries and Coasts* Springer 39(1), 123–137.

Chefaoui, R.M., Duarte, C.M., Serrão, E.A., (2018). Dramatic loss of seagrass habitat under projected climate change in the Mediterranean Sea, *Global change biology* Wiley Online Library 24(10), 4919–4928.

Christia, C., Giordani, G., Papastergiadou, E., (2018). Environmental variability and macrophyte assemblages in coastal lagoon types of Western Greece (Mediterranean Sea), *Water* Multidisciplinary Digital Publishing Institute 10(2), 151.

Cullen-Unsworth, L.C., Unsworth, R.K.F., (2016). Strategies to enhance the resilience of the world's seagrass meadows, *Journal of Applied Ecology* 53(4), 967–972. doi: 10.1111/1365-2664.12637.

Cunha, A.H., Araújo, A., (2009). New distribution limits of seagrass beds in West Africa, *Journal of biogeography* Wiley Online Library 36(8), 1621–1622.

Davis, R.C., Short, F.T., (1997). Restoring eelgrass, Zostera marina L., habitat using a new transplanting technique: the horizontal rhizome method, *Aquatic Botany* Elsevier 59(1–2), 1–15.

DeLeon, M.F., Durako, M.J., Shup, J.J., Daeschner, S., (1997). Real-world considerations for micropropagating Ruppia maritima L. (Widgeon grass) for seagrass restoration projects, in *Proceedings of the 22nd Annual Conference on Ecosystems Restoration and Creation. Tampa FL: Hillsborough Community College* , 27–38.

Descamp, P., Cornu, T., Bougerol, M., Boissery, P., Ferlat, C., Delaruelle, G., Deter, J., Gobert, S., Ozhan, E., (2017). Experimental transplantation of Posidonia oceanica, in *13th International*

MEDCOAST Congress on Coastal and Marine Sciences, Engineering, Management and Conservation, MEDCOAST 2017 Mediterranean Coastal Foundation.

Dhib, A., Brahim, M.B., Turki, S., Aleya, L., (2013). Contrasting key roles of Ruppia cirrhosa in a southern Mediterranean lagoon: reservoir for both biodiversity and harmful species and indicator of lagoon health status, *Marine pollution bulletin* Elsevier 76(1–2), 116–127.

Domínguez, M., Celdrán, D., Muñoz-Vera, A., Infantes, E., Martinez-Baños, P., Marín, A., Terrados, J., (2012). Experimental Evaluation of the Restoration Capacity of a Fish-Farm Impacted Area with Posidonia oceanica (L.) Delile Seedlings, *Restoration Ecology* Wiley Online Library 20(2), 180–187.

Duarte, B., Martins, I., Rosa, R., Matos, A.R., Roleda, M.Y., Reusch, T.B.H., Engelen, A.H., Serrão, E.A., Pearson, G.A., Marques, J.C., (2018). Climate change impacts on seagrass meadows and macroalgal forests: An integrative perspective on acclimation and adaptation potential, *Frontiers in Marine Science* 5. doi: 10.3389/fmars.2018.00190.

Duarte, C., Borum, J., Short, F.T., Walker, D., (2008). "Seagrass ecosystems: their global status and prospects", in: Polunin, N.V.C., *Aquatic ecosystems: trends and global prospects,* UK: Cambridge University Press , 281–294.

Duman, M., Borum, J., Short, F.T., Walker, D., (2019). Mapping Posidonia oceanica (Linnaeus) Meadows in the Eastern Aegean Sea Coastal Areas of Turkey: Evaluation of Habitat Maps Produced Using the Acoustic Ground Discrimination Systems, *International Journal of Environment and Geoinformatics* 6(1), 67–75.

Elso, M.Z., Manent, P., Robaina, R.R., (2018). Zostera noltii in the Canary Islands: A Genetic Description for Conservation Purposes, *Journal of Coastal Research* The Coastal Education and Research Foundation 34(5), 1129–1135.

Espel, D., Manent, P., Robaina, R.R. (2019). Dynamics of the seagrass Zostera noltei in a shallow Mediterranean lagoon exposed to chemical contamination and other stressors, *Estuarine, Coastal and Shelf Science* Elsevier 222, 1–12.

EUR-Lex (n.d.): EU Publications Office. Available at: https://eur-lex.europa.eu/homepage.html [Accessed on 17-06-2020]

European Commission (1981) “Council Decision 82/72/EEC of 3 December 1981 concerning the conclusion of the Convention on the conservation of European wildlife and natural habitats”.

European Commission (1992) “Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora”.

European Commission (2006) “Council Regulation (EC) No 1967/2006 of 21 December 2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, amending Regulation (EEC) No 2847/93 and repealing Regulation (EC) No 1626/94”

Fernández-Torquemada, Y., Sánchez-Lizaso, J.L., (2006). Effects of salinity on growth and survival of Cymodocea nodosa (Ucria) Ascherson and Zostera noltii Hornemann Società Italiana di Biologia Marina.

Fernández-Torquemada, Y., Sánchez-Lizaso, J.L., (2013). Effects of salinity on seed germination and early seedling growth of the Mediterranean seagrass Posidonia oceanica (L.) Delile, *Estuarine, Coastal and Shelf Science* Elsevier 119, 64–70.

García-Redondo, V., Bárbara, I., Díaz-Tapia, P., (2019). Zostera marina meadows in the northwestern Spain: distribution, characteristics and anthropogenic pressures, *Biodiversity and Conservation* Springer 28(7), 1743–1757.

Garmendia, J.M., Valle, M., Borja, Á., Chust, G., Lee, D.J., Rodríguez, J.G., Franco, J. (2017). Effect of trampling and digging from shellfishing on Zostera noltei (Zosteraceae) intertidal seagrass beds, *Scientia Marina* 81(1), 121–128.

Garrido, M., Lafabrie, C., Torre, F., Fernandez, C., Pasqualini, V., (2013). Resilience and stability of Cymodocea nodosa seagrass meadows over the last four decades in a Mediterranean lagoon, *Estuarine, Coastal and Shelf Science* Elsevier 130, 89–98.

