



## *Posidonia oceanica* restoration review: Factors affecting seedlings

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### ABSTRACT

Seagrasses like *Posidonia oceanica* provide a wide range of benefits for the ocean and for the planet. Efforts in conservation and restoration have increased in the last 20 years to preserve *P. oceanica* meadows; a plenty of works have been conducted to develop restoration and planting actions as a way to offset the regression of this seagrass in the Mediterranean Sea. We here compile the information on these studies to make a bibliographic review. The review results showed that seedlings grow better on dead mat and rock covered with algae in calm waters with moderate depths. We highlighted the gaps of knowledge on restoration actions of *Posidonia oceanica*. Furthermore, with intensive and integrative work these gaps could be filled in the upcoming years.

### 1. Introduction

Seagrasses are plants that colonized marine ecosystems, about 100 million years ago, evolving from terrestrial monocotyledons (Zenone et al., 2020). They are one of the most productive and valuable ecosystems on Earth (Murphy et al., 2021; Valdez et al., 2020; van Katwijk et al., 2016). Numerous studies have recognized its ecological and economic value, which would disappear along with them, as they provide important ecosystem services such as, nutrient cycling, sequestration of carbon, a habitat for thousands of fish and invertebrate species and a source of food for many of them, among others (Bidak et al., 2021; Terrados et al., 2013). Seagrasses are globally threatened by direct (dredging, fishing or anchoring) and indirect human impacts, e.g. global change. (Bidak et al., 2021; Duarte, 2002; Waycott et al., 2009). A global estimation found that 29% of the known surface area has disappeared since 1879. This results in rates of decline accelerating from an average of 0.9% year<sup>-1</sup> before 1940 to 7% year<sup>-1</sup> by the end of the 20<sup>th</sup> century (Waycott et al., 2009). In Europe, seagrasses showed a slowdown in the declining rates due to an improvement on the coastal management to reduce nutrient supply from urban waters and agricultural pollution (Grizzetti et al., 2021). These enhancements led the decadal rate of area loss to a deceleration: in the 1980s (27% decade<sup>-1</sup>), in the 1990s (16% decade<sup>-1</sup>) and in the 2000s (8.3% decade<sup>-1</sup>) (de los Santos et al., 2019). In the Balearic Islands, 70% of the seagrasses show stable or improving trends, but still several meadows show regression. This loss could be due to the fact that the sexual reproduction of phanerogams is very limited

(Balestri and Cinelli, 2003) and, therefore, recovery is very slow.

Seagrasses can reproduce both sexually and asexually (clonation), but prefer the latter (Duarte, 2002; Terrados et al., 2013). Vegetative propagation, which does not involve critical phases like germination and establishment, offers ecological advantages because it allows the colonization through ramets, which have the same genetic information as the mother plant (Ferrer et al., 2011). This lack of recombination could explain their regression and diminish their efficiency in migrating and evolving (Dodd and Douhovnikoff, 2016; Vangelisti et al., 2020). Sexual reproduction is more difficult (Meinesz et al., 1993) and depends on many factors (flower and fruit production and frequency, current directions, fruit buoyancy or prosperous anchorage of the seedling) (Balestri and Cinelli, 2003; Vermaat, 2009). The dispersal from a distant source leads to an establishment of the propagule and a prosperous recolonization. This recolonization leans on current directions and on the floating of seeds and fruits, which normally is unlikely to occur. In addition, seagrass with annual shoots may change its extension and density every year because the seed production and the recruitment varies every year (Vermaat, 2009). Although seagrasses are widespread all over the world, the requirements of each species influence reproductive success. Seagrasses can be found on muddy, sandy or rocky sediments and in shallow or platform waters, forming huge and dense meadows from 0 to 35 m depth (Duarte, 2002). Water movement alters the seedling establishment because of the sediment movement; the roots fix them to the soil to counteract the pressure waves make. However, water movement often leads to wear, tear and loss of plant tissue, mainly

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leaves, because the drag forces augment with leaf area (Infantes et al., 2011). They need nutrients, carbon and light to survive, so it's rare to see them in deep water (Vermaat, 2009).

