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Bottlenecks to seed-based seagrass restoration reveal opportunities for improvement

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ABSTRACT

Habitat restoration is becoming an increasingly prevalent tool in the armoury of marine conservation, particularly given the expanding interest in creating nature-based solutions to a changing climate. Seagrass restoration is a particular focus of increased numbers of projects and although there is a growing number of examples of successful seagrass restoration attempts, there remain extensive case studies of poor success or restoration failure from around the globe. To enable marine habitat restoration to happen at scale and make a genuine contribution to carbon sequestration rates globally, improved methods that are both practical and simple are required to foster higher rates of success and lower costs. Here, we present seven bottlenecks to achieving reliable seed-based seagrass restoration. In doing so, we also provide opportunities for practical and simple ways in which the knowledge gaps that underpin these bottlenecks can be filled. Seed collection needs to be easier and more efficient; the processing and storage of seeds more controlled; and germination and seedling survival more effective so that fewer seeds are required for more reliable planting. We conclude that further collaborative multidisciplinary science is required in all parts of the world to improve seagrass seed-based restoration through better incorporation of faunal and microbial ecology, more reliable modelling, improved reporting of restoration failures and a wider investigation of seed-based ecology beyond a handful of species.

1. Introduction

Seagrass restoration is increasingly a tool in the armoury of anthropogenic planetary support strategies, driven in part by expanding interest in creating nature-based solutions to a changing climate (Seddon et al., 2021; Rifai et al., 2022). Although successful seagrass restoration is increasing in prevalence, there are also many examples of poor success rates or restoration failure from around the globe (van Katwijk et al., 2016). The term restoration is broad and may include protecting existing populations or interventions to accelerate natural regeneration. Active restoration typically falls into two main; 1. seed-based restoration, and 2. transplantation of wild sourced or nursery-grown plants (van Katwijk et al., 2009; van Katwijk et al., 2021). For a variety of regulatory, logistical, environmental and

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ecological reasons, uptake of these methods varies (van Katwijk et al., 2016), and although there has been far more focus on seagrass transplantations, the use of seeds is expanding (Tan et al., 2020; Govers et al., 2022; Xu et al., 2023). A review of seagrass domestication found 44% of seagrass species have promising reproductive traits for seed-based restoration (van Katwijk et al., 2021). Due to high seed production (densities often above 10 million per hectare) (Marion and Orth, 2010) capacity exists to collect and utilise high numbers of seeds. A successful restoration project from the United States illustrates this potential, where over 70 million *Zostera marina* seeds were collected over a 20-year period (Orth et al., 2020) leading to the eventual recover of over 300 ha of seagrass. Examples of success at this scale however are rare. For successful restoration to become the dominant result requires a better understanding of the multiple factors that contribute to seed-based restoration success and sustainability.

In order to enable restoration to happen at scale, improved methods that are both practical and simple are required to foster higher success rates (in terms of germination rates and long term meadow development) and lower costs (Unsworth et al., 2019a). Where large-scale restoration has been possible, restoration methods have been simplistic (e.g. throwing seeds into the ocean) and low-cost (Orth et al., 2020). However such methods have proven difficult to replicate in varied environmental and biological conditions due to complex ecological feedbacks (Maxwell et al., 2017). Ultimately marine environments present considerable challenges for seedling establishment, such as strong tidal currents, waves, sediment movement, and the presence of predators (de Boer, 2007). Sediment properties influence germination, as well as seedling survival and development (de Boer, 2007). Larger scale restoration needs to rise to the challenges presented by the marine environment.

Here, we present seven bottlenecks (see Fig. 1) to achieving reliable seed-based seagrass restoration. In doing so, we also present opportunities for practical and simple ways in which the knowledge gaps that underpin these bottlenecks can be filled.

2. Bottlenecks for seed-based seagrass restoration

2.1. Site selection

Identifying suitable environments is one of the major challenges for seagrass restoration. Habitat suitability (HS) modelling is widely used to help direct site choice (Bertelli et al., 2022). HS modelling establishes a broad series of correlations between the presence of mature seagrass and a range of environmental variables such as depth, wave power and topography. The variables used are usually based on the availability of high-resolution spatial datasets rather than the exact requirements of seagrass. While this method is broadly successful, a key caveat is that it fails to recognise that the presence of a mature seagrass meadow does not necessarily equate to the physical conditions needed for emerging seedlings to survive. This arises typically because negative feedbacks exist, such as sediment movement being too high or hydrodynamics too strong (de Boer, 2007; Carr et al., 2012). As individual and isolated seedlings develop into mature plants and then create seagrass meadows, their emergent properties mitigate factors such as sediment movement and hydrodynamics making the meadow more resilient (Temmink et al., 2020). Habitat suitability models do not account for these developmental stages in meadow growth. Although some HS models have been created that consider some conditions necessary for seedling establishment, there is a lack of quantitative data available to drive the models (Erftemeijer et al., 2023). Creating HS models that are more reliable necessitates better understanding of the conditions required for seedling development (Bertelli et al., 2022).

