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Contrasting evolutionary histories from the warm Atlantic to the cold Pacific - overlooked seagrass diversity

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Declaração de autoria de trabalho

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Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

I declare to be the author of this work, which is original and unpublished. Authors and works consulted are duly cited in the text and are included in the list of references.

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To my grandpa Júlio,

for always asking me

“(…) are you ever going to stop studying?”

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Abstract

Marine connectivity and genetic differentiation among populations and species across different sides of oceans are hypothesized to be driven by contrasting processes in tropical versus cold and temperate regions. Tropical species occupy restricted warm ocean ranges where patterns of genetic differentiation might be driven more by oceanic connectivity and spatial habitat continuity and stepping-stone processes. In contrast, the genetic structure across the ranges of species occupying cold and temperate habitats might be more influenced by the past climate-driven range shifts that led to demographic effects (e.g., bottlenecks, founder events, local extinctions, and expansions) with strong consequences for evolution and extant genetic diversity. There is well supported evidence for the role of Quaternary ice ages that had crucial effects on present distributions of species genetic diversity. Recurrent glacial-interglacial cycles led to a series of events shaping the phylogeographic patterns of marine species, leaving evidence that the present geographical genetic structure of northern hemisphere species has been strongly affected by historical vicariance. Seagrasses of the genera *Halodule* and *Phyllospadix* are distributed along coastal shores on both sides of the tropical Atlantic and temperate Pacific, respectively. They are therefore excellent models to study the impact of past and future climatic fluctuations and physical oceanographic connectivity on the patterns of genetic diversity and differentiation along the distributional ranges of both tropical and temperate regions. Additionally, these species they offer valuable insights into past range shifts and contemporary connectivity dynamics. This thesis aims to understand the population genetic history of seagrasses in biogeographical settings with contrasting oceanographic and seascape characteristics.

Keywords: seagrasses; population connectivity; dispersal; genetic diversity; marine biogeography; climate change.

Resumo

A conectividade marinha e a diferenciação genética são teoricamente consideradas como impulsionadoras de processos que diferem entre regiões tropicais e temperadas/frias entre populações e espécies que estão distribuídas em lados opostos dos oceanos. Espécies tropicais ocupam regiões oceânicas quentes e mais restritas, onde padrões de diferenciação genética podem ser influenciados mais pela conectividade oceânica, continuidade espacial de habitat e processos de *stepping-stone*, em detrimento de oscilações climáticas. Por outro lado, a estrutura genética de espécies que ocupam regiões de habitats temperados e frios pode ser mais influenciada por mudanças climáticas passadas. Estas alterações climáticas deram origem a efeitos demográficos (por exemplo: *bottlenecks*, eventos fundadores, extinções e expansões) que tiveram uma grande influência na evolução e diversidade genética que hoje é observada. Existem fortes evidências sobre o papel dos períodos glaciais do Quaternário, nomeadamente os efeitos cruciais que tiveram nas distribuições atuais da diversidade genética das espécies. Estes ciclos glaciais e interglaciais levaram a uma série de eventos que moldaram os padrões filogeográficos das espécies marinhas, deixando evidências na sua estrutura genética e geográfica atual, principalmente no hemisfério norte foi fortemente afetado pela vicariância histórica. As ervas marinhas dos géneros *Halodule* e *Phyllospadix* estão distribuídas ao longo de zonas costeiras na zona tropical do Oceano Atlântico e na zona temperada fria do Oceano Pacífico, respetivamente. Assim, estas espécies são excelentes modelos para estudar os impactos das alterações climáticas passadas e futuras e conectividade oceânica nos padrões de diversidade e diferenciação genética ao longo da distribuição em regiões tropicais e temperadas. Mais ainda, estas espécies oferecem informações importantes sobre mudanças que ocorreram anteriormente na sua distribuição e sobre a dinâmica de conectividade atual. Esta tese tem como objetivo compreender a história populacional destas espécies de ervas marinhas em diferentes contextos biogeográficos com características oceanográficas diferentes.

Palavras-chave: ervas marinhas; conectividade populacional; dispersão; diversidade genética; biogeografia marinha; alterações climáticas.

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List of abbreviations

ºC: Degrees Celsius

µM: Micromolar

µl: Microliter

A: Allelic richness

Â: Standardize allelic richness

AMOVA: Analysis of Molecular Variance

AUC: Area Under the Curve

bp: base pairs

BP: Before Present

BRT: Boosted Regression Trees

DAPC: Discriminant Analysis of Principal Components

DNA: Deoxyribonucleic Acid

FCA: Factorial Correspondence Analysis

Fis: Inbreeding coefficient

FST: Fixation Index

G: Genet(s)

H_E: Expected Heterozygosity

H_O: Observed Heterozygosity

HYCOM: Hybrid Coordinate Ocean Model

ILS: Incomplete Lineage Sorting

IPCC: Intergovernmental Panel on Climate Change

KYA: Thousand Years Ago

LGM: Last Glacial Maximum

LLD: Long distance dispersal

MCMC: Markov Chain Monte Carlo

MLG: Multi-Locus Genotype

MYA: Million Years Ago

N: Sampling units

PA: Private alleles

PÂ: Standardize private alleles

PCA: Principal Component Analysis

PCR: Polymerase Chain Reaction

R: Genotypic richness index

RCP: Representative Concentration Pathway

SDM: Species Distribution Modeling

SNP: Single Nucleotide Polymorphism

Ta: Annealing temperature

TSS: True Skill Statistic

CHAPTER 1

GENERAL INTRODUCTION

1.1. Marine population connectivity

Marine population connectivity refers to the effective exchange of individuals and genes between geographically separated marine populations that comprise a metapopulation (Cowen et al., 2007). Population connectivity is important for the overall health and resilience of marine ecosystems, as it is a key factor in (meta)population dynamics and gene-flow, geographical distributions, population genetics, and species evolution (Roughgarden et al., 1988; Palumbi, 2003; Cowen et al., 2007; Pineda et al., 2007).

In the marine realm, populations can be spatially separated by unsuitable habitat, yet connected by the exchange of individuals via dispersal (i.e., gene flow; the proportion of genes from new immigrants that migrate and establish into a given population; Wright, 1949). Thus, populations can vary in both size and potentially in connection strength. Successful establishment of individuals into a new areas involves gene flow, which significantly contributes to population growth, persistence, and genetic evolution (Cowen and Sponaugle, 2009; Lowe and Allendorf, 2010). Gene flow, along with introgression (hybridization), genetic drift, and selection, can influence the intra-specific genetic structure of populations and variation among and within populations (Hellberg et al., 2002). Whereas introgression and genetic drift may cause populations to diverge, gene flow contributes to genetic homogenization of populations and the dilution of genetic structure/differences within species (Slatkin, 1987). Understanding the interplay and relative roles of the evolutionary forces acting on metapopulation dynamics is essential for understanding the genetic structure of populations as well as their biogeography, partitioning of intraspecific diversity and potential responses to selective pressures of ongoing climate change (e.g., (Jump and Peñuelas, 2005).

1.1.1. Drivers and barriers of marine connectivity

Previously, marine populations were assumed to be widely connected across broad spatial scales, as it was assumed there were no obvious and distinct physical barriers preventing connectivity (Cowen et al., 2000). However, dispersal in the marine environment is mediated by a series of biotic and abiotic interactions such as species traits (e.g., propagule buoyancy or longevity), physical processes (e.g., currents) or biological processes (e.g., competition; Goldberg and Lande, 2007). When successful, connectivity has the potential to significantly influence the structure of marine populations and ecosystem function.

The huge diversity of life histories among marine organisms means that dispersal capabilities and opportunities vary broadly. Some species produce heavier, non-motile propagules, such as seeds or plant fragments, which are less likely to disperse far from their parent population. Others, such as sessile organisms like corals or oysters, rely on the dispersal of their gametes or larvae to establish new colonies. The behavior of different dispersal units, such as seeds, spores, eggs, plant fragments, larvae, and juveniles, has therefore been investigated in several marine connectivity research studies (e.g., Tulipani and Lipcius, 2014; Wood et al., 2014; Green et al., 2015; Kendrick et al., 2017).

Abiotic drivers of marine connectivity include physical factors such as currents, tides, temperature, and salinity, that can either promote or hinder the dispersal of marine organisms (Cowen et al., 2007). For example, strong currents can help to transport marine organisms faster or over longer distances (Li et al., 2017), while temperature and precipitation can create barriers that prevent the movement of species across biogeographic regions (Belanger et al., 2012), for instance across the Arctic or the tropical equator for the generality of cold-temperate organisms. Similarly, changes in precipitation and wind patterns can affect the strength and direction of ocean currents, which can in turn influence the movement of marine organisms (Pinsky et al., 2020). Natural events such as tsunamis, volcanic eruptions, and hurricanes can disrupt marine ecosystems and cause temporary pulses or permanent changes in marine connectivity (Kendall et al., 2004; Bryan et al., 2012; Carlton et al., 2017). Other factors that can affect marine connectivity include human activities such as coastal development and fishing that destroy or fragment marine habitats, making it difficult for individuals to move between populations. Moreover, overfishing, and other forms of exploitation can reduce the abundance of certain species, which can disrupt natural patterns of dispersal and connectivity (Bindoff et al., 2019). However, human activities also influence the dispersal of marine organisms through ship hulls, ballast water, and recreational boating but also via construction of artificial seaways like the Suez and Panama canals (Waterkeyn et al., 2010; Crook et al., 2015; Castellanos-Galindo et al., 2020). These activities have enabled numerous species to breach natural biogeographic barriers, leading to their invasion and aggressive spread in new, alien ranges, exemplified by species such as *Sargassum muticum* and *Rugulopteryx* (Engelen et al., 2015; García-Gómez et al., 2020).

Biotic vectors (e.g., fish, seabirds, and marine mammals) can interact and enhance the transport of dispersal units from one location to another. This can occur through a variety of mechanisms, such as endozoochory (i.e., seed dispersal by animals after passage through their guts) (Wu et al., 2016; Tol et al., 2021). However, while the role of abiotic vectors dispersion has been increasingly acknowledged, biotic means of marine dispersal is still difficult to track (Viana et al., 2013).