*Posidonia oceanica* is an endemic seagrass in the Mediterranean Sea. Its meadows can be up to 100,000 years old, so it is considered a long-lived and slow-growing plant. It uses dispersal propagules for sexual reproduction by forming floating fruits with non-dormant seeds in its interior (Guerrero-Meseguer et al., 2018). Strategies based on seeds are an advantage for the donor because they depend on biological material, that in most cases don't survive, and provide genetic diversity for the new plant (Alagna et al., 2020). *Posidonia* seeds are moved by water currents after they leave the donor meadow, and they can be found as drift material on sandy or rocky beaches. The seed is embedded in a pulposus ovoid pericarp which will open and discharge the seed that will sink to the seabed (Terrados et al., 2013). The seeds have photosynthetic activity, thanks to chloroplasts found in the external layers of the fruit, to produce oxygen when the fruit is floating on the surface and will continue during seed germination (Guerrero-Meseguer et al., 2018). *Posidonia* seeds contain enough nutrients and carbon to survive from 6 to 8 months. (Terrados et al., 2013). When the seed reaches the seabed, it needs to establish, this phase between germination and establishment is the most difficult one (Pereda-Briones et al., 2020). In order to establish, seedlings must form a root system and enter deep into the substrate (Balestri et al., 2015). Adhesive root hairs must be in contact with the substrate to fix the seedling and grow, also increasing photosynthetic rates will help seedlings establish. Root hair morphology will rely on the seabed topography (Alagna et al., 2020; Balestri et al., 2015; Guerrero-Meseguer et al., 2018).

Broadly speaking, the main aim of this study is to improve the knowledge about *Posidonia oceanica* restoration methodology to enhance its protection and conservation. The main specific goal of this piece is to make a revision of papers about *Posidonia oceanica* culture by seedlings to increase our understanding about it. In fact, this revision is done to find the best way to grow *Posidonia* seedlings. This information will help restoration projects to move to larger scales, reducing information search time and getting better results.

## 2. Material and methods

The present study is based on a bibliographic research conducted between April and June 2022. The data was obtained from ResearchGate, Scopus and Google Scholar, a web search engine that works with citation indexing through papers, books and studies. The keywords applied to identify the most suitable works were: "*Posidonia oceanica*", "seedlings", "seed" and "restoration". All papers were included in this search, regardless of the date of publication. All the studies were analyzed, but only those focusing on restoration with seeds and not with *Posidonia* cuttings were retained. In particular, the greatest effort was focused on those that studied the factors involved in seedling culture. Later, parameters to study needed to be chosen. First, the author and year of publication were chosen to know better which authors are the most recurrent ones. Then, we start analyzing the experiment: factor/parameter, culture and experiment time, year of study, location, season, replicates and study area. The factor studied in the experiment was the most important issue, because it allows the reader to understand what and why it is done. The result could help others in new restoration projects. Culture time allows us to know the age of the seedlings at the time of the experiment. The experiment time is also important because a 1-year study is not the same as a 4-year study. A longer investigation makes it possible to verify that the seedlings have survived and grown after restoration, because survival and the factors that affect them can change over the years. The year of study indicates how much time has passed between the experiment and the publication of the article. The location gives an idea of the section of the Mediterranean that is being analyzed and, consequently, its contamination, exposure or regression of *Posidonia* in the area. The season will directly affect seawater

temperature and mixture. Replicates and study areas help to perceive the reliability of the study by the number of times an experiment has been repeated.

## 3. Results

The 27 studies included in the present study are shown in Suppl. Mat.. All the studies have been carried out in countries around the Mediterranean Sea (i.e., Italy, France and Spain), as the *Posidonia oceanica* is an endemic species. After the analysis, new factors were revealed to be of utmost importance such as, variables regarding the substratum, i.e., complexity, roughness or slope.