Garrote-Moreno, A., Fernández-Torquemada, Y., Sánchez-Lizaso, J.L., (2014). Salinity fluctuation of the brine discharge affects growth and survival of the seagrass Cymodocea nodosa, *Marine pollution bulletin* Elsevier 81(1), 61–68.

GBIF Secretariat (2019). GBIF Backbone Taxonomy. Available at: https://www.gbif.org/species/5328663 [Accessed on: 05-06-2020]

Githaiga, M.N., Gilpin, L., Kairo, J.G., Huxham, M. (2016). Biomass and productivity of seagrasses in Africa, *Botanica marina* De Gruyter.

González-Correa, J.M., Bayle, J.T., Sánchez-Lizaso, J.L., Valle, C., Sánchez-Jerez, P., Ruiz, J.M., (2005). Recovery of deep Posidonia oceanica meadows degraded by trawling, *Journal of*

Experimental Marine Biology and Ecology 320(1), 65–76. doi: 10.1016/j.jembe.2004.12.032.

González-Correa, J.M., Sempere, J.T.B., Sánchez-Jerez, P., Valle, C., (2007). Posidonia oceanica meadows are not declining globally. Analysis of population dynamics in marine protected areas of the Mediterranean Sea, *Marine Ecology Progress Series* 336, 111–119.

Grech, A., Chartrand-Miller, K., Erftemeijer, P., Fonseca, M., McKenzie, L., Rasheed, M., Taylor, H., Coles, R., (2012). A comparison of threats, vulnerabilities and management approaches in global seagrass bioregions, *Environmental Research Letters* 7(2). doi: 10.1088/1748-9326/7/2/024006.

Green, E.P., Short, F.T., Frederick, T., (2003). *World atlas of seagrasses,* Berkeley and Los Angeles: University of California Press.

Guerrero-Meseguer, L., Marín, A., Sanz-Lázaro, C., (2017). Future heat waves due to climate change threaten the survival of Posidonia oceanica seedlings, *Environmental Pollution* Elsevier 230, 40–45.

Guillén, J.E., Lizaso, J.L.S., Jiménez, S., Martínez, J., Codina, A., Montero, M., Triviño, A., Soler, G., Zubcoff, J.J., (2013). Evolution of Posidonia oceanica seagrass meadows and its implications for management, *Journal of sea research* Elsevier 83, 65–71.

Gutiérrez-Cánovas, C., Velasco, J., Millán, A., (2009). Effects of dilution stress on the functioning of a saline Mediterranean stream, *Hydrobiologia* Springer 619(1), 119–132.

Den Hartog, C., (1970). *The Sea grasses of the World*, Amsterdam London, North Holland Publishing Company. doi: 10.1002/iroh.19710560139.

Harwell, M.C., Orth, R.J., (1999). Eelgrass (Zostera marina L.) seed protection for field experiments and implications for large-scale restoration, *Aquatic Botany* Elsevier 64(1), 51–61.

Hemminga, M.A., Duarte, C.M., (2000). *Seagrass ecology,* London: Cambridge University Press.

Heywood, V.H., (2014). An overview of in situ conservation of plant species in the Mediterranean, *Flora Mediterranea*, 5–24.

Holon, F., Mouquet, N., Boissery, P., Bouchoucha, M., Delaruelle, G., Tribot, A.S., Deter, J., (2015). Fine scale cartography of human impacts along French Mediterranean coasts: a relevant map for the management of marine ecosystems, *PLoS One* Public Library of Science 10(8).

Hooper, D.U., Chapin, S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, J., Vandermeer, J., Wardle, D.A., (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge, *Ecological monographs* Wiley Online Library 75(1), 3–35.

Hunter, D., Heywood, V.H., (2011): *Crop Wild Relatives. A manual of in situ Conservation*, London, UK. EarthScan.

Infantes, E., Eriander, L., Moksnes, P.O., (2016). Eelgrass (Zostera marina) restoration on the west coast of Sweden using seeds, *Marine Ecology Progress Series,* 546, 31–45.

International Union for Conservation on Nature and Natural Resources (IUCN), (1980): First World Conservation Strategy.

Jahnke, M., Serra, I.A., Bernard, G., Procaccini, G., (2015). The importance of genetic make-up in seagrass restoration: a case study of the seagrass Zostera noltei, *Marine Ecology Progress Series* 532, 111–122.

Jiménez-Ramos, R., Egea, L.G., Ortega, M.J., Hernández, I., Vergara, J.J., Brun, F.G., (2017). Global and local disturbances interact to modify seagrass palatability, *PloS one* Public Library of Science San Francisco, CA USA 12(8), e0183256.

Jump, A.S., Marchant, R., Peñuelas, J., (2009). Environmental change and the option value of genetic diversity, *Trends in plant science* Elsevier 14(1), 51–58.

Kasso, M., Balakrishnan, M., (2013). Ex situ conservation of biodiversity with particular emphasis to Ethiopia, *ISRN Biodiversity* Hindawi Publishing Corporation 2013.

Kocak, F., Uluturhan, E., Gier, G.Y., Önen, S.A., (2011). Impact of environmental conditions on Posidonia oceanica meadows in the Eastern Mediterranean Sea, NISCAIR-CSIR, India.

Koch, E.W., Durako, M.J., (1991). In vitro studies of the submerged angiospermRuppia maritima: Auxin and cytokinin effects on plant growth and development, *Marine Biology* Springer 110(1), 1–6.

Kuo, J., Den Hartog, C., (2007). Seagrass morphology, anatomy, and ultrastructure, in *SEAGRASSES: BIOLOGY, ECOLOGYAND CONSERVATION* Springer , 51–87.

Laffoley, D., Grimsditch, G., (2009): *The management of natural coastal carbon sinks, Switzerland.* IUCN Gland.

Laguna, E., (1998). Flora rara, endémica o amenazada de la Comunidad Valenciana: Valencia: Consellería de Medio Ambiente de la Generalitat Valenciana.

Laguna, E., (2012): “Estrategias de conservación y planes de recuperación de especies amenazadas de flora silvestre”, at: Consellería de Infraestructuras, Territorio y Medio Ambiente, Generalitat Valenciana, Valencia, España.