1.1.2. Assessment of marine population connectivity

Understanding and estimating marine dispersal and connectivity is highly demanding due to the diversity of life traits exhibited by marine species as well as the vast range of geographical and temporal scales associated with marine environments. Nevertheless, there are several ways to measure marine connectivity. Some common methods include tracking the movements of individual organisms using tags or markers (e.g., Patrício et al., 2022). By tracking the signals of multiple tags, it is possible to create a connectivity map of individuals between different populations. However, it is often not possible to obtain direct measurements of the movement of marine species that produce vast numbers of propagules when they are subject to transport by ocean currents (Cowen and Sponaugle, 2009). Thus, population connectivity assessments may rely on more indirect measures such as genetic estimates (e.g., SNP; Evans et al., 2021 or microsatellite markers; Jahnke et al., 2019), biophysical models and simulation techniques to predict the flow of propagules between different areas. These methods allow a better understanding of connectivity patterns as they provide information on the spatial extent of population connectivity (Marko and Hart, 2018; Swearer et al., 2019; Jahnke and Jonsson, 2022). Additionally, remote sensing technologies, such as satellite imagery, can be used to monitor the physical characteristics of the seascape and identify potential barriers or corridors of marine connectivity (Johns et al., 2020). Overall, a combination of these approaches can provide a more comprehensive understanding of marine connectivity and help inform conservation and management efforts.

1.2. Species geographic distribution patterns

A crucial feature of every species is its geographical distribution, i.e., how is distributed across its range, and the limits that characterize the geographic boundaries of tolerance to environmental and biotic factors such as habitat suitability, competition and, dispersal ability (Gaston, 2009). In the context of species distributions, the leading edge is characterized by the expanding edge due to recent colonization or expansion events. This can occur when a species moves into a new area due to changing environmental conditions, such as a shift in climate or the availability of new habitat (Assis et al., 2018b; Song et al., 2021) or when it is introduced in a new area by humans (Engelen et al., 2015; Richardson et al., 2016). Thus, these populations might be critically important, allowing species to survive under climatic fluctuations (Gibson et al., 2009). However, it is essential to emphasize that while species leading edges played a crucial role during climatic shifts by allowing species to adjust to new climatic conditions, they are not static long term and may eventually exhibit limited diversity (Coyer et al., 2003; Assis et al., 2018b).

The range center of species distribution is theoretically where species are most abundant, have the most suitable habitat and where they can thrive. At the range center, species genetic diversity is also expected to be higher (when compared to perineal populations), with considerable gene flow, therefore limiting differentiation among populations (Jueterbock et al., 2018). This concept of species ranges is usually referred to as the “abundant center” model and it has influenced many questions in the field of ecology and evolution of species ranges (Brown, 1984; Brown et al., 1995). This model although well-established is yet controversial since species abundance and genetic diversity are determined by historical dynamics that this model does not consider, such as the periodic climatic shifts associated with the glacial/interglacial cycles interaction of contemporary environmental and historical drivers (Sagarin and Gaines, 2002; Eckert et al., 2008; Zardi et al., 2015).

Contrarily, species rear edge (or trailing edge) refers to the edge of a species range that is declining or disappearing. This can occur due to a variety of factors, including habitat loss or degradation, competition, or the impact of natural disasters or human activities (Buonomo et al., 2018; Duarte et al., 2018), and at global scales due to ongoing climatic changes. Due to decreased gene flow brought on by isolation or local adaptation, and also because of their role as central ranges during past glacial stages, modern rear edge populations are expected to exhibit higher ecological marginality and higher genetic divergence relative to other rear edge and more central populations (Eckert et al., 2008; Pearson et al., 2009). These populations may thus have less genetic diversity, but they also have a potential to be genetically distinct.

Although theory predicts these previous described patterns, evidence suggests species distributional patterns are more complex than theoretical models suggest (e.g., similar levels of genetic diversity along range center and peripheral populations of *Zostera marina*; Diekmann and Serrao, 2012 or, in *Saccorhiza polyschides,* an increase of genetic diversity towards the distributional edge; Assis et al., 2013). These ambiguities might be attributed to either historic (i.e., glacial cycles) or/and contemporary factors (e.g., population size, dispersal, bottlenecks, and habitat fragmentation).

1.2.1. Distributional range shifts

Species distributions change over time (range shifts) due to various factors such as climate change, habitat loss, or the introduction of invasive species (Gaston, 2003). These changes can happen at different scales and can have significant effects on both ecosystems and the species inhabiting them (Chen et al., 2011). Theory predicts that species will respond to changing climatic conditions by 1) migrating to areas with emerging favorable environmental conditions, and 2) acclimating (or perishing) wherever conditions become less favorable. Several studies have supported the hypothesis that species can experience range contractions during glacial cycles (Assis et al., 2014, 2016a), and conversely expand shifts towards higher latitudes (Neiva et al., 2018), or colonize greater depths (Fragkopoulou et al., 2021) during warming periods. However, not all species can shift their range in response to changing environmental conditions due to physical barriers, such as temperature or ocean currents, that restrict their ability to move to new areas (Assis et al., 2017; Kumagai et al., 2018). Some species are unable to adapt to new environmental conditions, which can make it impossible for them to survive in new areas. In such cases, range shifting may not be a viable option, and if conditions in their current range become unsuitable, there is a risk of genetic diversity loss and species regional extinction (Buonomo et al., 2018; Chefaoui et al., 2018). Therefore, it is essential to monitor these range shifts, identify the species most at risk, the most evolutionarily important populations (often confined to specific hotspot areas), and assess the rate at which these changes are happening to ensure the well-being of ecosystems and species (Bonebrake et al., 2018). However, marine taxa exhibit significant variation in their range shifts, making it challenging to develop effective management plans (Poloczanska et al., 2016).

Species distribution models (SDMs) are empirical models that quantify species-environment interactions, developed using species distribution data (such as occurrence or abundance) and environmental factors that affect species distribution. These models are valuable when gathering data on species distribution is unfeasible or when environmental factors that influence species distribution have changed (reviewed by Guisan and Thuiller, 2005). In recent years, these models have been used to forecast climate change-induced range expansions and contractions in several marine species (Assis et al., 2014; Martínez et al., 2018; Song et al., 2021). Furthermore, when coupled with genetic data, SDMs can help to identify unique gene pools that are crucial for conservation efforts (Alsos et al., 2009; Lovrenčić et al., 2022).

1.2.2. Climate-driven species range shifts

1.2.2.1. Historical events

There is well supported evidence for the role of Quaternary glacial cycles (~2.6 MYA to present) that had crucial effects on present-day species genetic diversity and population structure due to repeated shifts (expansions and contractions) in species ranges caused by changes in regional temperatures, sea level, and oceanographic circulation patterns (Provan and Bennett, 2008). The Last Glacial Maximum (LGM: ~21 KYA BP), was one of the most extreme periods, that affected species distributions and population sizes, resulting in severe reductions in the high latitude ranges of cold-temperate species (Maggs et al., 2008). This series of events shaped the phylogeographic patterns of many marine species, that have persisted in more suitable habitat conditions southwards, leading to isolation of populations in confined regions or refugia (Hewitt, 2004). In addition to species equatorial retreat and poleward expansion, glacial periods also induced allopatric speciation (or vicariance) in refugia, followed by potential admixture when subsequent warming conditions and ice retreat allow range expansions, forming evolutionarily unique contact zones, where divergent genotypes can recombine in rapid evolutionary events (Assis et al., 2014, 2018a; Neiva et al., 2016, 2018). There is evidence that the present geographical genetic structure of northern hemisphere marine species has been strongly affected by historical vicariance and long term-persistence during interglacial cycles with important implications for speciation (Maggs et al., 2008; Coyer et al., 2011; Neiva et al., 2018).

1.2.2.2. Contemporary drivers

At the onset of the inter-glacial period (Holocene; 12 KYA to the present), the melting of sea-ice and coastal ice-sheets resulting from warmer conditions at northern mid- and high latitudes allowed cold-temperate species to establish in northern regions (Provan and Bennett, 2008). However, the climate has been changing at an unparalleled pace. Indeed, by 2100 the planet is forecast to have warmed at least 2ºC (relative to the pre-industrial average) or up to 5°C depending on the greenhouse gas emissions trajectory (IPCC, 2022). This environmental warming is one of the many climate change conditions we are currently facing (Pinsky et al., 2020) that it is causing species to shifts their distributional ranges (Lima et al., 2007; Sudo et al., 2020) and theory predicts that it is one of the most important drivers for biodiversity loss, including at intra-specific genetic levels (Assis et al., 2018a; Román-Palacios and Wiens, 2020; Chefaoui et al., 2021). Also, extreme climatic events such as marine heatwaves, can lead to long lasting changes in habitat forming specie ecosystems such as seagrasses (Thomson et al., 2015). Warming trends are particularly relevant for populations that are near the edges of a species range where sub-optimal conditions may prevail. Although species can survive either through plasticity or adaptation (if time permits), high rates of climatic change may force relocation or local extinction in certain areas (Nicastro et al., 2013; Duarte et al., 2018). This may in turn result in the loss of ancient gene pools that survived in refugia during past climate extremes (Assis et al., 2014; Neiva et al., 2015), reducing the overall genetic diversity of species, with potential impacts on the ability of the species to evolve in the longer term (Pujol and Pannell, 2008).

1.3. Seagrasses: ecosystem engineers of the sea

Seagrasses are a group of marine flowering plants (Angiosperms) belonging to four families of monocots in the Alismatales (Arber and Arber, 1920; den Hartog, 1970; Les et al., 1997; Olsen et al., 2016). Seagrasses are considered a biological group, having arisen independently at least three times (i.e., a polyphyletic group; Les et al., 1997). The rbcL gene was used as a genetic marker by (Les et al., 1997) to identify three distinct origins of seagrasses, and further research supported these findings (Janssen and Bremer, 2004; Larkum et al., 2006). The evolutionary history of seagrasses is linked to freshwater aquatic ancestors. Around 70 to 100 million years ago, terrestrial alismatid (flowering) plants returned to the water and colonized the sea during the Cretaceous period, resulting in at least three distinct evolutionary processes that resulted in the modern seagrasses (den Hartog, 1970; Les et al., 1997; Hemminga and Duarte, 2000; Benzecry and Brack-Hanes, 2016). The several evolutionary events that seagrass lineages witnessed brought together a series of physiological, morphological, and genomic adaptive modifications that allow seagrass to survive in the world oceans (e.g., adaptations to high salinity, water movement, tidal oscillations, and capacity to survive and reproduce fully submerged in the marine environment) (Spalding et al., 2003; Olsen et al., 2016).