A wide range of substrata were compared: dead matte, *Posidonia oceanica* meadow, rock, sand, gravel, pebble, fiberglass or tiles with different complexity or shape. The most suitable substratum type is dead matte or consolidated ones like rocks (Piazzi et al., 1999; Pereda-Briones et al., 2020). Dead matte complexity allows the seedling to anchor in all dead and uprooted roots. The maximum survival rate was 92% on rock covered with algae and 75% on dead matte (Domínguez et al., 2012; Pereda-Briones et al., 2020). The minimum survival was recorded on sand, where seedlings are much more difficult to establish (Balestri and Lardicci, 2008; Castejón-Silvo and Terrados, 2021). Substratum complexity and roughness favors seedling anchorage, like the dead matte, where roots can penetrate in the interstitial space of the substratum. High complexity substrata had a survival rate as high as 55% (Zenone et al., 2022). Another study evaluated the influence of pre-experimental substratum comparing between hard glass and sand but found out that it does not affect seedlings survival, just seedlings growing (4 times higher growth rate on sand) (Guerrero-Meseguer et al., 2018).

Exposing seedlings to 25° (control), 27° and 29 °C revealed that at the control the survival rate was 94–100% (Hernán et al., 2017). Pazzaglia et al. (2022) proved the effects of thermal-printing in seedlings. Primed seedlings were exposed to 30.5 °C for 11 days and then exposed to 32 °C for 2 weeks. Non-primed seedlings were just exposed to 32 °C. "Priming" made the seedlings ready to withstand the extreme temperatures and, therefore, primed seedlings had more growth rates and pigment content. As the depth increases, *Posidonia oceanica* is becoming less abundant, so depth is an important factor for seedling culture. When cultured at 2 and 10 m depth, seedlings at 2 m died; only the ones cultured in matte at 10 m survived. The higher survival rate was on dead matte at 10 m (69%), this indicates that *Posidonia oceanica* needs to grow on moderate depths (Piazzi et al., 1999).

Studying the planting level (above or below ground) demonstrated that it does not influence seed and leaves growing (Terrados et al., 2013). Neither does natural anchoring nor with "mesh pots" on survival (Terrados et al., 2013). Seawater hydrodynamics hinders establishment; how much the seedling is affected by the drag force depends on the size of the leaves and the strength of the roots (Infantes et al., 2011). This is explained in a paper where seedlings with less exposure had a survival rate of about 80% (Pereda-Briones et al., 2020). Association with macroalgae like *Caulerpa cylindracea* or with the seagrass *Cymodocea nodosa* enhances seedling anchorage by augmenting the substratum roughness and complexity (Pereda-Briones et al., 2020). Rocks covered by crustose algae or brown seaweed, such as *Cystoseira* spp., reached a survival rate of 96 and 81%, respectively (Pereda-Briones et al., 2020).

Experiments in dark and light conditions were performed in order to understand how much photosynthesis affects the seedlings' growth. In illuminated conditions, leaves, roots and seeds grow more and better, with more nutrients mobilized. However, a study stated that seedlings that were exposed to direct sunlight >1 day showed irreparable morphological and physiological damage (Bedini, 1997). Herbivorism (i.e., fish *Salpa salpa* or sea urchin, *Paracentrotus lividus*) is a problem that may be important, but a study found out that protecting the seeds with cages or nets (67–85% and 70–90% survival) or leaving them unprotected (60–70% survival) do not influence seedling survival and

development (Balestri et al., 1998). Interestingly, when seedlings were in high pCO<sub>2</sub> conditions, more herbivore feeding by sea urchins was detected. In addition, these extreme conditions did not affect seedlings survival, but it produced larger seedlings with more photosynthetic activity (Hernán et al., 2016). For the purpose of knowing if nutrients are important, a study fertilized the sediments where seedlings were growing. Seedlings in fertilized sediments presented more leaf development, but it did not change survival that range between 55 and 65% (Pereda-Briones et al., 2018).