Published literature about favourable or even necessary conditions for seedling emergence is limited. Survival is likely influenced

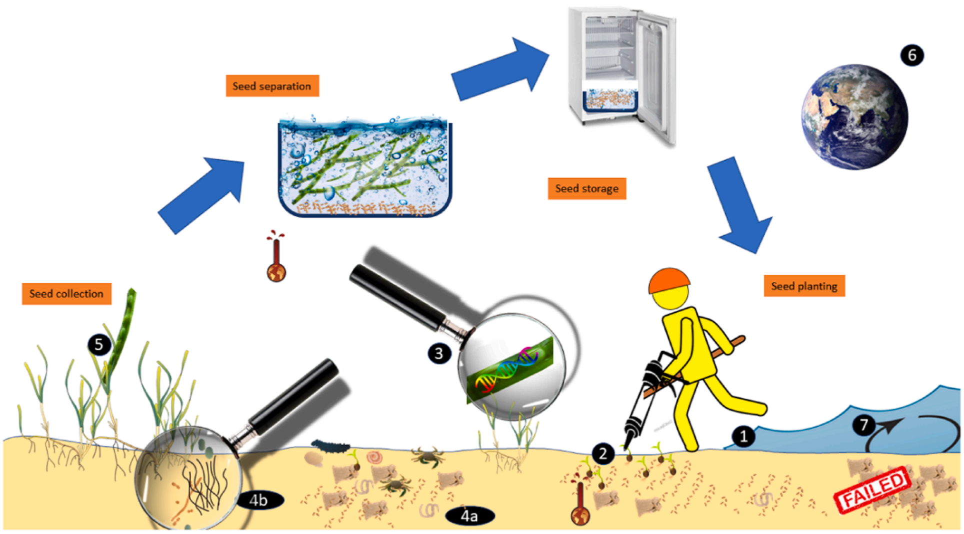

Fig. 1. The process of seagrass seed collection, separation and replanting showing seven proposed bottlenecks for seagrass seed based restoration:1. Finding a suitable environment for restoration; 2. Seed germination and emergence; 3. Understanding of the Conservation genetics of seagrass; 4. Role of biological interactions, a) Predation and competition on seed and seedling survival, b) The significance of the seagrass holobiont; 5. The sustainable supply of seagrass propagules; 6. Knowledge gaps within and across species and environments; 7. Learning from failure.

by multiple environmental parameters such as pore water nutrient concentrations, sulphide concentrations and sediment type, as well as biological parameters such as grazing and bioturbation. We need to better understand the response of seedlings to these parameters. While targeted research is needed to quantify seed and seedling responses in realistic field-based conditions, opportunities may exist to integrate expert opinion to fill these knowledge gaps (e.g. Bayesian Network approach). Regardless of method, quantifiable responses are needed for HS models to reliably predict where seed-based restoration is most likely to succeed. Although research is emerging on how we can begin to mitigate some environmental stresses using physical structures, these factors remain bottlenecks to expanding approaches to restoration at large scale (Temmink et al., 2020).

Various structures have been used successfully to improve the physical and biological conditions for restoration, for example biodegradable plastic mesh structures (BESE) to improve the physical stability of sediments (Temmink et al., 2020). These structures, however, are not feasible for use at large scales and have so far only been successfully applied to seagrass transplanting rather than seed-based restoration. In some locations hessian bags have been anchored to the sediment to prevent seed loss, but to varying degrees of success (Unsworth et al., 2019a). To deal with sulphide feedbacks the potential of mutualistic interactions of seagrass with bivalves have been explored with mixed results (Donaher et al., 2021). Improving our knowledge of these physical and chemical environmental requirements of seedlings and the relative interactions these may have with biological conditions will help improve mitigative methods.

2.2. Seed germination and emergence

Large-scale seed-based restoration is often limited by generally low rates of field-based seedling emergence. While terminology related to germination and emergence are often inconsistent in the literature, here we refer to emergence as being the emergence of a shoot above the sediment and germination as the rupture of the seed coat and emergence of the cotyledon.

Field-based studies have commonly reported low seedling emergence rates of < 5% (Robert et al., 2003), while laboratory and mesocosm studies have demonstrated emergence rates as high as 60% (Balestri and Lardicci, 2012; Jørgensen et al., 2019). This low field-based rate of emergence is largely considered to be the result of poor seed germination, but actual germination rate is rarely determined in field-based experiments, as it requires close observation or recovery of the seeds. In addition, seed loss due to sediment movement, predation, and other factors may result in perceived low germination rates, further complicating restoration efforts.

Earlier studies have demonstrated that an assortment of environmental factors influence seed germination; these include nutrients, light, temperature, salinity, sediment type and anoxia, and sulphide (Probert and Brenchley, 1999; Orth et al., 2000; Cumming et al., 2017; Xu et al., 2021). Additionally, pathogens can reduce germination rates (Govers et al., 2017). For logistical reasons, most studies introduce seeds into bare sediment, but we know that seagrasses alter their physical environment, and so should need to learn from recruitment rates and conditions within an existing seagrass meadow. We also maintain limited understanding of how environmental cues operate within the field, particularly as it is impossible to control environmental factors in field-based studies. Within the context of a rapidly changing climate that often brings unpredictable local weather patterns we need to develop better understanding of these cues to ensure seed planting happens at the most appropriate time, reducing the likelihood of environmental stressors.