1.3.1. Seagrasses taxonomy

Even today there is still much debate associated with seagrass taxonomy and phylogenetics. According to recent phylogenetic classification, about 70 seagrass species have been assigned to 5 families within the order Alismatales (den Hartog and Kuo, 2007); Zosteraceae (*Zostera and Phyllospadix*), Cymodoceaceae (*Amphibolis, Halodule, Syringodium, Cymodocea,* *Thalassodendron*), Posidoniaceae (*Posidonia*) and Hydrocharitaceae *(Enhalus, Halophila, Thalassia*). The inclusion of the family Ruppiaceae (*Ruppia*) is still under debate as some species are not yet considered true seagrasses. A rbcL gene-based phylogenetic tree, which includes all main families is still the most comprehensive molecular analysis of alismatids based on 70 species to date (Les and Tippery, 2013). The same phylogenetic relationships were also supported by other studies, meaning that the fundamental relationships related to seagrasses were unaffected (Petersen et al., 2006; Iles, 2013; Iles et al., 2015).

1.3.2. Distribution of seagrasses

Seagrasses have a worldwide distribution (except Antarctica), with species occupying coastal areas across multiple bioregions (Short et al., 2007). There are six bioregions established to define seagrass species distribution (Figure 1), Temperate North Atlantic, Tropical Atlantic, Mediterranean, Temperate North Pacific, Tropical Indo-Pacific and Temperate Southern Oceans (den Hartog, 1970; Green et al., 2003; Spalding et al., 2003). Across these bioregions, seagrass species diversity is unevenly distributed with tropical regions holding seven of the current eleven genera (*Halodule*, *Thalassia*, *Halophila, Enhalus, Syringodium, Cymodocea* and *Ruppia*) and the remaining four (*Zostera*, *Posidonia*, *Phyllospadix* and *Amphibolis*) are confined to temperate areas (Short et al., 2007, 2016; Waycott et al., 2014). Along these regions, seagrass meadows

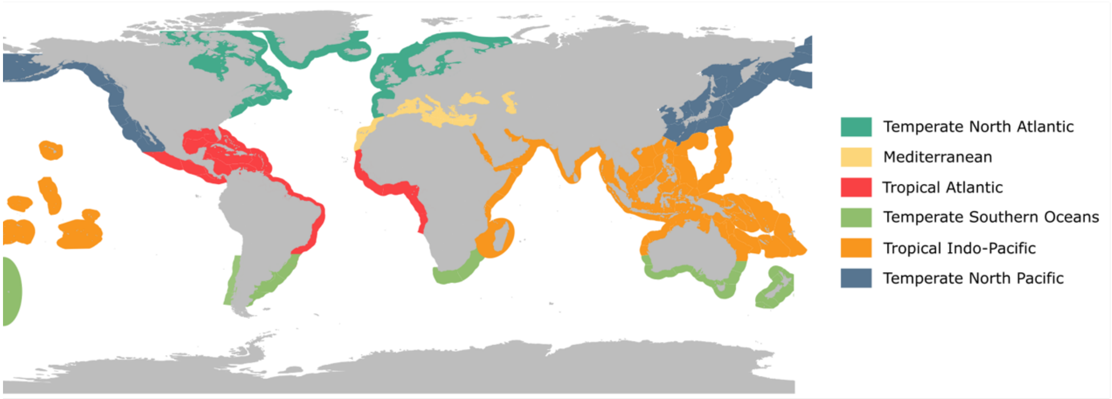

Figure 1 – World seagrass geographic bioregions (Short et al., 2007).

that can be found in muddy or sandy bottoms (except the genus *Phyllospadix* that grows on rocky substrates) in several different conditions ranging from estuarine habitats, typically along gently sloping, protected coastlines in clear, shallow waters where light penetrates, allowing photosynthesis in habitats with strong currents. Seagrass species can grow in environments exposed to air during low tide or down to 90 meters depth (Duarte, 1991). However, temperature, tides, currents, salinity, light, or nutrients are some factors that influence seagrass survival and growth (Greve and Binzer, 2004), resulting in different spatial species distributions.

1.3.3. Morphology of seagrasses

Seagrass morphology varies among species, but common features include three main distinct segments: rhizomes, roots, and leaves (Figure 2). As below-ground sections, roots function in anchoring and nutrient acquisition, and rhizomes/stems provide mechanical support and nutrient transport. Above the sediment surface, shoots have several leaves where each leaf usually has a basal sheath that protects the apical meristem and where new leaves grow (Kuo and Hartog, 2007). Seagrass species share similar morphological features, such as reduced cuticle, lack of stomata, chloroplasts, and structures to resist wave-action, such as thickened shoot bases (Ackerman, 2005; Larkum et al., 2006). Due to the lack of stoma, gas exchange in seagrasses occurs through permeable leaf cuticles as well as aerenchyma, i.e., gas-filles spaces in the tissues that provide low resistance gas diffusion in the water. This adaptation is found in the roots and rhizomes, facilitating oxygen exchange under anaerobiotic conditions (Larkum et al., 2006). Nonetheless, depending on the environmental conditions or location, seagrass species display a variety of different morphological characteristics (e.g., *Zostera* has ribbonlike leaves and *Halophila* has paddlelike leaves; Kuo and Hartog, 2007) and adaptations (e.g., tolerance to different salinities as seen in *Halophila ovalis* (Tyerman, 1982) versus *Halodule wrightii* (Biber, 2022).

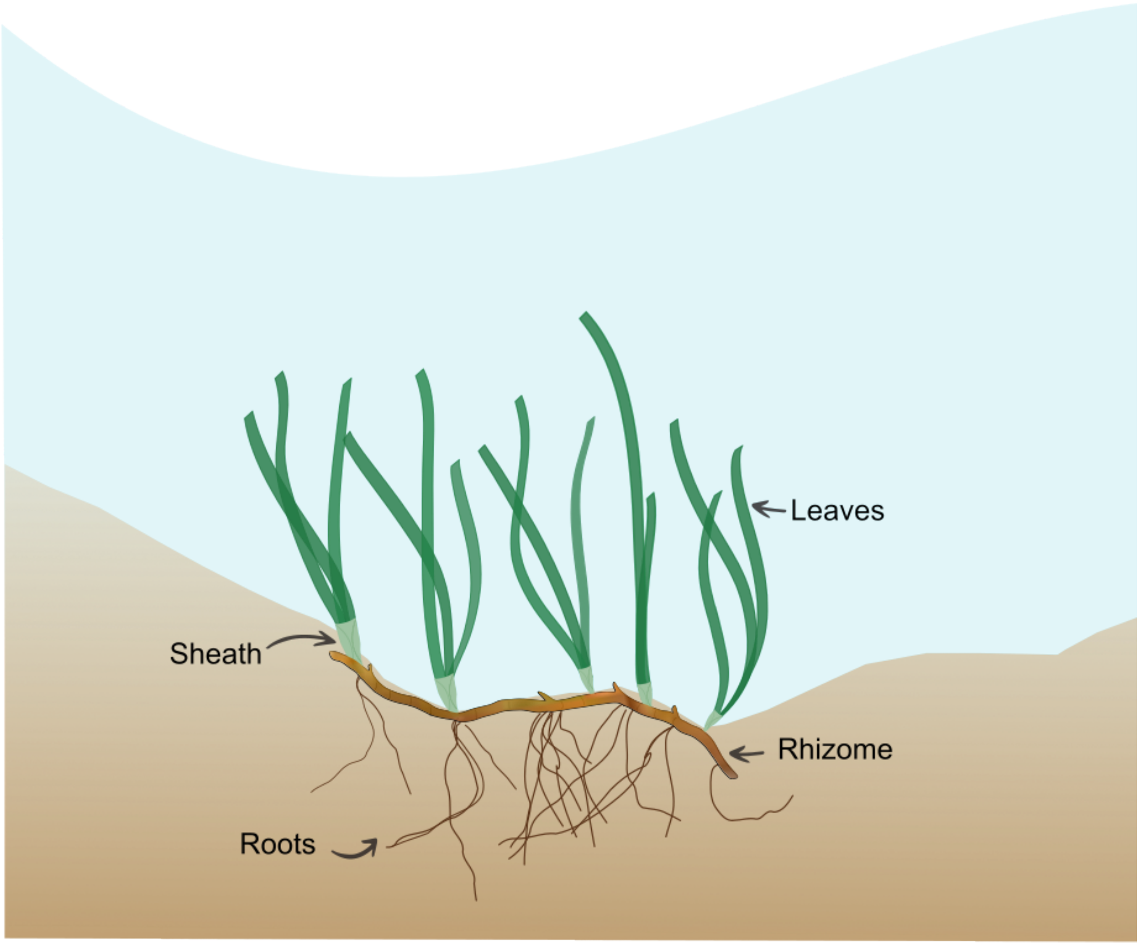

Figure 2 - Schematic representation of the general morphology of seagrasses.

1.3.4. Growth and reproduction

Seagrasses grow vertically and horizontally; their leaves reach upwards, to catch sunlight and their roots down and sideways to capture nutrients from the water and sediment. To achieve population growth, seagrasses have adopted two different means of reproduction, sexual and asexual (or clonal) propagation (Ackerman, 2007). Seagrass species reproduce sexually through hydrophilous fertilization of flowers and seed production and asexually by producing vegetative propagules (ramets) through rhizome horizontal growth (Hemminga and Duarte, 2000). Ramets are the dispersal stage, reproductively independent and genetically identical to the parent plant (genet). In fact, marine plant clones can grow for many years such of the case of *Posidonia oceanica* (Arnaud-Haond et al., 2012), *Thalassia testudinum* (Bricker et al., 2018) or *Posidonia australis* (Edgeloe et al., 2022). Clonal growth allows seagrass species to get access to light and nutrients easily due to fast spreading of new rhizomes, that can reach new environments easily (Hemminga & Duarte, 2000; Larkum et al., 2006). Also, clonal reproduction has a lower energy cost and happens when sexual reproduction is not viable either due to lack of mating partners or non-suitable ecological conditions for seed dispersal and establishment (Kendrick et al., 2012). Despite these advantages, it is sexual reproduction that confers populations resilience by maintaining high levels of genetic diversity and genetic variation, through the combination of genes from distinct parent plants. (Ackerman, 2007; Reynolds et al., 2013). This way, sexual reproduction provides also other means of dispersal and colonization to new areas or establishment to new patches (Larkum et al., 2006; Kendrick et al., 2012; McMahon et al., 2014). Most seagrass species exhibit separate male and female plants (dioecious), while some species are monoecious, allowing for self-pollination by having both sexes on the same plant (van Tussenbroek and Helgueras, 2010). The combination of both sexual and asexual reproductive strategies helps ensure seagrass meadow development and promotes healthy and dynamic coastal environments (Olsen et al., 2004; Arnaud-Haond et al., 2005).