Salinity is also a factor of utmost importance in *Posidonia oceanica* culture. An experiment proved the effects of salinity in seeds germination and growth. In the germination period, salinity did not significantly affect survival (90% in the control salinity (37 PSU) and 63–80% in greater salinities (40–49 PSU)) (Fernández-Torquemada and Sánchez-Lizaso, 2013). Yet, in 50 days of growth, leaves in the control situation showed significantly more leaves and leaf and root proliferation.

#### 4. Discussion

This study comprised a bibliographic review of the papers concerning *Posidonia oceanica* restoration by seedlings. Sexual reproduction is difficult, and it is not likely to succeed (Meinesz et al., 1993), because it has a lot of critical phases (i.e., germination and establishment) so, the factor affecting seedlings in these phases need to be studied (Alagna et al., 2020; Ferrer et al., 2011). The most suitable substratum are consolidated substrata (dead matte or rocks), where survival is higher than 75%. Association with algae or seagrasses increases substratum roughness and complexity and, therefore, helps establishment. Moderate depths (10 m) proved to be the most appropriate for seedling planting. Temperatures over 25 °C showed an increase of seedling mortality and herbivorism whilst at 25 °C survival rates were at least 95%. Hydrodynamics also affects seedling establishment and survival, low exposed areas had an 80% of survival rate. Higher salinities (40–49 PSU) decreased leaves and roots proliferation. Our analysis gave an overall view to help others understand all the parameters that could affect seedlings in any way.

Improving coastal ecosystems protection and preservation can palliate the losses on a small scale, but *Posidonia* is disappearing faster than it is growing (van Katwijk et al., 2016). Therefore, *P. oceanica* restoration is an important instrument to balance losses and recovery rates. Our review could help understand how to manage this restoration by seedlings on a larger scale, understanding what can affect their growing and survival. The most studied parameter in the analyzed papers was the substratum, which is one of the most important parameters during seedling anchorage; 15 of the 27 studies (55.6%) analyzed proved the effects of the substratum type. Pereda-Briones et al. (2020) compared seedlings growth in consolidated substratum (rock and dead matte) and in unconsolidated ones (sand and gravel) and found that consolidated ones are the most suitable. They also found that macroalgae cover (crustose algae) improves the conditions for seedling success, because with its complexity and roughness facilitates seedling settlement. Alagna et al. (2013) also observed this trend, where higher seedling survival was observed in rocks covered with *Cystoseira* spp. Other studies compared rock with sand or gravel, sand with gravel, fiberglass with sand or pebble or matte with vegetated pebbles and reinforce the idea that more consolidated substrata help seedlings anchorage and establishment (Balestri et al., 1998, 2015; Domínguez et al., 2012; Guerrero-Meseguer et al., 2018; Piazzi et al., 1999). Balestri and Lardicci (2008) do not approve this idea and establish that seedlings can grow in consolidated and unconsolidated substratum, because they found that there were no significant differences in seedlings establishment and abundance between substrata (rock vs sand). Donor populations play an important role in restoration, healthy meadows are needed for survival of seedlings and shoots, and for this reason, protection of *Posidonia* is also necessary. Donor meadows can improve seedlings survival if they accomplish some criteria. The transplant site

must be located close to the donor plant, since its presence indicates that it is a suitable site for its development. In addition, transplanted plants must have proper gene attributes to survive on a long-term scale that can be obtained by gene flow from nearby plants (van Katwijk et al., 2016).