Larkum, A.W.D., Davey, P., Kuo, J., Raplh, P., Raven, J., (2017). Carbon-concentrating mechanisms in seagrasses, *Journal of Experimental Botany* Oxford University Press UK 68(14), 3773–3784.

Larkum, A.W D., Orth, R. J., Duarte, C.M., (2006). Seagrasses: Biology, ecology and conservation, *Seagrasses: Biology, Ecology and Conservation*, 1–691.

Liu, X., Zhou, Y., Liu, B., Zhang, X., (2019). Temporal dynamics of the natural and trimmed angiosperm Zostera marina L. (Potamogetonales: Zosteraceae), and an effective technique for transplantation of long shoots in a temperate tidal zone (northern China), *Wetlands* Springer 39(5), 1043–1056.

Loques, F., Caye, G., Meinesz, A., (1990A). Axenic culture of selected tissue of Posidonia oceanica, *Aquatic botany* Elsevier 37(2), 171–188.

Loques, F., Caye, G., Meinesz, A., (1990B). Germination in the marine phanerogam Zostera noltii Hornemann at Golfe Juan, French Mediterranean, *Aquatic Botany* Elsevier 38(2–3), 249–260.

de los Santos, C.B., Krause-Jensen, D., Alcoverro, T., Marbà, N., Duarte, C.M., van Katwijk, M.M., Pérez, M., Romero, J., Sánchez-Lizaso, J.L., Roca, G., (2019). Recent trend reversal for declining European seagrass meadows, *Nature communications* Nature Publishing Group 10(1), 1–8.

Magni, P., Draredja, B., Melouah, K., Como, S., (2015). Patterns of seasonal variation in lagoonal macrozoobenthic assemblages (Mellah lagoon, Algeria), *Marine Environmental Research* Elsevier 109, 168–176.

Malea, P., Boubonari, T., Kevrekidis, T., (2008). Iron, zinc, copper, lead and cadmium contents in Ruppia maritima from a Mediterranean coastal lagoon: monthly variation and distribution in different plant fractions, *Botanica Marina* De Gruyter 51(4), 320–330.

Malea, P., Kevrekidis, T., Mogias, A., (2004). Annual versus perennial growth cycle in Ruppia maritima L.: temporal variation in population characteristics in Mediterranean lagoons (Monolimni and Drana Lagoons, Northern Aegean Sea), *Botanica marina* De Gruyter 47(5), 357–366.

Manent, P., Bañolas, G., Alberto, F., Curbelo, L., Espino, F., Tuya, F., (2020). Long-term seagrass degradation: Integrating landscape, demographic, and genetic responses, *Aquatic Conservation: Marine and Freshwater Ecosystems* Wiley Online Library.

Mannino, A.M., Menéndez, M., Obrador, B., Sfriso, A., Triest, L., (2015). The genus Ruppia L. (Ruppiaceae) in the Mediterranean region: an overview, *Aquatic Botany* Elsevier 124, 1–9.

Mannino, A.M., Geraci, A., (2016). Diploid Ruppia cirrhosa populations from a southern Mediterranean shallow system, *Aquatic Botany* Elsevier 132, 37–40.

Mannino, A.M., Graziano, M., (2016). Differences in the growth cycle of Ruppia cirrhosa (Petagna) Grande in a Mediterranean shallow system, *Plant Biosystems, An International Journal Dealing with all Aspects of Plant Biology* Taylor & Francis 150(1), 54–61.

Mannino, A.M., Sara, G., (2006). The effect of Ruppia cirrhosa features on macroalgae and suspended matter in a Mediterranean shallow system, *Marine Ecology* Wiley Online Library 27(4), 350–360.

Marba, N., Díaz-Almela, E., Duarte, C.M., (2014). Mediterranean seagrass (Posidonia oceanica) loss between 1842 and 2009, *Biological Conservation* Elsevier 176, 183–190.

Marion, S.R., Orth, R.J., (2010). Innovative Techniques for large-scale seagrass restoration using Zostera marina (eelgrass) seeds, *Restoration Ecology* Wiley Online Library 18(4), 514–526.

McCormick, K., (2018). *Fish Assemblages in South Puget Sound Z. marina,* Thesis, Evergreen State College, USA.

Meinesz, A., Caye, G., Loques, F., Molenaar, H., (1991). Growth and development in culture of orthotropic rhizomes of Posidonia oceanica, *Aquatic botany* Elsevier 39(3–4), 367–377.

Meinesz, A., Caye, G., Loques, F., Molenaar, H., (1993). Polymorphism and development of Posidonia oceanica transplanted from different parts of the Mediterranean into the National Park of Port-Cros, *Botanica Marina* Walter de Gruyter, Berlin/New York 36(3), 209–216.

Menéndez, M., Abril, M., Colls, Miriam., Quintana, X.D., (2019). Decomposition processes in coastal lagoons and their implications for the assessment of ecological health, *Aquatic Conservation: Marine and Freshwater Ecosystems* Wiley Online Library 29(3), 450–460.

Menéndez, M., Hernandez, O., Comin, F. A., (2002). Spatial distribution and ecophysiological characteristics of macrophytes in a Mediterranean coastal lagoon, *Estuarine, Coastal and Shelf Science* Elsevier 55(3), 403–413.

Molenaar, H., Meinesz, A., (1995). Vegetative reproduction in Posidonia oceanica: survival and development of transplanted cuttings according to different spacings, arrangements and substrates, *Botanica Marina* Walter de Gruyter, Berlin/New York 38(1–6), 313–322.

Montefalcone, M., Lasagna, R., Bianchi, C.N., Morri, C., Albertelli, G., (2006). Anchoring damage on Posidonia oceanica meadow cover: a case study in Prelo Cove (Ligurian Sea, NW Mediterranean), *Chemistry and Ecology* Taylor & Francis 22(sup1), S207–S217.