1.3.5. Seagrasses dispersal mechanisms

Seagrass species can colonize new environments via three main mechanisms; 1) the dispersal of sexual propagules such as seeds or pollen, 2) through clonal growth of rhizomes, enabling the spread of roots to new areas or 3) via detachment of vegetative fragments that can be dispersed by currents to new locations where they can establish new populations (Kendrick et al., 2012; McMahon et al., 2014). Different reproductive modes produce propagules with diverse dispersal abilities (McMahon et al., 2014; Kendrick et al., 2017).

Seeds can have negative, neutral, or positive buoyancy (Orth et al., 2007). Positive and neutrally bouyant seeds may float great distances, facilitating the dispersal and further colonization of species (e.g. *Posidonia oceanica*). However, species of the genera *Cymodocea* and *Halodule*, due to their negatively bouyant seeds, largely lack this mean of dispersal via current transport (McMahon et al., 2014). An additional feature of sexual reproduction is that seeds can become dormant, forming seed banks that provide a source of continuous recruitment and resilience over time (Unsworth et al., 2015).

Pollen is also a crucial component of seagrasses dispersal mechanism. The dispersal of seagrass pollen is influenced by several factors, including water currents and tides, but also meadow features such dentisity or the proximity betwenn male and female plants (Verduin and Backhaus, 2000). The same way as for seeds, abiotic and biotic factors can play a vital role in the dispersal of the pollen grains either within the immediate vicinity of the plants or over longer distances (Kendrick et al., 2012; van Tussenbroek et al., 2012). Seagrass pollen dispersal has been a research subject of several studies (e.g., Ackerman, 2002; Smith and Walker, 2002; van Tussenbroek et al., 2008b; Van Tussenbroek et al., 2016).

The vegetative growth of rhizomes is a crucial mechanism through which seagrasses expand and colonize new areas. This growth pattern facilitates the colonization of previously unoccupied habitats, as new shoots and roots establish in suitable areas, creating dense meadows and enhancing their ecological impact. This growth strategy not only promotes the persistence of seagrass populations but also contributes to the stability and structural integrity of sediments, preventing erosion and providing habitat for various marine organisms (Kendrick et al., 2012, 2017).

Detachment of vegatative fragments is an important mechanism by which seagrasses can propagate and establish new populations. Once detached, these fragmented shoots become free-floating in the water column asthey are then subjected to the movement of ocean currents, which can transport them over long distances (Kendrick et al., 2012). The journey of these floating fragments can be influenced by factors such as current strength, direction, as well as the shape and buoyancy of the shoots. Also, depending on the species, fragments can travel through the water column at different rates (Hall et al., 2006). When the fragmented shoots reach suitable substrates, they have the potential to take root and establish new seagrass populations (Campbell, 2003; Di Carlo et al., 2005). Once the fragmented shoots successfully establish roots, they can grow into mature seagrass plants over time. This mechanism allows seagrasses to respond to environmental changes, such as disturbances or the availability of suitable habitats in different locations. By dispersing through fragmentation, seagrasses can maintain genetic diversity, increase their resilience, and enhance their capacity to adapt to changing conditions in coastal ecosystems.

In general, there are several circumstances that can drive the different seagrass propagules dispersal routes over time. Abiotic factors such as wind, waves, tides, or currents can lead seagrass-detached fragments to be easily transported away from the source location (Ruiz-Montoya et al., 2015). Although seagrass propagules have been reported to be transported by ocean currents, tides, wind, and sediment movement (Ruiz-Montoya et al., 2012, 2015), their consequent arrival in a suitable habitat and effective recruitment, although possible (Berković et al., 2014), is not fully understood. In contrast, biotic vectors such as seabirds, fish, sea turtles, manatees and dugongs may mediate seagrass dispersal and gene flow by transporting clonal propagules or dispersing seeds after passing through their digestive system (Sumoski and Orth, 2012; Tol et al., 2017), increasing the chances, or even enriching, their germination success (Tol et al., 2021). These biophysical interactions (between propagule features and transport mechanisms) are predicted to shape the extent of connectivity among seagrass meadows over large geographical scales and to determine population longevity.

1.3.6. Ecological importance

Seagrasses are not only key primary producers but also foundation species (i.e., species that have a strong role in structuring a community) by providing a wide variety of ecosystem services (de los Santos et al., 2020). Seagrasses can influence the ecosystem structure by forming a three-dimensional landscape structure (so called meadows). Seagrass meadows support some of the most productive and diverse coastal marine ecosystems on the planet by creating suitable conditions and habitat for associated organisms and communities such as invertebrates, fishes, or marine mammals, most of which could not persist in the absence of seagrass meadows (Green et al., 2003; Sievers et al., 2019). Seagrasses have the capacity to remove toxins, pollutants, bacteria, and nutrients from water, hence improving water quality (e.g., (Serrano et al., 2011; Lamb et al., 2017). Seagrass meadows are important global carbon sinks with a high capacity for absorbing and storing carbon in the sediment (i.e., blue carbon;

Nellemann and Corcoran, 2009), thus helping to alleviate some effects of climate change (de los Santos et al., 2020). Additionally, seagrasses offer coastal protection against erosion, floods, and storms (Duarte et al., 2013; Ondiviela et al., 2014) which is crucial in the context of climate change. Seagrass meadows also have a socio-economic importance including tourism and recreation opportunities (Plummer et al., 2013; Cullen-Unsworth et al., 2014; Wahyudin et al., 2018).

1.3.7. Climate change impacts on seagrasses

Despite the multiple benefits and important ecosystem functions of seagrasses, meadows are in decline worldwide, due to pressure from several changing environmental conditions (Waycott et al., 2009; Duarte et al., 2018). Some of these changes include sea level rise, increments in sea surface temperature, salinity changes, extreme weather events, sedimentation, changes in currents or wave dynamics, and increased levels of contaminants (Orth et al., 2006b; Zimmerman, 2021). Also, seagrasses meadow can be affected by biological factors such as eutrophication, algae blooms, diseases, or competition (Short and Wyllie-Echeverria, 1996). Moreover, seagrass loss can be associated with the decline of associated animal species such as dugongs, seahorses, or commercially targeted species (Scott et al., 2018; Sievers et al., 2019). Unfortunately, several seagrass species are declining at a rapid pace worldwide, posing a threat to their survival and endangering both the productivity and health of coastal ecosystems as well as human activities dependent on coastal resources (Kendrick et al., 2019). Nevertheless, climatic changes have been a trigger not just for the loss of seagrass species (Chefaoui et al., 2018) but also for shifting their limits of distribution (Chefaoui et al., 2021). This is especially concerning in temperate locations, where populations have already been impacted by ocean warming near the low-latitude margins of their distributions (Valle et al., 2014; Hyndes et al., 2017). These shifts may impact seagrass species by reducing meadow sizes and consequently their genetic diversity, species richness, biomass, and productivity (Zimmerman, 2021). Given the global ecological importance of seagrass species, predictions of distribution patterns in the face of climate change are essential. However, when it comes to range shift predictions, seagrasses are one of the least-studied taxa (Melo-Merino et al., 2020), and there is a clear and pressing need to assess and forecast the effects of these changes.

1.4. Focal species

In this thesis, I investigate the evolutionary history of three seagrass species, with contrasting biogeographical, oceanographic and seascape characteristics. This thesis aims to infer the patterns of genetic diversity and differentiation along their distributional ranges while also seeking to understand past range shifts and contemporary connectivity.

Halodule wrightii, Ascherson, 1868 (Cymodoceaceae) is a dioecious (i.e., separate males and females) and perennial species. *H. wrightii* has a tropical distribution with the most extensive geographic range across the Eastern and Western Tropical Atlantic Ocean (den Hartog and Kuo, 2007). It can be found in the low intertidal of coastal areas down to 30m depth (Gallegos et al., 1994; den Hartog and Kuo, 2007) and consequently tolerates a wide range of environmental conditions (Bercovich et al., 2019). Morphologically, *H. wrightii* has a root system that grows on the soil surface, between 5 cm to 9 cm depth depending on current strength or wave action. From the horizonal rhizomes, between 3 to 5 unbranched roots emerge that are whitish and end in a dark tip (Pangallo and Bell, 1988; Kuo and Hartog, 2007; van Tussenbroek and Helgueras, 2010). This species has strap-shaped leaves, and each shoot (from which they emerge) contains between 2 to 4 leaves with a length ranging from 3 to 32 cm and a width of 0.2 to 0.5 cm (see Figure 3) (Rivera-Guzmán et al., 2017). *H. wrightii* has a distinctive leaf apex that is used for morphological identification and has two small lateral projections and a small medium one in the middle. Like all seagrasses, *H. wrightii* can reproduce sexually or through clonal growth. It is known to have low dispersal capacity via seed dispersion, which is limited in common with other species of the same family (e.g., *Cymodocea nodosa*). Also, *H. wrightii* seeds can remain dormant for long periods (up to 4 years; Orth et al., 2007), allowing the species to endure after environmental disturbances (Rivera-Guzmán et al., 2017).