Marine restoration is a new challenge for scientists, but it has proved its potential to restore destroyed ecosystems (Duarte et al., 2020; McAfee et al., 2021). The small-scale success in restoration projects is what contributes to building a solid foundation of models, political disposition and agreement to advance to a larger scale, like global scale (McAfee et al., 2021). Ecological restoration as a way for conservation is on the rise, so biotic and abiotic factors and culture methods need to be studied and understood to fill the gaps in restoration knowledge (Valdez et al., 2020). Our review found that, even if *Posidonia oceanica* restoration by seedlings started about 20 years ago, there are gaps in the literature that need to be reinforced since there are parameters that have been overlooked or poorly studied by former researchers. As mentioned above, the most studied parameter was the substratum, followed by seawater temperature, but the remaining parameters were poorly studied. Two studies compared seedlings growing at three temperatures (25, 27 and 29 °C) and found out that seedlings showed the highest growth at 25 °C. Higher temperatures decreased success in seedlings establishment and their carbohydrate stock. In warmer conditions, more herbivorism was seen, grazers selected seedlings with lower content of fiber in its leaves (Hernán et al., 2017; Pereda-Briones et al., 2019). Another study proved that thermo-printing helps seedlings to be ready to extreme conditions, i.e., if the seedlings had an adaptation period with progressive temperature increases, they would be able to adapt to high temperatures (Pazzaglia et al., 2022). Increased concern about global warming may explain why it is the second most studied parameter, but the results are not encouraging. Association with macroalgae and seagrasses was also evaluated in four papers, and was proven to increase the probability of survival. Algae coverage (crustose algae, *Cystoseira* spp. and *Caulerpa cylindracea*) increased rate of survival (Alagna et al., 2013; Pereda-Briones et al., 2020; Pereda-Briones et al., 2018), as did the presence of seagrasses, like *C. nodosa* (Balestri et al., 2021). Planting level and seed burial, exposure to seawater hydrodynamics and effects of nutrients and hormones have been previously studied. Planting level and seed burial were not significant in seedlings cultures (Domínguez et al., 2012; Guerrero-Meseguer et al., 2017; Terrados et al., 2013). The studies evaluating hydrodynamics showed that low exposure areas are appropriate for seedling culture, because drag forces could uproot them (Infantes et al., 2011; Pereda-Briones et al., 2020; Zenone et al., 2022). Studying the effects of nutrients in the sediments revealed that they improve leaf development (Pereda-Briones et al., 2018). Another study proved that seed germination stimulant (Sprintene) does not make the seed germinate earlier, however, the presence of this hormone caused the roots to emerge earlier and to grow longer (Balestri and Bertini, 2003). The other parameters taken into account in this review (effects of the presence of invasive algae, photosynthesis, light, herbivorism, high pCO<sub>2</sub>, epiphyte cover, salinity, depth and seedling density) have been scarcely studied to date.

Our work allowed us to sum up the information about *P. oceanica* seedlings culture, with many studies carried out in the Mediterranean Sea. However, there are still too many topics to study and understand about this seagrass. Restoration projects with seedlings are being carried out in order to fill these gaps of knowledge and improve restoration success. Restoration projects, like the one conducted in Formentera by “Asociación Vellmarí” with *Posidonia* seedlings (<https://seagrassrestorationnetwork.com/seed-based-restoration-1>), or the one carried out by researchers from the University of Western Australia *Posidonia australis* seedlings (<https://asociacionvellmari.com/siembra-de-posidonia>) a subject on which there are still no published results will provide solid information to reach strong conclusions and fill the gap of knowledge. This analysis is only the beginning of a project that could help us learn some important parameters about *Posidonia* sexual reproduction. To fill the gaps in *Posidonia* restoration knowledge, more years and efforts are



necessary. We need to explore thoroughly about *Posidonia* flowering and its variability, to understand how it works and why seedlings showed interannual variability. Parameters affecting seedling culture, establishment and its further development should be further studied with field and laboratory experiments to fill the gaps or to reinforce the existing information. This study is a first step that could be strengthened with integrative work in the next few years.

## 5. Conclusions

- Our analysis stated that for the best seedling growth, they need to establish in consolidated substratum (dead matte or rock).
- Algae or seagrass coverage enhances seedlings establishment by increasing substratum roughness and complexity.
- Low exposed areas with moderate depths are more suitable for seedlings to grow.
- High temperatures (>25 °C) and salinities (>39 PSU) decrease seedlings' success.
- Future studies will have to increase the number of years of study to have a clear representation of what is happening.
- There are a lot of gaps to fill in this study area, *Posidonia oceanica* seedlings culture must be thoroughly studied in order to fill these gaps.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Data availability

No data was used for the research described in the article.

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