Direct seed injection methods, such as Dispenser Injection Seeding (DIS), might improve success as a more precise tool for placing

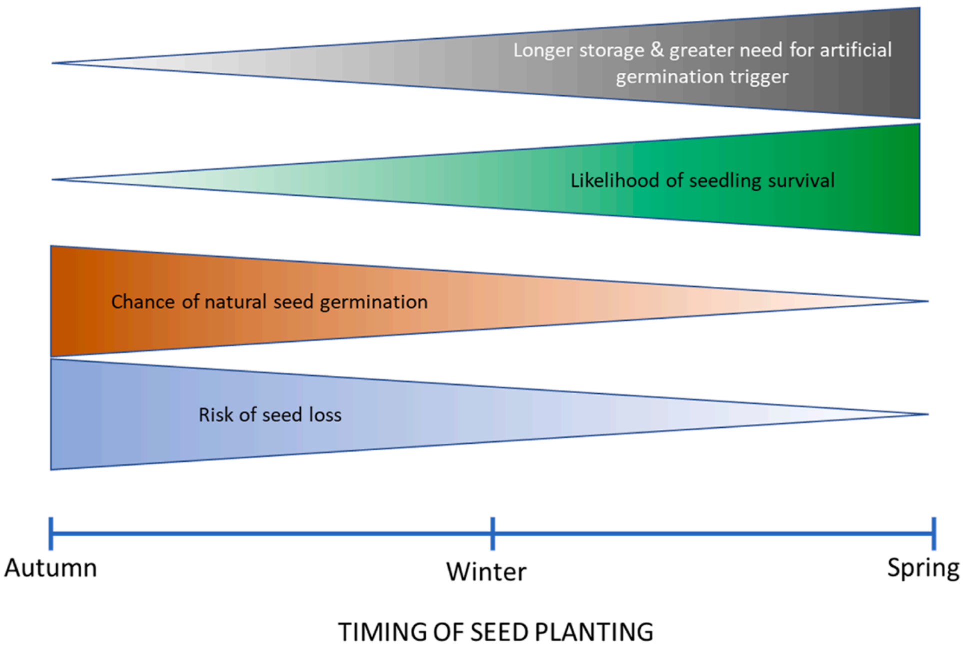

Fig. 2. Hypothesised relationships between seagrass seed planting time, seedling germination and survival.

seeds into the right field-based environment (Traber et al., 2003; Govers et al., 2022). Adaption to local environmental conditions can increase efficiency of direct seed injection methods (Gräfnings et al., 2023) but difficulties remain prominent within subtidal environments and there are knowledge gaps around the appropriate receiving environment as well as the sediment mixture within which seeds are dispensed. In some localities different populations have also shown local variation in their germination time in spite of having similar maturation times suggesting that understanding of local adaptation patterns may also play a role in improving success of planting (Xu et al., 2018; Zhang et al., 2022). The timing and growth of seedlings prior to experiencing stressful periods (e.g. summer environmental conditions) could be critical to their survival (Johnson et al., 2020). These environmental drivers of seedling development are key knoweldge gaps in our understanding of seed based seagrass restoration.Restoration projects often use pre-planting germination triggers, such as freshwater soaking or scarification, to alleviate dormancy and increase germination rates (Stafford--Bell et al., 2016; Xu et al., 2016). The use of these triggers is largely based on research that simulated possible natural events in laboratory settings, however it is not clear what relative impact these treatments have on improving field-based emergence. Given the wide variation in environmental conditions within which seagrasses proliferate, and that the natural presence of these triggers may vary spatiotemporally, it is unsurprising that field-based seed germination rates can often be low and variable. Winter ecology is well documented to be a huge knowledge chasm in plant ecology (Kreyling, 2010) and this is also true of seagrasses. We hypothesise that within temperate climates many of the triggers that improve germination in laboratory settings are more likely to occur in the winter (e.g., freshwater pulse events and scarification from moving sediment) (see Fig. 1), and in tropical climates that similar processes exist within the wet season. In line with this, our experience from temperate climates (e.g., the United Kingdom) suggest that field-based seedling germination rates may be higher if seeds overwinter in their natural environment. This aligns with studies of ruderal (*Halodule univervis*) tropical species that indicate that freshwater inputs (that may act as seed triggers) may have a positive impact on populations (Rasheed and Unsworth, 2011). Fig. 2.

Natural conditions, however, might also provide a combination of different triggers, which are harder to simulate in laboratory settings, and may even have cumulative additive or antagonistic responses, but we have limited understanding of these interactions. For instance, in locations where seagrass is no longer present, we hypothesise that such overwintering poses a trade-off: while winter conditions could assist germination through scarification and freshwater pulses, there is a high probability that seeds are washed away or buried by winter storms and floods. Given these trade-offs, it is evident that we need to improve our understanding of how to transfer the laboratory-based knowledge of seed germination to the field to maximise its effectiveness. This needs to happen beyond local case studies with a wider uptake of network-based research that elicits findings which transcend local environmental parameters.