Phyllospadix torreyi, S.Watson, 1879 and *Phyllospadix scouleri*, Hook, 1838 (Zosteraceae) are both perennial, dioecious angiosperms. Although both species

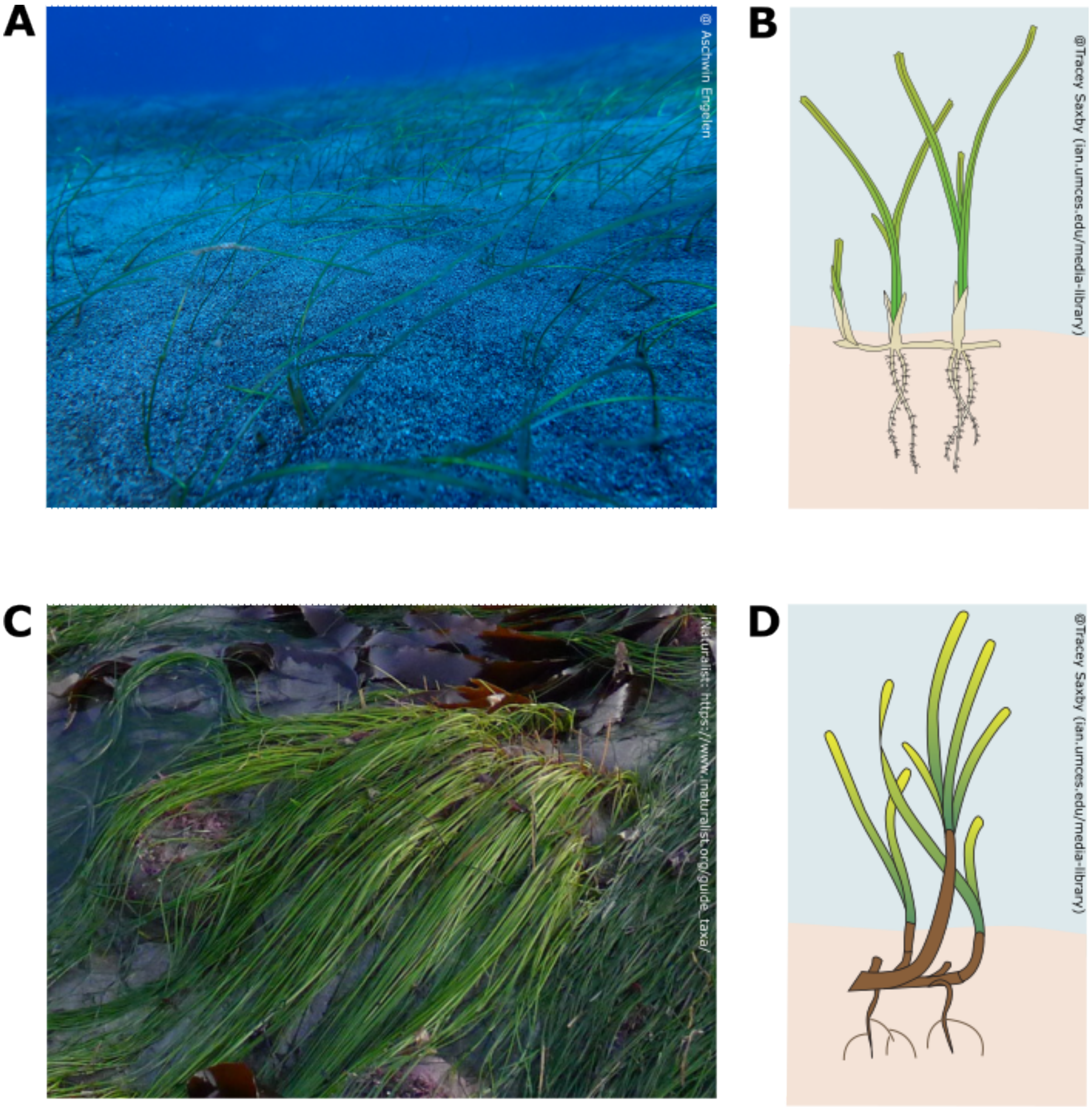

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Figure 3 – Pictures of the seagrasses and its schematic representation: **A**); **B**) *Halodule wrightii* and **C**); **D**) *Phyllospadix* spp.

are present along the Northeast Pacific coast, they have different distributional limits. *P. torreyi* can be found from Baja California to Vancouver Island, while *P. scouleri* has a broader latitudinal range, from Baja California to Sitka, Alaska (Ramirez-Garcia et al., 2002). Unlike most seagrass species that grow in soft and sandy bottoms, these species grow in the rocky intertidal with roots that grow attached to rocks (den Hartog, 1970; Hemminga and Duarte, 2000). This is possible due to rhizome nodes that are very short with many root hairs that allow them to attach to hard surfaces. Once established, these two seagrass species can trap large amounts of sediment from the water column and play a role in the stabilization of rocky substrata against waves and erosion (Gibbs, 1902). *P.*

torreyi and *P. scouleri* are morphological different since *P. scouleri* leaves are flat and wider compared with *P. torreyi* leaves that have a cylindrical shape (see Figure 3). *P. scouleri* stems are unbranched with a length of 5-40 cm and width of approximately 2 mm, whereas *P. torreyi* stems are branched, 40-120 cm long and 0.5-2 mm wide. Both species produce negatively buoyant seeds with two downward projecting lobes. These two seagrass species also show differences in bathymetric distribution, with *P. torreyi* growing deeper than *P. scouleri*, with the latter usually found in in the low intertidal zone on emergent substrate or in tidepools (Turner and Lucas, 1985). This segregation is due to the capacity of *P. scouleri* to resist air-exposure during low tides (Phillips, 1979; Ramírez-García et al., 1998).

1.5. Aims and thesis structure

Despite the ecological and economic importance of seagrasses and the extensive research on their ecology, there is still a surprising lack of knowledge about 1) connectivity of seagrass populations, 2) the distribution of intra-specific genetic diversity across seagrass ranges, and 3) the main historical and contemporary drivers that play the most important role in 1 and 2. Similar to other groups, range shifts associated to climatic oscillations, geographic settings, and modern oceanographic patterns are probable factors influencing seagrass populations. Nevertheless, seagrasses present a unique challenge due to their diverse reproductive and dispersal traits, habitat, and biogeography. Consequently, the drivers shaping their genetic structure can greatly vary and may be unique to each species. Thus, understanding the range shifts of seagrasses in response to climatic oscillations, geographic settings, and modern oceanographic patterns is vital. However, the complexity of seagrass genetic structure demands a comprehensive approach that considers various factors such as biogeographic histories, life-history traits, metapopulation dynamics, and ocean currents. To gain a more accurate understanding, it is essential to study multiple seagrass species with contrasting biological, ecological, and biogeographic contexts. By doing so, we can better capture the diversity and uniqueness of each species and the drivers shaping their genetic structure. In this line of thinking, several seagrass models that exhibit contrasting biological, ecological, and biogeographic contexts were selected, to gain a more comprehensive understanding of the factors shaping the connectivity of seagrass populations and the distribution of intra-specific genetic diversity across their ranges.

This thesis is composed by 3 peer-reviewed, scientific journal publications that have either been published or are now under review. Each refer to a specific chapter. The general goal of this thesis is to explore the population genetic history of seagrasses in biogeographical settings characterized by contrasting oceanographic and seascape characteristics. The specific aims are to investigate the structure of genetic connectivity along the distributional ranges of the target species and to assess the impacts of past and future climatic events on the distribution and genetic diversity of these species in both tropical and temperate seagrass habitats. The main goal of this thesis is to shed light on the factors that influence the genetic diversity, population structure and geographic distribution of marine foundation species. Specifically, this thesis aims to:

1) Investigate *Halodule wrightii* differentiation between distinct sides of the oceans and infer the differentiation level in species and populations in which stepping-stone connectivity paths are lacking (Chapter 2).
2) Estimate genetic connectivity of *Halodule wrightii* to assess the influence of ocean currents and biotic vectors on the observed genetic patterns (Chapter 3).
3) Investigate how temporal scale (palaeo-historical *vs* contemporary) processes shaped population differentiation between species in the genus *Phyllospadix* along the North-East Pacific coast (Chapter 4).

The key findings from this research works are covered in a general discussion in the last chapter (Chapter 5). Overall, a thorough review of the findings given here and their implications for the evolutionary history, connectivity, conservation, and management of the seagrass species under study are covered. Finally, potential directions for future research are also addressed.

CHAPTER 2

Long range gene flow beyond predictions from oceanographic transport in a tropical marine foundation species

Long range gene flow beyond predictions from oceanographic transport in a tropical marine foundation species

Tavares AI, Assis J, Larkin PD, Creed JC, Magalhães K, Horta P, Engelen A, Cardoso N, Barbosa C, Pontes S, Regalla A, Almada C, Ferreira R, Abdoul BM, Ebaye S, Bourweiss M, Van-Dúnem dos Santos C, Patrício AR, Teodósio MA, Santos R, Pearson GA & Serrao EA (2023). Long range gene flow beyond predictions from oceanographic transport in a tropical marine foundation species. *Sci Rep* 13, 9112. doi: 10.1038/s41598-023-36367-y

Chapter 2

2.1. Abstract

The transport of passively dispersed organisms across tropical margins remains poorly understood. Hypotheses of oceanographic transportation potential lack testing with large scale empirical data. To address this gap, we used the seagrass species, *Halodule wrightii*, which is unique in spanning the entire tropical Atlantic. We tested the hypothesis that genetic differentiation estimated across its large-scale biogeographic range can be predicted by simulated oceanographic transport. The alternative hypothesis posits that dispersal is independent of ocean currents, such as transport by grazers. We compared empirical genetic estimates and modelled predictions of dispersal along the distribution of *H. wrightii*. We genotyped 8 microsatellite loci on 19 populations distributed across Atlantic Africa, Gulf of Mexico, Caribbean, Brazil and developed a biophysical model with high-resolution ocean currents. Genetic data revealed low gene flow and highest differentiation between 1) the Gulf of Mexico and two other regions: 2) Caribbean-Brazil and 3) Atlantic Africa. These two were more genetically similar despite separation by an ocean. The biophysical model indicated low or no probability of passive dispersal among populations and did not match the empirical genetic data. The results support the alternative hypothesis of a role for active dispersal vectors like grazers.

2.2. Introduction

Long-range dispersal of marine organisms with passively transported propagules is often hypothesized to be mediated by ocean currents, a hypothesis that lacks much empirical testing despite its major ecological and evolutionary implications (Cowen et al., 2007; Cowen and Sponaugle, 2009). Challenges related with empirically tracking the movement and outcome of passive dispersal units have narrowed our knowledge on population connectivity across most marine biodiversity (Pineda et al., 2007). Indeed, most marine species, particularly habitat-forming species (macroalgae, corals, or seagrasses), migrate via passively-dispersed propagules (Kinlan and Gaines, 2003; McMahon et al., 2014). Thus, these species, are expected to form metapopulations that are passively connected by the transportation of such dispersal stages (Harris, 2012), but the processes mediating such transportation are poorly understood. Many such marine species, including seagrasses, have wide geographical distribution ranges, which contrasts with their predicted dispersal capacity or ability to maintain regular long-distance dispersal throughout their range (Wu et al., 2016; Tavares et al., 2022). Biological features of the dispersal phase, its interaction with a range of abiotic, historical, and biotic factors, determine species range limits and gene flow among populations (Kendrick et al., 2017), which significantly impact a species' distribution and persistence (Palumbi, 2003).