Montefalcone, M., Albertelli, G., Morri, C., Parravicini, V., Bianchi, C.N. (2009). Legal protection is not enough: Posidonia oceanica meadows in marine protected areas are not healthier than those in unprotected areas of the northwest Mediterranean Sea, *Marine Pollution Bulletin* Elsevier Ltd 58(4), 515–519. doi: 10.1016/j.marpolbul.2008.12.001.

Mosbahi, N., Boudaya, L., Neifar, L., Dauvin, J.C., (2020). Do intertidal Zostera noltei meadows represent a favourable habitat for amphipods? The case of the Kneiss Islands (Gulf of Gabès: Central Mediterranean Sea), *Marine Ecology* Wiley Online Library.

Mosbahi, N., Dauvin, J.C., Neifar, L., (2018). Molluscs associated with intertidal Zostera noltei Hornemann beds in southern Tunisia (central Mediterranean): seasonal dynamics and environmental drivers.

Novak, A.B., Plaisted, H.K., Hays, C.G., Hughes, R.A., (2017). Limited effects of source population identity and number on seagrass transplant performance, *PeerJ* PeerJ.

Obrador, B., Pretus, J.L., (2010). Spatiotemporal dynamics of submerged macrophytes in a Mediterranean coastal lagoon, *Estuarine, Coastal and Shelf Science* Elsevier 87(1), 145–155.

Obrador, B., Pretus, J.L., Menéndez, M., (2007). Spatial distribution and biomass of aquatic rooted macrophytes and their relevance in the metabolism of a Mediterranean coastal lagoon, *Scientia Marina* 71(1), 57–64.

Ondiviela, B., Ondiviela, A., Losada, I.J., Lara, J.L., Maza, M., Galván, C., Bouma, T., van Belzen, T., (2014) . The role of seagrasses in coastal protection in a changing climate, *Coastal Engineering* Elsevier 87, 158–168.

Ontoria, Y., Gonzalez-Guedes, E., Sanmartí, N., Bernardeau-Esteller, J., Ruiz, J.M., Romero, J., Pérez, M., (2019). Interactive effects of global warming and eutrophication on a fast-growing Mediterranean seagrass, *Marine environmental research* Elsevier 145, 27–38.

Orfanidis, S., Pinna, M., Sabetta, L., Stamatis, N., Nakou, K., (2008). Variation of structural and functional metrics in macrophyte communities within two habitats of eastern Mediterranean coastal lagoons: natural versus human effects, *Aquatic Conservation: Marine and Freshwater Ecosystems* Wiley Online Library 18, 45–61.

Ort, B.S., Cohen, C.S., Boyer, K.E., Reynolds, L.K., Tam, S.M., Wyllie-Echeverria, S., (2014). Conservation of eelgrass (Zostera marina) genetic diversity in a mesocosm-based restoration experiment, *Plos One* Public Library of Science 9.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., (2006). A Global Crisis for Seagrass Ecosystems, *BioScience* 56(12), 987.

Orth, R.J., Harwell, M.C., Fishman, J.R., (1999). A rapid and simple method for transplanting eelgrass using single, unanchored shoots, *Aquatic Botany* Elsevier 64(1), 77–85.

Palomar, P., Losada, I.J., (2010). Desalination in Spain: recent developments and recommendations, *Desalination* Elsevier 255(1–3), 97–106.

Park, J.I., Lee, K.S., (2007). Effects of the loess coating on seed germination and seedling growths of the eelgrass, Zostera marina, *Algae* The Korean Society of Phycology 22(2), 141–146.

Pasqualini, V., Pergent-Martini, C., Fernandez, C., Ferrat, L., Tomaszewski, J.E., Pergent, G., (2006). Wetland monitoring: aquatic plant changes in two Corsican coastal lagoons (Western Mediterranean Sea), *Aquatic Conservation: Marine and Freshwater Ecosystems* Wiley-Blackwell 16(1), 43–60.

Pasqualini, V., Derolez, V., Garrido, M., Orsoni, V., Baldi, Y., Etourneau, S., Leoni, V., Rébillout, P., Laugier, T., Souchu, P., (2017). Spatiotemporal dynamics of submerged macrophyte status and watershed exploitation in a Mediterranean coastal lagoon: Understanding critical factors in ecosystem degradation and restoration, *Ecological Engineering* Elsevier 102, 1–14.

Paulo, D., Cunha, A.H., Boavida, J., Serrão, E.A., Gonçalves, E.J., Fonseca, M., (2019). Open coast seagrass restoration. Can we do it? Large scale seagrass transplants, *Frontiers in Marine Science* Frontiers 6, 52.

Pereda-Briones, L., Tomas, F., Terrados, J., (2018). Field transplantation of seagrass

(Posidonia oceanica) seedlings: Effects of invasive algae and nutrients, *Marine pollution bulletin* Elsevier 134, 160–165.

Pérez-Lloréns, J.L., Vergara, J.J., Olivé, I., Mercado, J.M., Conde-Álvarez, R., Pérez-Ruzafa, Á., Figueroa, F.L., (2014). Autochthonous seagrasses, in *The Mediterranean Sea* Springer, 137–158.

Pérez-Ruzafa, A., Marcos, C., Bernal, C.M., Quintino, V., Freitas, R., Rodrigues, A.M., García-Sánchez, M., Pérez-Ruzafa, I.M., (2012). Cymodocea nodosa vs. Caulerpa prolifera: Causes and consequences of a long term history of interaction in macrophyte meadows in the Mar Menor coastal lagoon (Spain, southwestern Mediterranean), *Estuarine, Coastal and Shelf Science* Elsevier 110, 101–115.

Pergent-Martini, C., Buia, M., Sghaier, Y., Fernández Torquemada, Y., Pergent, G. & Gerakaris, V. (2015): *Cymodocea nodosa*. *The IUCN Red List of Threatened Species*. Available at: https://www.iucnredlist.org/species/164508/5897205 [Accessed on: 10-04-2020].

Pergent-Martini, C., Buia, M. & Fernández Torquemada, Y. (2015): *Zostera noltii*. *The IUCN Red List of Threatened Species*. Available at: https://www.iucnredlist.org/species/164508/5897205 [Accessed on: 10-04-2020].