To overcome the trade-offs associated with overwintering, some restoration activities delay planting until the onset of spring by storing seeds. However, an increase of seed storage time also presents trade-offs in terms of labour, energy, seed quality, and seed loss. Extending seed storage time increases the risk of seed loss due to early germination, parasites, and disease, and generally decreases germination potential (Dooley et al., 2013; Sullivan et al., 2022). Seed treatments, such copper sulphate or bleach, have been used to help mitigate these trade-offs with no noticeable negative effect on germination rates (Govers et al., 2017). However, it is unclear whether these treatments have wider impacts on the biological environment, such as the microbiome (see section on Biological Interactions). Moreover, data on the effect of short (weeks to months) to long-term (several years) storage of seeds are mostly limited to laboratory trials (Conacher et al., 1994; Cumming et al., 2017; Govers et al., 2017) and rarely lead to actual assays of field planting.

There is a growing body of evidence around the need for nutrient additions within seed-based restoration, as pore waters are commonly thought to be nutrient limiting in bare sediment (Lee et al., 2007; MacDonnell et al., 2022; Unsworth et al., 2022). Numerous studies have recorded observations of nutrient limitation within seagrass restoration and recovery (Peralta et al., 2003; Balestri and Lardicci, 2014), indicating that a lack of nutrients in some localities may be a bottleneck to high success rates. Whilst some studies have linked limitation to particular nutrients, we know little of the exact nutrient needs of seeds and seedlings, particularly in the context of their relative concentrations and the availability of micronutrients. Simple and practical methods to add nutrients have been applied to seed-based restoration attempts, such as the addition of Osmocote (Fonseca et al., 1994), the addition of struvite (MacDonnell et al., 2022), and by adding seagrass detritus as a form of organic enrichment (Unsworth et al., 2022). Whilst many of these attempts have been successful, the use of these additives is not ubiquitous due to limited understanding of where their window of opportunity may exist for their effective utilisation.

In other plant sciences fields, managing nutrient balance and the provision of micronutrients is critical for maximizing crop productivity (Pasala et al., 2022). We argue that improved plant science management techniques, particularly for macronutrients, are required to improve germination and growth rates to mitigate against ecological feedbacks (Maxwell et al., 2017). Rapid seagrass growth may help to stabilise sediments, trap nutrients, and kick-start the positive feedbacks (e.g. positive density dependence) (Valdez et al., 2020), that promote seagrasses to spread (van der Heide et al., 2011). For seed-based restoration to be effective, these positive feedbacks must initiate within a short time window.

2.3. Understanding of the conservation genetics of seagrass

Climate adjusted provenancing is widely used, and encouraged, in terrestrial restoration (Prober et al., 2015), but marine restoration lags behind. Seed-based seagrass restoration requires not only a sustainable supply of seeds, but an appropriate source of seeds adapted to and resilient to the local environment. Current reliance on locally sourced seeds in restoration, in combination with rapidly changing climatic conditions, presents risks of maladaptation (Smale et al., 2019). Many seagrasses, including *Zostera spp.,* are unlikely to be able to follow their climatic niche via migration due to dispersal limitations (Sherman et al., 2018). Given the rapidly changing climate and nature of our coastal seas we need to consider not just current conditions but those predicted for the future, and how appropriate adaptive strategies might be integrated into seagrass restoration (Unsworth et al., 2019b; Wood et al., 2019).

Within and between seagrass species, seeds are highly variable in anatomy, germination strategy and nutrient storage (Orth et al., 2000), ultimately leading to differences in their emergence potential and their capacity to be resilient to particular environments (Smith et al., 2022). Recent re-examination of the disappearance of seagrass from a lagoon in the Netherlands highlighted how environmental changes at the site that led to the seagrass loss necessitate that restoration may require donor seeds of an ecotype adapted to a high salinity environment rather than the low salinity lagoon that the seagrass once thrived within (van Katwijk et al., 2023).

The challenge in seed-based restoration is to be able to readily integrate understanding of conservation genetics, local adaptation and observable seed traits (e.g., condition and size) in the context of variability within and between populations to maximise restoration effectiveness (McKay et al., 2005). This is critical in order to plant seeds appropriate for current environments as well as future environments and do so within a commonly complex regulatory environment that attempts to manage seed provenance and restrict where and to what extent donor seeds can be collected (Kramer and Havens, 2009). Better understanding of populations genetics is required that can be placed not only in the context of genetic variability but in the context of adaptation to facilitate more resilient restored populations of seagrass.

2.4. Role of biological interactions

2.4.1. Predation and competition on seed and seedling survival

There is increasing evidence that other biota (animals and algae) are components of both top-down and bottom-up processes within seagrass meadows (Moksnes et al., 2008). For example, by consuming sprouted seagrass seeds, ragworms may impede seed-based restoration efforts (Kwakernaak et al., 2023). Seed predation by multiple species of crustaceans, reducing seedling growth and survival, also reduces potential seagrass restoration success (Fishman and Orth, 1996; Darnell and Dunton, 2015; Infantes et al., 2016). In addition, we know that the excess growth of algae can lead to the suffocation of seagrass seedlings and reduce germination due to shading (Thomsen et al., 2012). In Denmark for example, (Valdemarsen et al., 2010) found that seedling mortality was significantly correlated with the presence of drift macroalgae, highlighting the problems that this sort of interaction can create.