Large-scale studies focused on seagrass population connectivity have been developed (Arriesgado et al., 2015; Sinclair et al., 2018; Triest et al., 2018) but none have addressed the long-distance cross-ocean dispersal in the tropical Atlantic region (Short et al., 2007). Along this wide geographical region, seagrass populations might be isolated by biogeographic barriers such as vast oceanic distances (e.g., the thousands of kilometres that separate east and west Atlantic), lack of suitable habitat (e.g., freshwater discharge near the Amazon or Congo rivers), or main oceanographic currents (e.g., Caribbean Current and South Equatorial Current (van Dijk et al., 2018; Jahnke et al., 2019). Leading hypotheses predict that oceanographic currents determine most connectivity of passively dispersed stages, functioning as both barriers and promoters of gene flow. The tropical Atlantic region, rich in seagrass diversity (Short et al., 2007), has only local-scale assessments focused on seagrass genetic diversity, dispersal and connectivity (Larkin et al., 2017; Bijak et al., 2018; van Dijk et al., 2018). Four seagrass genera are known to dominate the tropical Atlantic - *Thalassia*, *Syringodium*, *Halophila,* and *Halodule* - which either occur as single species or intermixed (Short et al., 2016). However, only *Halodule wrightii* is distributed on both east and west tropical Atlantic coastal shores, serving as an optimal model for range-wide connectivity studies across pan-Atlantic spatial scales.

Seagrasses form one of the most productive and important ecological groups along with mangroves and corals. They are flowering marine angiosperms that can form dense meadows in shallow coastal waters around the world (Green et al., 2003; Larkum et al., 2006). Seagrasses play a strong role in structuring communities and have received much attention due to their large ecological and social significance (Nordlund et al., 2018). However, they are under increasing pressure from anthropogenic activities and climate change (Micheli et al., 2008; Waycott et al., 2009). As a result, seagrass habitats are declining globally (Waycott et al., 2009), becoming lost or fragmented, and further extinctions are forecasted under future climate change scenarios (Jordà et al., 2012).

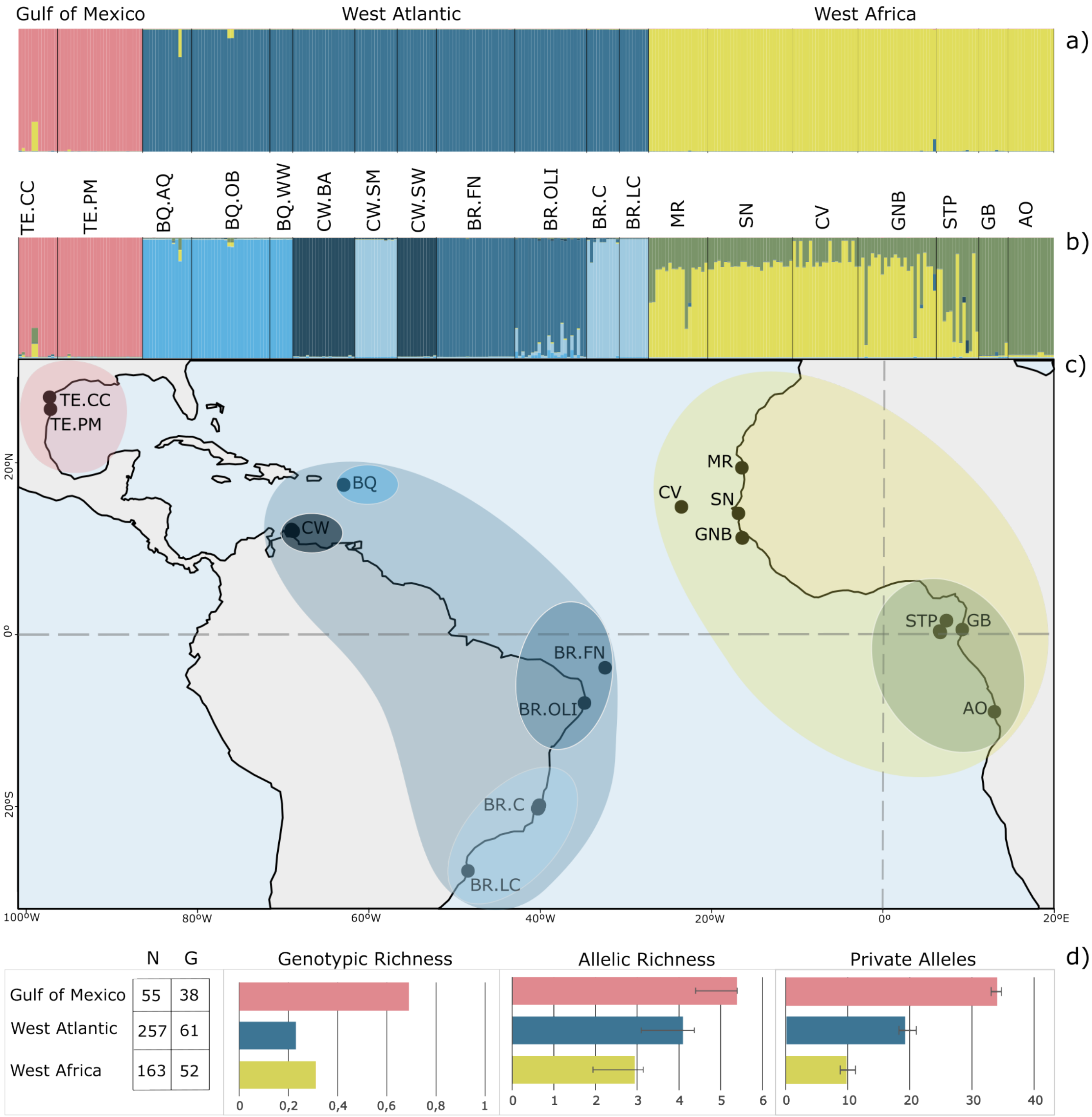
Seagrasses can reproduce sexually by seed production and asexually by vegetative clonal development (Ackerman, 2007) and each reproductive mode produces different types of propagules with diverse dispersal abilities (McMahon et al., 2014; Kendrick et al., 2017). There are several circumstances that can drive seagrass propagule dispersal routes over time. Abiotic factors such as wind, waves, tides or currents can lead seagrass-detached fragments to be easily transported away from the source location (Ruiz-Montoya et al., 2015). In contrast, seabirds, fish, sea turtles, and dugongs are examples of biotic vectors that may mediate seagrass migration and gene flow by transporting clonal propagules or dispersing seeds after passing through their digestive system (Sumoski and Orth, 2012; Tol et al., 2017), increasing the chances, or even boosting, their germination success (Schupp and Fuentes, 1995). Moreover, propagule features such as seed buoyancy can mediate dispersal scenarios (McMahon et al., 2014). These biophysical interactions (between propagule features and transport mechanisms) are predicted to shape the extent of connectivity among seagrass meadows over large geographical scales and to determine population longevity (McMahon et al., 2014; Tavares et al., 2022).

To address this knowledge gap, this study aims to compare predictions and empirical data on large scale connectivity for the seagrass species *Halodule wrightii*, the tropical species with the most extensive geographic range across the Eastern and Western Tropical Atlantic Ocean (den Hartog and Kuo, 2007). As for all in most other seagrass species, *H. wrightii* reproduces asexually and sexually, and the extent of each reproduction mode influences dispersal, genetic diversity, and biogeography. Despite its widespread distribution, the species produces seeds that are neutrally to negatively buoyant, with an apparent lack of long-distance dispersal means (Darnell et al., 2015a). Seeds of *H. wrightii* are able to form persistent seed banks that can remain dormant up to 4 years (McMillan, 1981). Therefore, seeds play an important role in the persistence of the species. *H. wrightii* can also colonize new areas through by asexual clonal growth of detached fragments that can survive in the water column for up to one month (Hall et al., 2006). Previous studies have explored the genetic structure and connectivity of *H. wrightii* (Larkin et al., 2017; Digiantonio et al., 2020; Tavares et al., 2022) but only focused on specific areas of its distribution. To date, there is not a comprehensive study that encompasses this tropical amphi-Atlantic seagrass, in contrast with other seagrass species located on a single side of this ocean, such as *Cymodocea nodosa* (Alberto et al., 2008).

Here, we aim to test the hypothesis of ocean currents as the main vectors for dispersal of *H. wrightii* in the Atlantic Ocean by comparing oceanographic predictors with genetic information. This is the first study that considers the distributional range of *H. wrightii*. The main goal of the present study was to uncover the population genetic structure and connectivity of this habitat-forming seagrass species in the tropical Atlantic region. This species is an ideal model for range-wide connectivity studies due to its vast geographical distribution. Here, we ask if: 1) there is genetic structure among *H. wrightii* populations across distant biogeographic regions along the tropical Atlantic; 2) levels of genetic and genotypic diversity are similar among populations; 3) the main ocean currents explain the present genetic structure; or 4) if the latter could be explained by biotic transportation. These questions and hypotheses were approached by using microsatellite markers and biophysical modelling based on oceanographic transport to model connectivity along the vast *H. wrightii* distributional range.

2.3. Results

No evidence of null alleles nor linkage disequilibrium was found. For the *H. wrightii* ramet dataset, we obtained a total of 475 sampling units (ramets) successfully genotyped for 8 microsatellite loci across 19 populations. For the genet-level dataset, after the removal of repeated copies of each genet, 176 individual genotypes remained for analysis. Measures of genetic diversity such as allelic richness (i.e., the mean number of alleles per locus within a population)



	N	G
Gulf of Mexico	55	38
West Atlantic	257	61
West Africa	163	52

Figure 1 – **a, b**): Assignment of individuals to genetic groups that minimize Hardy-Weinberg and linkage disequilibria, estimated by STRUCTURE; colors depict the genetic subdivision based on K=3 and K=7 levels of subdivisions; Site names and coordinates are listed in Table S1 and S4; **c**): Sampling sites of *Halodule wriightii* with colors depicting the main highest genetic differentiation inferred with STRUCTURE (K = 3 groups); **d)** Sample size (N); number of unique genotypes (G); genotypic richness, standardized allelic richness and standardized number of private alleles for the smallest common sample size. The figure was generated using R (version 4.2.2) and Inkscape 1.2.1 (https://inkscape.org/).

and gene diversity, or expected heterozygosity (i.e., the probability that two randomly selected alleles at a particular locus will be different in a population) were generally low for ramet and genet level datasets within all populations (Figure 1D; Table S1 and S2), except for both populations in the Gulf of Mexico. Likewise, private alleles ranged from high values in the same region to nearly no private diversity in several sites of the Caribbean region but also in West Africa (see Figure 1D; Tables S1, S2). All the genetic diversity parameters when analyzed for the first hierarchical level of genetic structure (k=3) revealed high estimates higher in Gulf of Mexico than the other clusters (Table S4).