Pergent, G., Gerakaris, V., Sghaier, Y.R., Zakhama-Sraier, R., Fernández Torquemada, Y., Pergent-Martini, C. (2016): *Posidonia oceanica* (errata version published in 2018). *The IUCN Red List of Threatened Species*. Available at: https://www.iucnredlist.org/species/164508/5897205 [Accessed on: 10-04-2020].

Pergent-Martini, C., Pasqualini, V., Ferrat, L., Pergent, G., Fernandez, C., (2005). Seasonal dynamics of Zostera noltii Hornem. in two Mediterranean lagoons, *Hydrobiologia* Springer 543(1), 233–243.

Pergent, G., Djellouli, A., Hamza, A.A., Ettayeb, K.S., El Mansouri, A.A., Talha, F.M., Hamza, M.A., Pergent-Martini, C., Platini, F., (2002). Characterization of the benthic vegetation in the Farwà Lagoon (Libya), *Journal of Coastal Conservation* Springer 8(2), 119.

Pergent, G., Pasqualini, V., Pergent-Martini, C., Ferrat, L., Fernandez, C., (2006). Variability of Ruppia cirrhosa in two coastal lagoons with differing anthropogenic stresses, *Botanica marina* De Gruyter 49(2), 103–110.

Pergent, G., Bazairi, H., Bianchi, C.N., Boudouresque, C.F., Buia, M.C., Calvo, S., Clabaut, P., Harmelin-Vivien, M., Mateo, M.A., Montefalcone, M., (2014). Climate change and Mediterranean seagrass meadows: a synopsis for environmental managers, *Mediterranean Marine Science* 15(2), 462–473.

Phillips, R.C., (1974). Transplantation of seagrasses, with special emphasis on eelgrass, Zostera marina L., *Aquaculture* Elsevier 4, 161–176.

Phillips, R.C., Meñez, E.G., (1988). *Seagrasses,* Washington, D.C.: Smithsonian Insititution Press.

Piazzi, L., Balestri, E., Magri, M., Cinelli, F., (1998). Experimental transplanting of Posidonia oceanica (L.) Delile into a disturbed habitat in the Mediterranean Sea, *Botanica marina* Walter de Gruyter, Berlin/New York 41(1–6), 593–602.

Pirrotta, M., Tomasello, A., Scannavino, A., Di Maida, G., Luzzu, F., Bellissimo, G., Bellavia, C., Costantini, C., Orestano, C., Sclafani, G., (2015). Transplantation assessment of degraded Posidonia oceanica habitats: site selection and long-term monitoring, *Mediterranean Marine Science* 16(3), 591–604.

Prado, P., Caiola, N., Ibáñez, C., (2013). Spatio-temporal patterns of submerged macrophytes in three hydrologically altered Mediterranean coastal lagoons, *Estuaries and coasts* Springer 36(2), 414–429.

Rajpurohit, D., Jhang, T., (2015). In situ and ex situ conservation of plant genetic resources and traditional knowledge, in *Plant Genetic Resources and Traditional Knowledge for Food Security* Springer , 137–162.

Ramos-Esplá, A., Cebrián, D., Demetropoulos, A., (2007). Integrated coastal area management in Cyprus: biodiversity concerns on the coastal area management programme of Cyprus, *RAC/SPA, Tunis*.

Reynolds, L.K., McGlathery, K.J., Waycott, M., (2012). Genetic diversity enhances restoration success by augmenting ecosystem services, *PloS one* Public Library of Science 7(6), e38397.

Rova, S., Pastres, R., Zucchetta, M., Pranovi, F., (2018). Ecosystem services' mapping in data-poor coastal areas: Which are the monitoring priorities?, *Ocean & Coastal Management* Elsevier 153, 168–175.

Rueda, J.L., Gofas, S., Urra, J., Salas, C., (2009). A highly diverse molluscan assemblage associated with eelgrass beds (Zostera marina L.) in the Alboran Sea: Micro-habitat preference, feeding guilds and biogeographical distribution, *Scientia Marina* 73(4), 679–700.

Rueda, J.L., Salas, C., Marina, P., (2008). Seasonal variation in a deep subtidal Zostera marina L. bed in southern Spain (western Mediterranean Sea), *Botanica Marina* De Gruyter 51(2), 92–102.

Ruíz, J.M., Boudouresque, C.F., Enríquez, S., (2009). Mediterranean seagrasses. *Botanica Marina* Walter de Gruyter 52(5), 369–381.

Ruiz-Fernández, J. M., Pérez, M., Romero, J., Tomas, F., (2009). The importance of herbivory in the decline of a seagrass (Posidonia oceanica) meadow near a fish farm: an experimental approach.

Ruiz, J. M., Guillén, E., Ramos Segura, A., Otero, M., (2015): *Atlas de las praderas marinas de España,* España. Instituto Español de Oceanografía.

Sánchez-Carnero, N., Rodríguez-Pérez, D., Couñago, E., Aceña, S., Freire, J., (2012). Using vertical sidescan sonar as a tool for seagrass cartography, *Estuarine, Coastal and Shelf Science* Elsevier 115, 334–344.

Sánchez-Lizaso, J. L., Romero, J., Ruiz, J., Gacia, E., Buceta, J.L., Invers, O., Torquemada, Y.F., Mas, J., Ruiz-Mateo, A., Manzanera, M., (2008). Salinity tolerance of the Mediterranean seagrass Posidonia oceanica: recommendations to minimize the impact of brine discharges from desalination plants, *Desalination* Elsevier 221(1–3), 602–607.

Sanchez-Lizaso, J.L., Fernandez-Torquemada, Y., Gonzalez-Correa, J.M., (2009). Evaluation of the viability of Posidonia oceanica transplants associated with a marina expansion, *Botanica Marina* De Gruyter 52(5), 471–476.

Seeliger, U., Cordazzo, C., Koch, E.W., (1984). Germination and algal-free laboratory culture of widgeon grass, Ruppia maritima, *Estuaries* Springer 7(2), 176–178.