With increasing levels of nutrients in our coastal seas, the spread of invasive species and the reduction in top predators available to control lower order crustacean populations, these biological interactions are potential major bottlenecks for seed-based restoration activities (Duffy et al., 2015). Understanding and learning how to manage these interactions that are thought to often create negative feedbacks, preventing seagrass emergence and growth, remains one of the major challenges to seagrass restoration in general – not just for seed-based methods.

Whilst controlling eutrophication (although this should be key), associated algal overgrowth and nuisance macroalgae is unrealistic within a seagrass restoration project, anecdotal information exists of projects seeking to minimize algal build up through site maintenance. Whether this has been successful is less clear. Although such clearance activities are unlikely to solve the overall nutrient issue, it may enable seedlings to survive long enough and grow to a point that they are able to overcome such negative feedbacks. Removal of macroalgae is also a direct way of removing nutrients from the system, whilst also providing the additional benefit of reducing stress from shading (Duarte and Krause-Jensen, 2018). Although this approach is potentially useful in the short term (e.g., commencing restoration), it may be a temporary solution that is ineffective and unsustainable for enhancing restoration in the long term.

Whilst small scale attempts have been made to control seed predators such as crabs using cages (Infantes et al., 2016), this is not a suitable option for larger scale restoration. A whole series of restoration methods have been proposed that seek to reduce the impact of predators, although the specific evidence around these remains limited. For example, researchers in Korea have utilized seeds coated in loess to reduce predation (Park and Lee, 2007), and in the US, Knox Gelatine (as a matrix for seed injection) has been used knowing a side benefit was reduced crab predation (Traber et al., 2003; Marion and Orth, 2010). Although in both cases these coatings had an impact on seed success, it is unclear whether this related to improved germination, reduced crab predation or other factors, highlighting the need to empirically test such factors. For example, within terrestrial environments, the addition of gelatine has improved seedling survival and growth by acting as a stimulant to improve plant performance (Wilson et al., 2018). Some evidence exists that placing seeds inside vesicles such as hessian bags may provide protection from predation (Harwell and Orth, 1999), but this is also largely unquantified, especially within field trials.

Confounding these methodical challenges to reduce predation is the problem that we do not understand the full breadth of predators that feed upon seagrass seeds. For example recent observational evidence (pers obs) has highlighted that nematodes may attack seagrass seeds, leading to their rapid degradation. Given the ever expanding range of invasive species such as the Green Crab that act as seed predators in seagrass systems (Carlton and Cohen, 2003), the trophic shifts in coastal food webs (Maureaud et al., 2017) and the ever present problem of eutrophication (Smith et al., 2006), seed-based seagrass restoration methods need to be innovative in order to break the negative feedbacks that many biological interactions present. In summary, we need improved, applied, 'solution driven' research in this field to solve these bottlenecks and create simple solutions that can be deployed at scale.

2.4.2. The significance of the seagrass holobiont

Seagrass restoration is more successful in close proximity to existing seagrass (van Katwijk et al., 2016). While this may merely be due to the fact that the environmental conditions within those meadows are more suitable for seagrass, we believe that this is not the full explanation. Instead, we hypothesise that localities further away from existing seagrass are likely to have negative feedbacks existing that involve critical functions of the microbial community that associates to seagrass. We know that unique microbial assemblages are present on seagrass roots and rhizomes relative to surrounding sediments (Fahimipour et al., 2017; Ugarelli et al., 2019), and that these root associated communities are enriched in species known to play functional roles in altering the sedimentary biochemistry, particularly sulphur and nitrogen cycling bacteria (Ugarelli et al., 2019).

Recent studies examining the microbiome associated with seagrass over individuals of varied age structure, and within experimental transplants, indicate that the microbiome changes (Sanders-Smith et al., 2020; Wang et al., 2021). We also know that various changes in the environmental and physical conditions of the sediment alter the seagrass microbiome (Martin et al., 2018; Zhou et al., 2021). This microbiome, in association with the plant roots, is increasingly being referred to as the holobiont. A holobiont is an assemblage of a host and the many other species living in or around it, which together form a discrete ecological unit through symbiosis. Although the exact nature of such an association between microbes and plants is poorly known, and its association as a symbiotic one mere speculation, it is fair to expect that the microbiome plays a key role in enabling seagrass to inhabit a stressful environment of low oxygen, high sulphide and limited nutrients (Tarquinio et al., 2019). We hypothesise that the seagrass microbiome and its formation of the seagrass holobiont is a key element of why some seagrass plants and seedlings will establish and survive in an environment or not. For example, nutrient levels associated to *Zostera mulleri* were correlated to enhance the abundance of putatively beneficial microbial taxa (e.g. sulphide- oxidising Beggiatoaceae and denitrifying *Geofilum rubicundum*) associated with seagrass roots, leading to the suggestions that under more stressful conditions positive plant-root microorganism interactions are of greater importance (Fuggle et al., 2023).