Most of the inbreeding coefficients (F_{IS}) estimates were significantly negative for both datasets (Table S1, S2 and S3), showing heterozygotes to be more prevalent than expected under Hardy-Weinberg equilibrium. The opposite scenario was observed for Gulf of Mexico, where heterozygote deficiency was prevalent (Table S1 and S2). Also, F_{IS} values across the different locations in both datasets exhibit a wide range of negative values dispersion (Figure S2). Genotypic richness (R) was highly variable among populations on both sides of the Atlantic, ranging across both extremes (Tables S1 and S2), from close to maximal contribution of sexual propagation (in the east Atlantic 88% of sampled shoots were distinct genets in Santana and Gabon, and in the west 93% were distinct in the Gulf of Mexico) to predominance of a single clone (only 8% were distinct genets in Angola in the east and in Curaçao in the west).

Pairwise differentiation (FST) between groups showed higher differentiation between West Africa against the other groups but differentiation was lower among West Africa populations (Figure S3).

The factorial correspondence analysis (FCA) revealed a first major genetic differentiation between the Gulf of Mexico and the remaining sites, and these latter also split into east and west, resulting in three genetic clusters: Gulf of Mexico, east Atlantic (West Africa), remaining west Atlantic (Brazil and Caribbean) (Figure 2). The STRUCTURE analysis, along with DAPC and PCA indicated the same three genetic clusters and at a more fine-scale differentiation these were subdivided into seven clusters (Figure 1A and S4). These revealed a differentiation between southern and northern populations in Africa, admixed in Sao Tome and Principe, and a differentiation into four clusters in the Caribbean-Brazil region, admixed in Curaçao (Figures 1B, 1C). Also, STRUCTURE plot for K=7, revealed more similarities between one of the Curacau populations (CW.SM) and the ones on south Brazil (Figure 1).

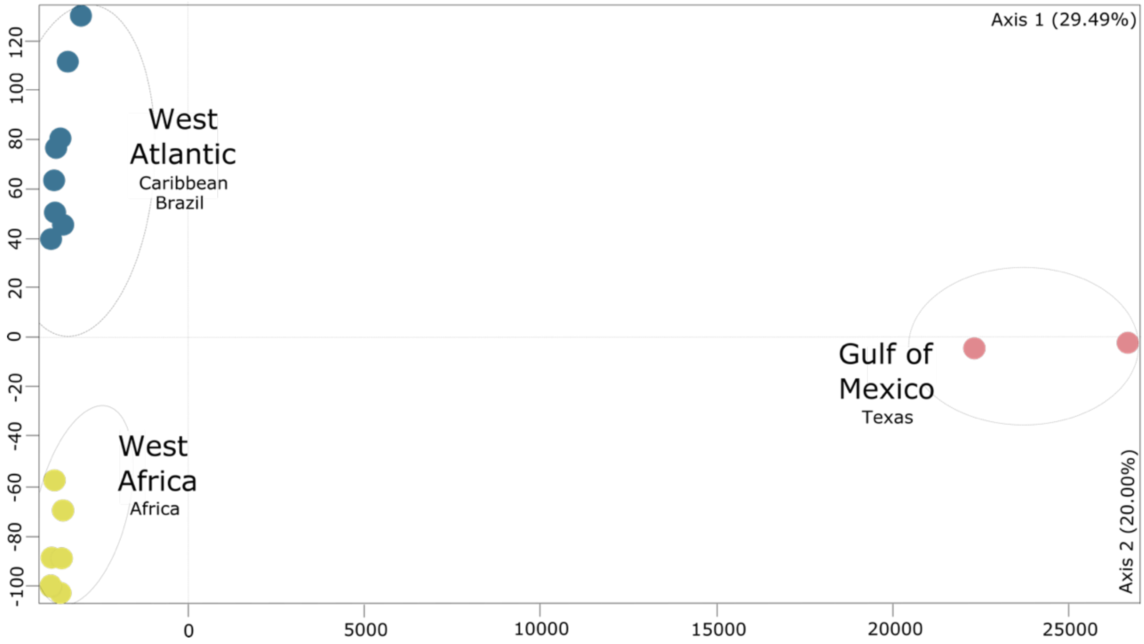


Figure 2 – Genetic differentiation of populations of *Halodule wrightii* illustrated by factorial correspondence analysis (FCA). Distances in the plot are proportional to genetic divergence, illustrating that the divergence between the Gulf of Mexico and the other populations is much higher than that among any other populations.

The data compiled from literature (Table S5, supplemental material) and biodiversity databases, yielded 1815 known locations for the species. This resulted in 183 distinct source/sink sites aggregated at 1 km, which were included in the biophysical modeling. The particle simulations delivered 668,499 particles over a 10-year period. These revealed a sharp decline in potential connectivity with distance (Figure S1), with high probabilities for propagule retention near the source locations (Figure S1). Most connectivity events were predicted to take place only at regional scales (average distance of connectivity events: 330.42±450.11 km; maximum: 3766.80 km), with low probabilities of connectivity (average probability of connectivity: 0.10±0.19) due to few pair-wise site connectivity events (average number of events: 36.67±70.65) (see Supplementary Information). The models predicted large scale oceanic transportation from Fernando Noronha (offshore Brazil) to Barbados in the Caribbean Sea (Figure 3). Geographic distant regions had no probability of connectivity mediated by ocean currents (i.e., no connectivity events occurred at all from / to these sites in the 10 years of simulations), indicating zero probability

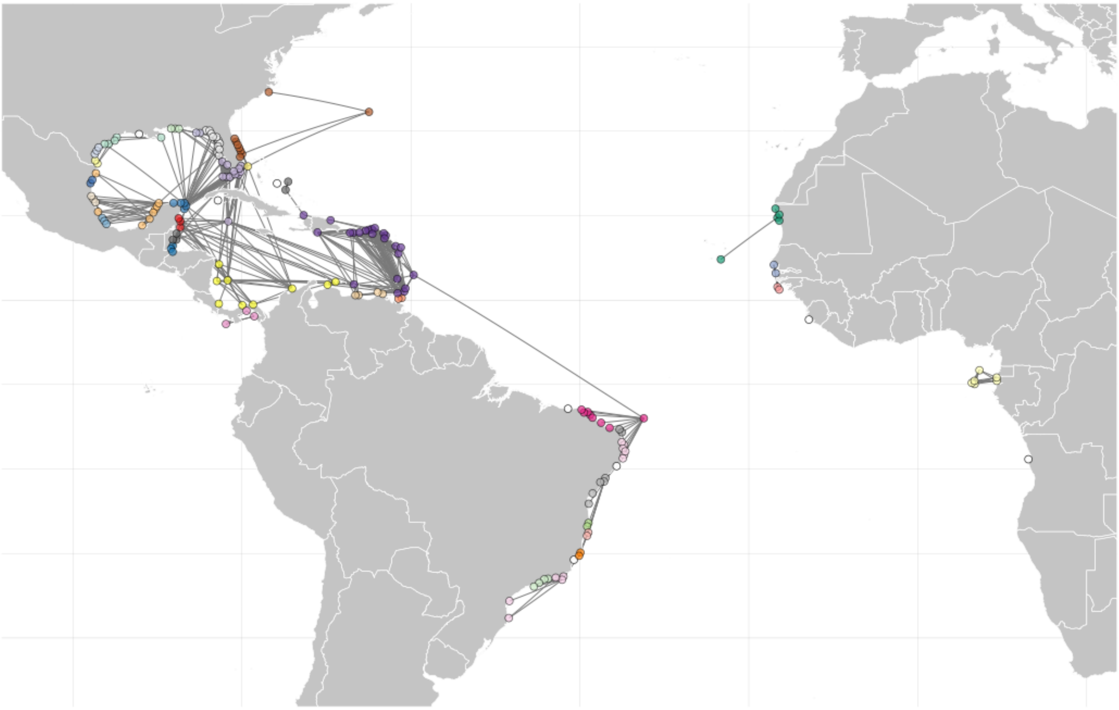


Figure 3 – Potential connectivity among *Halodule wrightii* populations that have been reported in literature and databases (see Table S5), estimated from simulations of transport by ocean currents data. The figure was generated using R (version 4.2.2).

that currents could be predominantly responsible for the dispersal and genetic differentiation of *H. wrightii* across such spatial and temporal scales.

2.4. Discussion

The genetic structure of the seagrass *H. wrightii* over its distributional range revealed three main genetic groups separated geographically at different spatial scales (from a broad to a regional scale), that did not completely match predictions from oceanographic transport of propagules (seeds or shoots) by currents. These results showed that although ocean circulation is an important factor in marine population structure (Cowen et al., 2007), long-distance dispersal is likely a very rare event. The results support the hypothesis that rare events of occasional biotic transportation might have been responsible for connectivity between *H. wrightii* populations that belong to distinct genetic clusters.