Sfriso, A., Buosi, A., Tomio, Y., Juhmani, A.S., Facca, C., Sfriso, A.A., Franzoi, P., Scapin, L., Bonometto, A., Ponis, E., (2019). Aquatic angiosperm transplantation: a tool for environmental management and restoring in transitional water systems, *Water* Multidisciplinary Digital Publishing Institute 11(10), 2135.

Shili, A., Maïz, N.B., Boudouresque, C.F., Trabelsi, E.B., (2007). Abrupt changes in Potamogeton and Ruppia beds in a Mediterranean lagoon, *Aquatic Botany* Elsevier 87(3), 181–188.

Short, F., Carruthers, T., Dennison, W., Waycott, M., (2007). Global seagrass distribution and diversity: A bioregional model, *Journal of Experimental Marine Biology and Ecology* 350(1–2), 3–20.

Short, F.T., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J., Sidik Calumpong, H.P., Carruthers, T.J.B., Coles, R.G., Dennison, W.C., (2011). Extinction risk assessment of the world's seagrass species, *Biological Conservation* Elsevier 144(7), 1961–1971.

Short, F.T., Short, C.A., Novak, A.B., (2016). Seagrasses, *The Wetland Book II: Distribution, Description, and Conservation* 1(34), 73–91.

Short, F.T., Carruthers, T.J.R., Waycott, M., Kendrick, G.A., Fourqurean, J.W., Callabine, A., Kenworthy, W.J. & Dennison, W.C. (2010): *Ruppia cirrhosa*. *The IUCN Red List of Threatened Species.* Available at: https://www.iucnredlist.org/species/164508/5897205 [Accessed on: 10-04-2020].

Short, F.T., Carruthers, T.J.R., Waycott, M., Kendrick, G.A., Fourqurean, J.W., Callabine, A., Kenworthy, W.J. & Dennison, W.C. (2010): *Ruppia cirrhosa*. *The IUCN Red List of Threatened Species.* Available at: https://www.iucnredlist.org/species/164508/5897205 [Accessed on: 10-04-2020].

Signorini, A., Massini, G., Migliore, G., Tosoni, M., Varrone, C., Izzo, G., (2008). Sediment biogeochemical differences in two pristine Mediterranean coastal lagoons (in Italy) characterized by different phanerogam dominance-A comparative approach, *Aquatic Conservation: Marine and Freshwater Ecosystems* 18(1), S27.

Stramska, M., Aniskiewicz, P., (2019). Recent large scale environmental changes in the Mediterranean Sea and their potential impacts on Posidonia oceanica, *Remote Sensing* 11(2).

Suykerbuyk, W., Van Der Heide, T., Faust, C., Govers, L.L., Giesen, W.B.J.T., De Jong, D.J., Van Katwijk, M.M., (2012). Suppressing antagonistic bioengineering feedbacks doubles restoration success, *Ecological Applications* Wiley Online Library 22(4), 1224–1231.

Suykerbuyk, W., Govers, L.L., Bouma, T.J., Giesen, W.B.J.T., de Jong, D.J., van de Voort, R., Giesen, K., Giesen, P.T., van Katwijk, M.M., (2016). Unpredictability in seagrass restoration: analysing the role of positive feedback and environmental stress on Z ostera noltii transplants, *Journal of Applied Ecology* Wiley Online Library 53(3), 774–784.

Tanner, C.E., Parham, T., (2010). Growing Zostera marina (eelgrass) from seeds in land-based culture systems for use in restoration projects, *Restoration Ecology* Wiley Online Library 18(4), 527–537.

Telesca, L., Belluscio, A., Criscoli, A., Ardizzone, G., Apostolaki, E.T., Fraschetti, S., Gristina, M., Knittweis, L., Martin, C.S., Pergent, G., (2015). Seagrass meadows (Posidonia oceanica) distribution and trajectories of change, *Scientific reports* Nature Publishing Group 5, 12505.

Terrados, J., Marin, A., Celdrán, D., (2013). Use of Posidonia oceanica seedlings from beach-cast fruits for seagrass planting, *Botanica Marina* De Gruyter 56(2), 185–195.

Thom, R., Gaeckle, J., Buenau, K., Borde, A., Vavrinec, J., Aston, L., Woodruff, D., Khangaonkar, T., Kaldy, J., (2018). Eelgrass (Zostera marina L.) restoration in Puget Sound: development of a site suitability assessment process, *Restoration ecology* Wiley Online Library 26(6), 1066–1074.

Thursby, G.B., (1984). Nutritional requirements of the submerged angiosperm Ruppia maritima in algae-free culture., *Marine ecology progress series. Oldendorf* 16(12), 45–50.

Traganos, D., Reinartz, P., (2018A). Interannual change detection of Mediterranean seagrasses using RapidEye image time series, *Frontiers in plant science* Frontiers 9, 96.

Traganos, D., Reinartz, P., (2018B). Mapping Mediterranean seagrasses with Sentinel-2 imagery, *Marine pollution bulletin* Elsevier 134, 197–209.

Triest, L., Sierens, T., Menemenlis, D., Van der Stocken, T., (2018). Inferring connectivity range in submerged aquatic populations (Ruppia L.) along european coastal lagoons from genetic imprint and simulated dispersal trajectories, *Frontiers in plant science* Frontiers 9, 806.

Triest, L., Sierens, T., (2009). High diversity of Ruppia meadows in saline ponds and lakes of the western Mediterranean, in *Pond Conservation in Europe* Springer , 253–261.

Tsioli, S., Orfanidis, S., Papathanasiou, V., Katsaros, C., Exadactylos, A., (2019). Effects of salinity and temperature on the performance of Cymodocea nodosa and Ruppia cirrhosa: a medium-term laboratory study, *Botanica Marina* De Gruyter 62(2), 97–108.

Tuya, F., Hernandez-Zerpa, H., Espino, F., Haroun, R., (2013). Drastic decadal decline of the seagrass Cymodocea nodosa at Gran Canaria (eastern Atlantic): interactions with the green algae Caulerpa prolifera, *Aquatic Botany*Elsevier 105, 1–6.