Although the available evidence indicates the high importance of the microbiome in facilitating adaption to difficult environments, we know very little about the role this plays in seagrass restoration or how we can use microorganisms to improve restoration success. Recent studies have begun to show how sedimentary additions, such as biochar, can improve overall restoration success (Zhang et al., 2021), and we speculate that this may be due to an increase of surface area for microbial populations to colonize. Overall, we argue that this association of seagrass and its microbiome as a holobiont is of such significant importance for seagrass that lack of knowledge in this area is a bottleneck to improving the reliability of seed-based seagrass restoration.

2.5. The sustainable supply of seagrass seeds

Sustainable seed supply is a major global issue for restoration projects across many varied ecosystems (Nevill et al., 2018). To enable scalable seed-based restoration of seagrass the issue is the same, asustainable and abundant supply of seagrass seeds is required, and to date this supply is almost exclusively collected from wild populations. To date no study that the authors are aware of explicitly tests the impact of the collection of seeds on wild seagrass populations at scale creating a bottleneck which is driven by a dearth of knowledge in the area of seagrass reproduction.

Studies indicate a varied role of seeds in the maintenance of the meadow, however its thought likely that only a very small proportion of the seeds of some species may lead to seedling development (Johnson et al., 2020). Although seeds may not always lead to rapid germination, in many instances their role is also to confer resilience upon the population to future perturbations through the development of a seed bank (Jarvis and Moore, 2010; Unsworth et al., 2015). Given the high energetic investment that plants make in seed production (Fridley, 2017), removal of significant proportions of this investment has the potential to have wider ecosystem impacts. In addition, this biomass may in some instances have a major role in supporting associated fauna such as wildfowl (Kollars et al., 2017).

Successful long-term seagrass restoration programs such as those in the coastal lagoons of Virginia, USA, have used in excess of 70 million wild collected seagrass seeds over many years (Orth et al., 2020). As projects expand globally there is understandably a lot of caution within many regulators as to the sustainability of the widespread collection of seagrass propagules at such scale, be it for seed- or shoot-based restoration. This caution is exacerbated by the low levels of success commonly observed across the restoration activities (van Katwijk et al., 2016). Although programmes of successful restoration, such as those in Chesapeake Bay have indeed collected large volumes of seagrass seeds over long periods of time, and the expectation is that such collection has negligible impacts, the evidence for this remains limited. However, collection of seagrass plants is not a new process, and Indigenous Peoples, particularly in North America previously harvested both roots and seeds as a traditional food source (Cullis-Suzuki et al., 2015). In many parts of the Indo-Pacific seagrass seeds from the species *Enhalus acoroides* remain a commonly used food source available for purchase at many local markets (McKenzie et al., 2021). Early experiments into the effects of small-scale harvesting, revealed that traditional harvesting did not negatively impact *Z. marina* meadows, and that instead harvesting likely increased shoot productivity and reproduction (Cullis-Suzuki et al., 2015). To ensure that collection of seagrass propagules for restoration is conducted in a sustainable manner requires both increased evidence of the impact of such activities, as well as a broader understanding of the factors driving reproductive effort. In the absence of experimental data, seagrass science should look to other wild plant harvesting guidelines and take a largely cautionary approach.

The millennium Seed Bank in their guidelines for seed collection suggest that no more than 20% of the annual reproductive effort of a population should be collected each year. The Centre for Plant Conservation in their guidelines to support species survival in the wild for endangered species recommend collecting a maximum of 10% of the annual reproductive effort of a population based on the models of 25 species of varied life history (Menges et al., 2004).

Given the varied life history traits of different seagrass species, this abundance of seeds is not always available, for example species of Posidonia may produce 4–13 fruits per m^2 per year (Balestri and Cinelli, 2003), whilst Zostera species commonly produce over 2000 seeds per m^2 per year and sometimes up to 9000 per m^2 (Larkum et al., 2006). While some data on seagrass flowering and seed production exists, this is generally limited at spatial scales and across different species, and very little data exists on the presence of seed banks as alternative sources of seed.

We know that flowering across many seagrass species is a temperature driven process (Inglis and Lincoln Smith, 1998; Diaz-Almela et al., 2006; Qin et al., 2020) and there is good evidence that environmental triggers are key to the whole reproductive process, but we are currently unable to reliably predict reproductive effort in seagrass. That said, evidence from North America suggests that populations existing close to their thermal limits, or within intertidal zones, are more characteristic of r-strategists, producing vast amounts of seeds, whereas subtidal populations existing within temperature limits are more characteristic of K-strategists where population maintenance and expansion is through asexual reproduction (Phillips et al., 1983). Such knowledge needs to be expanded upon across geographies to determine whether this is generalisable.

Numerous papers suggest that physical disturbance can positively influence seagrass reproduction (Cabaço and Santos, 2012), possibly as an evolutionary response to sexually reproduce to mitigate stress. For example, digging by sea otters has been shown to increase genetic diversity, likely as a result of sexual reproduction and the production of seeds (Foster et al., 2021), whereas physical disturbance by ice scouring in the Bering Sea has been shown to increase sexual reproductive effort (Phillips et al., 1983). Such positive influences of stress on reproductive effort also extend to water quality (Qin et al., 2021).

Given that our knowledge on reproductive performance is limited, our ability to model reproductive effort is limited. These knowledge gaps then limit our capacity for targeted collection of seagrass seeds and shoots, not only in order to ensure sustainability, but also to maximize the efficiency of collection and ensure that seeds are collected at the most effective point of maturation. Once sufficient knowledge is developed around the factors that influence reproductive effort, spatial models could be developed, much like those used for habitat suitability, that pinpoint seagrass populations, both within and across meadows, that are likely to produce high quantities of seeds.

Collection of seed is also limited by labour capacity. Globally, seeds are mostly collected by hand. Mechanized collection of *Zostera marina* seeds has previously been conducted in the US (Marion and Orth, 2010), yet the sustainability of such collection methods has never been assessed, leading to a limited uptake of this means of wild seed collection. The wider use of innovative techniques requires not just research on their effectiveness, but also on their sustainability in order to facilitate evidence-based decision making by regulators.

The majority of seagrass restoration activity to date has been conducted through collection of wild seeds or plants, however some projects have utilized seedlings grown in the laboratory under controlled conditions. Based on our knowledge and feedback from the Global Seagrass Nursery Network, we know that majority of nursery facilities and laboratory experiments have not facilitated sexual seagrass reproduction, and when they have, it has not produced seeds in noteworthy numbers. Some laboratories are also now using tissue culture techniques to expand the availability of such plants. The production of domesticated plant populations in laboratory and aquaculture type operations also provides opportunities for manipulating the environment and resources (e.g., use of hormones, and stimulants) to potentially improve seed yield, both in terms of seed quantity and quality. Seed production in nurseries or seagrass farms would be a logical step forwards to sustainable restoration efforts, but this needs to be in balance with the significance of the microbiome that we outline above. Creating seagrass nurseries also has large potential energetic costs, the sustainability of such operations needs to be considered in this context too.

2.6. Knowledge gaps within and across species and environments

To improve seed-based seagrass restoration there is a need for improved knowledge both within and across species, and over the full range of environments that seagrasses occupy. This is due to the large range in biological traits (e.g., life history, morphology) of those species and consequential adaptations to living in an environmental range that extends from shallow water super heating lagoons (Massa et al., 2011), to ice scoured coastlines (Robertson and Mann, 1984) and the depths of Indian ocean (Esteban et al., 2018). Although we know that 44% of seagrass species have promising life history traits for restoration by seeds (van Katwijk et al., 2021), and that advances in seagrass seed-based restoration have been spread globally, particular centres of research effort have biased our

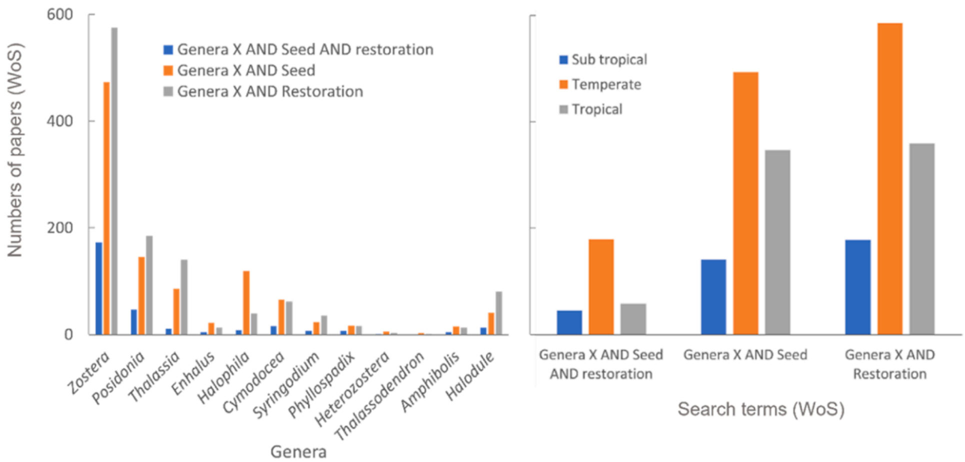

Fig. 3. Numbers of academic papers (as searched using Web of Science) on different aspects of seed based seagrass restoration (see Appendix 1).

knowledge, creating a significant bottleneck for the widespread uptake of seed-based restoration. Although restoration research has spread across all seagrass genera, a review (see Appendix 1) using Web of Science (see Fig. 3a and b) revealed that the overwhelming majority of published seed-based restoration literature was focused on the genera Zostera and Posidonia (and mostly *Zostera marina* and *Posidonia oceanica*). Some of the tropical genera (e.g., *Enhlaus, Syringodium, Thalassodendron*) of seagrass have received very limited research on their seeds, let alone the use of these seeds in restoration – however, publication practices also limit the uptake of this knowledge since research from emerging economies is routinely published in journals that are not indexed in Scopus (e.g., Western Indian Ocean Journal of Marine Science).

Between species differences highlight an additional bias of restoration effort towards the temperate seas relative to the tropical seas. However, such bias also exists within species. For example, *Zostera marina* occupies a latitudinal range of ~5000 km, existing from the cold waters of the Arctic at 70° N to subtropics at 26° N, yet seed-based restoration research has been restricted to ~60–30° N. While progress has commenced on seed-based seagrass restoration in the tropics (conversely, restoration via transplantation is far more common), a recent guideline document to seagrass restoration in the Western Indian Ocean highlights the lack of experimental knowledge on tropical species as it uses temperate case studies and data to explain many of the principles described (U.N.E.P, 2020).