Tuya, F., Vila, F., Bergasa, O., Zarranz, M., Espino, F., Robaina, R.R., (2017). Artificial seagrass leaves shield transplanted seagrass seedlings and increase their survivorship, *Aquatic Botany* Elsevier 136, 31–34.

United Nations, (1992): “United Nations Conference on Environment and Development (UNCED)”, in: Rio de Janeiro.

UNEP-RAC/SPA, (2012): Action Plan For The Conservation Of Marine Vegetation In The Mediterranean Sea, Tunis. UNEP.

UNEP-RAC/SPA, (2018): Protocol Concerning Specially Protected Areas and Biological Diversity in the Mediterranean. UNEP.

UNEP-RAC/SPA (n.d.): SPAMI Collaborative Platform. Available at: http://spami.medchm.net/en [Accessed on: 09-06-2020]

UNEP-RAC/SPA, (1995): The Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean, Barcelona. UNEP.

Unsworth, R.K.F., Collier, C.J., Waycott, M., Mckenzie, L.J., Cullen-Unsworth, L.C., (2015). A framework for the resilience of seagrass ecosystems, *Marine Pollution Bulletin* Elsevier Ltd 100(1), 34–46.

Unsworth, R.K.F., McKenzie, L.J., Collier, C.J., Cullen-Unsworth, L.C., Duarte, C.M., Eklöf, J.S., Jarvis, J.C., Jones, B.L., Nordlund, L.M., (2018). Global challenges for seagrass conservation, *Ambio* 48(8), 801–815.

Unsworth, R.K.F., Bertelli, C., Cullen-Unsworth, L., Esteban, N., Lilley, R., Jones, B.L., Lowe, C., Nuuttila, H., Rees, S., (2019). Sowing the seeds of seagrass recovery using hessian bags, *Frontiers in Ecology and Evolution* Frontiers 7, 311.

Vacchi, M., De Falco, G., Simeone, S., Montefalcone, M., Morri, C., Ferrari, M., Bianchi, C.N., (2017). Biogeomorphology of the Mediterranean Posidonia oceanica seagrass meadows, *Earth Surface Processes and Landforms* Wiley Online Library 42(1), 42–54.

Valle, M., Chust, G., del Campo, A., Wisz, M.S., Olsen, S.M., Garmendia, J.M., Borja, Á., (2014). Projecting future distribution of the seagrass Zostera noltii under global warming and sea level rise, *Biological Conservation* Elsevier 170, 74–85.

Valle, M., Garmendia, J.M., Chust, G., Franco, J., Borja, Á., (2015). Increasing the chance of a successful restoration of Zostera noltii meadows, *Aquatic Botany* Elsevier 127, 12–19.

Vieira, R., Martin, A., Engelen, A.H., Thomsen, M.S., Arenas, F., (2020). Interactive effects of co-occurring anthropogenic stressors on the seagrass, Zostera noltei, *Ecological Indicators* Elsevier 109, 105780.

Van Vierssen, W., Van Kessel, C.M., Van Der Zee, J.R., (1984). On the germination of Ruppia taxa in western Europe, *Aquatic Botany* Elsevier 19(3–4), 381–393.

Wang, M., Wang, Y., Guo, X., Sha, J., Zhang, H., Tang, X., Zhou, B.I., (2016). Reproductive properties of Zostera marina and effects of sediment type and burial depth on seed germination and seedling establishment, *Aquatic Botany* Elsevier 134, 68–74.

Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems, *Proceedings of the National Academy of Sciences of the United States of America* 106(30), 12377–12381.

WFO (2020): World Flora Online. Available at: http://www.worldfloraonline.org. [Accessed on: 03-05-2020]

Yang, C.J., Liu, Y.S., Liu, J., Xu, Q., Li, W.T., Zhang, P.D., (2016). Assessment of the establishment success of Zostera marina (eelgrass) from seeds in natural waters: implications for large-scale restoration, *Ecological Engineering* Elsevier 92, 1–9.

Zarranz, M.E., González-Henríquez, N., García-Jiménez, P., Robaina, R.R., (2010). Restoration of Cymodocea nodosa seagrass meadows through seed propagation: germination in vitro, seedling culture and field transplants, *Botanica Marina* De Gruyter 53(2), 173–181.

Zhang, P.D., Fang, C., Liu, J., Xu, Q., Li, W.T., Liu, Y.S., (2015). An effective seed protection method for planting Zostera marina (eelgrass) seeds: implications for their large-scale restoration, *Marine Pollution Bulletin* Elsevier 95(1), 89–99.

Zhang, Q., Liu, J., Zhang, P.D., Liu, Y.S., Xu, Q., (2015). Effect of silt and clay percentage in sediment on the survival and growth of eelgrass Zostera marina: transplantation experiment in Swan Lake on the eastern coast of Shandong Peninsula, China, *Aquatic Botany* Elsevier 122, 15–19.

Zharova, N., Sfriso, A., Voinov, A., Pavoni, B., (2001). A simulation model for the annual fluctuation of Zostera marina biomass in the Venice lagoon, *Aquatic Botany* 70(2), 135–150.

8. ANNEXES

ANNEX I

Table I.1. Seagrass species, family and bioregion. 1. Temperate North Atlantic, 2. Tropical Atlantic, 3. Mediterranean, 4. Temperate North Pacific, 5. Tropical Indo-Pacific, 6. Temperate Southern Oceans. Source: Short *et al.* (2016).

Species	Family	Bioregion
Amphibolis antarctica	Cymodoceaceae	6
Amphibolis griffithii	"	6
Cymodocea angustata	"	5
Cymodocea nodosa	"	1, 3
Cymodocea rotundata	"	5
Cymodocea serrulata	"	5
*Halodule beaudettei**	"	2
*Halodule bermudensis**	"	2
Halodule ciliate	"	2
Halodule emarginata	"	2
Halodule pinifolia	"	5
Halodule uninervis	"	5
Halodule wrightii	"	1, 2, 3, 4, 5
Syringodium filiforme	"	2, 3
Syringodium isoetifolium	"	5, 6
Thalassodendron ciliatum	"	5, 6
Thalassodendron pachyrhizum	"	6
Enhalus acoroides	Hydrocharitaceae	5
Halophila australis	"	6
Halophila baillonii	"	2
Halophila beccarii	"	5
Halophila capricorni	"	5
Halophila decipiens	"	2, 3, 4, 5, 6
Halophila engelmannii	"	2
Halophila euphlebia	"	4
Halophila hawaiiana	"	5
*Halophila johnsonii**	"	2
*Halophila minor**	"	5
Halophila nipponica	"	4
Halophila ovalis	"	4, 5, 6
*Halophila ovata**	"	5

Halophila spinulosa	“	5
Halophila stipulacea	“	2, 3, 5
Halophila sulawesii	“	5
Halophila tricostata	“	5
Thalassia hemprichii	“	5
Thalassia testudinum	“	2
Posidonia angustifolia	Posidoniaceae	6
Posidonia australis	“	6
Posidonia coriacea	“	6
Posidonia denhartogii	“	6
Posidonia kirkmanii	“	6
Posidonia oceanica	“	3
Posidonia ostenfeldii	“	6
Posidonia sinuosa	“	6
*Ruppia cirrhosa**	Ruppiaceae	3
Ruppia filifolia	“	6
*Ruppia maritima**	“	1, 2, 3, 4, 5, 6
Ruppia megacarpa	“	6
Ruppia polycarpa	“	6
Ruppia tuberosa	“	6
Lepilaena australis	Zannichelliaceae	6
Lepilaena marina	“	6
Phyllospadix iwatensis	Zosteraceae	4
Phyllospadix japonicus	“	4
Phyllospadix scouleri	“	4
Phyllospadix serrulatus	“	4
Phyllospadix torreyi	“	4
Zostera asiatica	“	4
Zostera caespitosa	“	4
Zostera capensis	“	5, 6
Zostera caulescens	“	4
*Zostera chilensis**	“	6
*Zostera geojeensis**	“	4
Zostera japonica	“	4, 5
Zostera marina	“	1, 3, 4
Zostera muelleri	“	5, 6
*Zostera nigricaulis**	“	6
Zostera noltii	“	1, 3
Zostera pacifica	“	4
*Zostera polychlamys**	“	6
*Zostera tasmanica**	“	6

* Species status under review

ANNEX II

Table II.1. Complete SPAMIs list. ("-" indicates the absence of all of the 6 species discussed in this study).

Country	SPAMI	Species
Albania	1.Karaburun Sazan National Marine Park	1. *P. oceanica*
Algeria	1. Banc Des Kabyles Marine Reserve 2. Habibas islands	1. – 2. –
Cyprus	1. Lara-Toxeftra Turtle Reserve	1. *P. oceanica* and *C. nodosa*
France	1. The Blue Coast Marine Park 2. Natural Reserve of Bouches de Bonifacio 3. Cerbère-Banyuls Marine Nature Reserve 4. The embiez Archipelago (Six Fours) 5. Calanques National Park 6. Port Cros National Park	1. *C. nodosa* and *P. oceanica* 2. *C. nodosa* and *P. oceanica* 3. *P. oceanica* 4. *P. oceanica, C. nodosa* and *Z. noltii* 5. *C. nodosa* and *P. oceanica* 6. *C. nodosa* and *P. oceanica*
Lebanon	1. Palm Islands Natural Reserve 2. Tyre Coast Nature reserve	1. *C. nodosa*, *Z. noltii* and *Z. marina* 2. *Z. marina* and *Z. noltii*
Monaco, Italy, France	1. Pelagos Sanctuary	1. *P. oceanica* and *Z. noltii*
Morocco	1. Al Hoceima National Park	1. –
Slovenia	1. Strunjan Landscape Park	1. *R. maritima*, *R. cirrhosa*, *Z. marina*, *Z. noltii* and *C. nodosa*
Italy	1. Capo Caccia- Isola Piana Marine Protected Area 2. Plemmirio Marine Protected Area 3. Capo carbonara Marine Protected Area 4. Portofino Marine Protected Area	1. *P. oceanica* 2. *C. nodosa* and *P. oceanica* 3. *C. nodosa* and *P. oceanica* 4. *P. oceanica* and *C. nodosa*

	5. Egadi Islands Marine Protected Area	5. *P. oceanica* and *C. nodosa*
	6. Miramare Marine Protected Area	6. *P. oceanica*, *Z. noltii* and *C. nodosa*
	7. Penisola del Sinis – Isola di Mal di Ventre Marine Protected Area	7. *P. oceanica*, *C. nodosa*, *Z. noltii*
	8. Porto Cesareo Marine Protected Area	8. *P. oceanica* and *C. nodosa*
	9. Punta Campanella Marine Protected Area	9. *P. oceanica*
	10. Torre Guaceto Marine Protected Area and Natural Reserve	10. *P. oceanica*, *C. nodosa* and *Z. noltii*
	11. Tavolara Punta Coda Cavallo Marine Protected Area	11. *P. oceanica*, *Z. noltii* and *Ruppia spp*.
Spain	1. Alboran Island	1. –
	2. Cabo de gata- Nijar Natural Park	2. *P. oceanica* and *C. nodosa*
	3. Cap de Creus Natural Park	3. *P. oceanica*, *C. nodosa*, *Z. marina*, *Z. noltii*
	4. Columbretes Islands	4. –
	5. Sea Bottom of the Levante of Almeria	5. *P. oceanica*
	6. Mar Menor and Oriental zone of the Region of Murcia coast	6. *P. oceanica*, *Z. noltii* and *C. nodosa*
	7. Medes Islands	7. *P. oceanica*
	8. Archipelago of Cabrera National Park	8. *P. oceanica* and *C. nodosa*
	9. Maro -Cerro Gordo Cliffs	9. *P. oceanica*, *C. nodosa* and *Z. marina*
	10. Cetaceans Migration Corridor in the Mediterranean	10. –
Tunisia	1. Zembra and Zembretta National Park	1. *P. oceanica*
	2. La Galite Archipelago	2. *P. oceanica* and *C. nodosa*
	3. Kneirr Islands	3. *P. oceanica* and *C. nodosa*