In addition to the species and latitudinal bias, there is also bias towards environments that are more readily accessible and easier to work in. In general, shallow, coastal and lagoon locations are the target of most restoration efforts and there is no reference in the literature to restoration of deep-water seagrasses. In addition to this, seed-based restoration efforts in low salinity environments are also sparse despite large historic losses in these types of environments. Seed-based restoration in such environments is challenging and requires greater research. For example, in the northern Baltic Sea (<7 psu), flowering shoots are rare, and seeds do not ripen (Boström et al., 2004) presenting a significant bottleneck. To restore seagrass at scales representative of historic loss and to recognize its potential as a Nature Based Solution requires expanding seed-based seagrass restoration work to new locations, species and environments.

The fact that seagrass restoration can be conducted using two fundamentally different approaches (seed vs transplants) raises key questions as to under what conditions either or both are appropriate or most effective. The use of seeds appears more favoured in some locations due to regulatory restrictions (e.g., the UK) rather than the availability of scientific evidence, whereas seeds have been favoured in some localities due to their apparent success (e.g., Waddenzee and Virginia, USA) (Orth et al., 2020; Govers et al., 2022). The literature contains many examples of inter method comparisons, but these do not consider the question of under what conditions the relative merit of each method has its strengths, this may be of particular value in overcoming negative feedbacks in a system whereby for example seedlings from field planted seed may on their own insufficient to break that feedback. To make seagrass restoration more successful we need knowledge that can truly drive appropriate decision making about the most appropriate method for the specific set of conditions.

2.7. Learning from failure

There are significant structural problems related to scientific outputs linked to the limited and largely absent reporting of experiments that produce so called negative results, e.g. those that don't support the tabled hypothesis (Fanelli, 2012). This problem is well documented and thought to be a growing problem across the sciences (Fanelli, 2012). In seagrass restoration, poor data sharing practice results in bottlenecks remaining even though solutions may have already been developed. Projects that are unsuccessful or not considered 'novel' commonly are not written up, and the knowledge gained from those projects is not shared with the wider scientific community. In the British Isles, we are aware of seagrass restoration projects that have been undertaken across a range of locations, environments and methods, however reports and papers are only available from two of them (Dale in South Wales and Lowestoft in Norfolk) (Ranwell et al., 1974; Unsworth et al., 2022).

Many of the now successful seed-based projects around the world have taken decades to establish (Orth et al., 2020), however, there are limited detailed descriptions of the projects, activities and methods that took place in the years that led to the outwardly shared success. Although many informative and excellent experiments were run alongside these projects over decades, the actual restoration activities at scale, particularly the aspects that failed or were discarded for probably key reasons at the time, remain unknown to external scientists.

Within the seagrass restoration community there needs to be greater transparency about the iterative nature of restoration projects, the decision-making processes, and results from each stage of projects as they develop. Such information would allow the local participants to the wider restoration community to learn not just from the successes, but importantly from the failures in order to more rapidly improve our restoration toolkit. The recent establishment of a Global Seagrass Nursery Network provides a model for how such knowledge can be shared, where partners are able to openly discuss the many facets of success and failure within the ongoing and past projects.

Wider data sharing of positive and negative results in ecological restoration improves (Fanelli, 2012) predictive capacity and can help to provide better information for evidence-based decision-making, and scale-up approaches to meet ambitious targets set for restoration (Ladouceur et al., 2022).

3. Moving forward to overcome these bottlenecks

In this paper, we present seven bottlenecks impeding reliable and successful seed-based seagrass restoration at scale. These are 1) Finding a suitable environment for restoration, 2) Poor seed germination and emergence, 3) Understanding of the conservation genetics of seagrass, 4) The role biological interactions, 5) The sustainable supply of seagrass propagules, 6) Knowledge gaps within and across species and environments, and 7) Learning from failure.

Given the evidence we present here, we argue that there is an urgent need for systematic, targeted, and applied multidisciplinary research into seagrass seed-based restoration. Such research would enable us to overcome, at least in part, the first six bottlenecks. Such research need not be grand and could instead be focused on a series of small field-based experiments and surveys across broad geographies, much like the Zostera Experimental Network or the Indo-Pacific Seagrass Network. Advances have already been made in this nature with the Global Seagrass Nursery Network, but we need to urgently apply and test.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

APPENDIX 1

Web of Science (WoS) was used as an approximation of the relative research effort in individual elements of seagrass seed-based restoration science. Each of the 12 seagrass genera was searched in WoS together with and without the terms 'seed' and 'restoration'. Studies just on seeds with the specific genera ranged from 473 for Zostera to 3 for Thalassadendron. The mean number per genera was 85 $\pm$ 130. When adding the term 'restoration, this reduced the availability of papers somewhat with 173 found for Zostera and 0 for Thalassadendron (mean 24 $\pm$ 48). Data was then grouped relative to the dominant distribution of that genera (temperate, sub-tropical and tropical).

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