2.4.1. Genetic structure and connectivity

2.4.1.1. Large scale connectivity

The significant genetic differentiation between West Atlantic, Gulf of Mexico, and West Africa, indicates restrictions to gene flow between these three main genetic lineages of *H. wrightii*. This was also supported by the biophysical modelling that revealed a null or very low probability of connectivity mediated by ocean currents between distant geographic regions (e.g., between east and west Atlantic coastlines) and suggested that most of the gene flow mediated by currents occurs at a regional scale (up to ~1230 km, Figure S1). Such skewed relationship between oceanographic connectivity and distance has been previously reported from biophysical model predictions, regardless of the dispersal potential of the species of interested. Additional studies using biophysical modelling to address the role of oceanographic connectivity to the distribution of, e.g., mangrove forests (high dispersal) and macroalgae (reduced dispersal) also showed null or very low predicted probability of connectivity across large water masses (Assis et al., 2018b, 2022; Gouvêa et al., 2023). Besides the null probability of transport by currents, distance and habitat discontinuity are likely to cause isolation between east and west Atlantic shores, as well as other physical processes, such as waves and direct forcing by winds, that are not accounted for in the HYCOM model. Predictions of long-distance connectivity patterns through ocean currents benefit from knowledge of key life history traits such as seed or shoot viability times and establishment success (McMahon et al., 2014). Some of these details are unknown for *H. wrightii*, although there is information on such life traits for other seagrass species (Harwell and Orth, 2002; van Tussenbroek et al., 2007), few of which facilitate oceanic rafting such as positively buoyant seeds or fruits (van Dijk et al., 2009; Ruiz-Montoya et al., 2015). However, *H. wrightii* has low seed dispersal potential because its seeds develop near the sediment at the base of the shoots and are negatively buoyant. Detached fragments may therefore be more likely to raft with ocean currents and they can survive floating in the water column up to four weeks (Hall et al., 2006), although the subsequent establishment success has not been determined. Yet the ability of seeds to remain in a dormancy stage for at least up to 46 months (McMillan, 1981) supports the possibility that they might be remain viable during occasional long-range dispersal, by either abiotic or biotic vectors.

Abiotic transport by ocean currents may not require the capacity to float if seeds become entangled on other floating rafts, such as those formed by *Sargassum*, that can cross the Atlantic Ocean in less than two weeks (Renner, 2004), a timeframe shorter than the viability of *H. wrightii* seeds and detached shoots. Rafting *Sargassum* have been recorded to move up the North Brazil current to the Caribbean and eastward towards western Africa (Putman et al., 2018; Wang et al., 2019) and seagrass fragments have been found among *Sargassum* rafts (Witherington et al., 2012; Salter et al., 2020). Moreover, migratory species like sea turtles (Witherington et al., 2012) and seabirds (Moser and Lee, 2012) use *Sargassum* mats and may facilitate dispersal. Alternative or complementary to ocean currents, seagrass propagules may also be transported to suitable habitat by grazers that use these mats as foraging sites.

Despite its low abiotic dispersal potential, *H. wrightii* has a wide distribution with variable genetic differentiation, and among all tropical seagrasses of the western Atlantic it is the only species that has colonized the eastern side of this ocean, which suggests long-distance dispersal capabilities. This apparent paradox supports the hypothesis of an important role for biotic transportation of its seeds by grazers, as proposed in other studies (Tavares et al., 2022). Megaherbivores, including dugongs, green turtles, some birds and fish, use seagrass as one of their food sources, and promote dispersal of seagrass seeds (Heck and Valentine, 2006). Successful seagrass endozoochory (i.e., seed dispersal by animals after passage through their guts) has been reported for fish, sea turtles and birds (Figuerola et al., 2002; Sumoski and Orth, 2012; Wu et al., 2016; Tol et al., 2021). The dispersal distances of such animals known to feed among seagrass meadows would allow long-ranging dispersal (e.g., 277 to 652 km in green turtles or 173 to 234 km by dugong (Tol et al., 2017). The reported digestion times for such herbivores, 6 – 7 days for dugongs (Lanyon and Marsh, 1995; Tol et al., 2017) and 1-2 weeks for green turtles(Brand et al., 1999), are compatible with a moderate scale of long-range transport. Satellite tracking of green turtles in Atlantic Africa shows travel of 40 to >1000 km between *H. wrightii* sites (Patrício et al., 2022) and genetic data indicate cross Atlantic green turtle migration (Patrício et al., 2017). Still, direct cross-Atlantic biotic dispersal seems unlikely or at least uncommon within average digestion time scales of grazers.

Migratory routes of green turtles that feed on *H. wrightii* (Hernández and van Tussenbroek, 2014) include several mid-Atlantic islands that are nesting grounds (Patrício et al., 2017), and could serve as occasional stepping-stones, although seagrass presence is mainly unknown in such islands (Tsiamis et al., 2017). It is relevant that a green turtle found dead in Senegal had been nesting in Trindade Island 5 months before (Marcovaldi et al., 2000). All this information and our data indicate that transoceanic dispersal must be a very rare event, because the distances may be too vast to allow continuous gene flow even by biotic vectors, and there is a remote hypothesis that stepping stone islands could facilitate occasional dispersal of viable seeds. However, even if propagule dispersal is successful, post-settlement survival may be low, dependent on ending dispersal in a favorable environment (Hall et al., 2006).

2.4.1.2. Fine scale connectivity

On the western Atlantic coastline, the biophysical model revealed high probability of oceanographic connectivity between populations, but the genetic differentiation into distinct clusters showed that gene flow is restricted among them. Genetic data revealed isolation between Gulf of Mexico, Caribbean, and Brazil despite the high probability of transport by oceanographic currents, particularly between the Caribbean and Gulf of Mexico. However, no apparent gene flow barrier exists between the Caribbean and the Gulf of Mexico, in contrast with the genetic structure shown in our findings and in previous seagrass studies using microsatellites (van Dijk et al., 2009, 2018). This is suggesting that this genetic differentiation might be due to recolonization events that occurred in the Caribbean and Gulf of Mexico after the last glacial maximum (Avise, 1992).

The low probability of connectivity between the Caribbean and Brazil coincides with a biogeographical barrier created by the discharge from the deltas of the Amazon and Orinoco rivers, among others (Briggs, 1995). This barrier, which runs for around 2,300 kilometers, separates the Brazilian coast from the Caribbean region and affects the genetic structure and dispersal of different marine animal organisms as fish or coral species (Volk et al., 2021; Giachini Tosetto et al., 2022). Yet, this barrier is not matched in our genetic data, that suggests gene flow among these regions. Thus, it is plausible to hypothesize that biotic dispersal might also play a role in increasing *H. wrightii* dispersal potential between these regions. Sea turtles and manatees are known to feed on *H. wrightii* meadows in these regions (de Meirelles et al., 2018), and for some species, these areas are part of their migratory pathways (Chambault et al., 2018). Moreover, our data also suggests a closer level of similarity between population of these two regions (k=7 structure plot; Figure 1). However, this result must be interpreted with caution as it might be attributed to homoplasy (i.e., individuals with different ancestries that mutate at a locus to the same allele). Homoplasy due to mutation is expected to occur for microsatellite markers due to their allele size and high mutation rates. Therefore, it is important to consider the potential for homoplasy when using microsatellites to infer relationships among individuals or populations.

Despite the restricted ocean-driven dispersal potential of *H. wrightii*, population genetic differentiation was non-significant across most populations in West Africa, indicating significant inter-population connectivity. The genetic structure of *H. wrightii* in Africa and in general, the genetic evidence indicating long-distance migration where it is not predicted, and high differentiation in some places at short distances, support the hypothesis of animal-mediated transportation (Tavares et al., 2022).

2.4.2. Genetic diversity

Genetic diversity contains the footprints of population stability, mating, and dispersal ecology, and is therefore used here as a proxy of the historical ecology of the populations. The higher genetic diversity of *H. wrightii* in the Gulf of Mexico supports previous reports (Larkin et al., 2017; Digiantonio et al., 2020) suggesting the Gulf of Mexico should be a genetic hotspot for seagrass conservation. This suggests long-term stability without major bottlenecks.

Additionally, genetic diversity decreased in range-edge populations in both the east and west Atlantic (Angola and south Brazil, respectively), as reported for other seagrass, mangrove, and coral species. Low diversity at range edges is common (Arnaud-Haond et al., 2006; Arriesgado et al., 2015) and might be influenced by less suitable habitat increasing the reliance on clonal propagation, population bottlenecks or a relatively recent founder event during colonization of these regions from a larger more central population, resulting in genetic variation loss.

In comparison to other tropical seagrass species, the general microsatellite genetic diversity of *H. wrightii* was lower than *Enhalus acoroides* (Nguyen and Jutta, 2019), *Thalassia testudinum* (van Dijk et al., 2018) and *Zostera japonica* (Jiang et al., 2018), but equivalent to *Halophila beccarii* (Phan et al., 2017), *Cymodocea serrulata* (Arriesgado et al., 2015), *Syringodium filiforme* (Bijak et al., 2018) and *Zostera marina* (Tanaka et al., 2011). Across the *H. wrightii* populations, negative inbreeding coefficients (F_{IS}) were obtained, which is common in seagrass species (Larkin et al., 2017; van Dijk et al., 2018; Yu et al., 2018) and supports the hypothesis of selection favoring heterozygous individuals.

2.4.3. Genotypic richness

Although*, H. wrightii* has been able to achieve such a vast distribution, its dispersal success alone cannot ensure establishment, because that is also influenced by survival, viability, and growth capacity of plants originated from seeds and fragments. The balance between sexual reproduction and clonal growth can be represented by the genotypic richness. Despite the small sample sizes in some populations, our findings revealed that genotypic richness of *H. wrightii* is very variable among locations. Higher genotypic richness values were found in populations located at the center of the distribution. Higher genotypic diversity (i.e., a population with a larger number of distinct genotypes or clones) may enhance productivity and community recovery (Reusch et al., 2005; Crutsinger et al., 2006). Seagrass genotypic diversity can range from near monoclonal (Alberto et al., 2008) to very high (Van Dijk and van Tussenbroek, 2010).

Reduced genotypic richness can be a common scenario for coastal populations found at the species' range edge (Alberto et al., 2008; Bricker et al., 2018). Indeed, clonal propagation was the dominant reproductive mode prevalent in southern marginal populations across east and west Atlantic, showing edge-of-range reduced sexual reproduction relative to populations at center of the species' range (geographic parthenogenesis) (Eckert et al., 2008; Tilquin and

Kokko, 2016). Such a pattern could be attributed to limited population sizes, geographical isolation, climate oscillations, and poor seed dispersal from other source populations. However, the northern marginal population in West Africa (Banc d'Arguin, Mauritania), revealed a much higher genotypic richness compared to other marginal populations. This may be due to the Banc d'Arguin's habitat suitability for seagrass species, as opposed to what would be predicted for a marginal range. The Banc d'Arguin is huge, protected, shallow, and nutrient-rich, with inputs of desert dust from the east and coastal upwelling from the northwest (Araujo and Campredon, 2016). Furthermore, this tropical seagrass species is projected to expand there with climate change (Chefaoui et al., 2021).

Our findings showed that *H. wrightii* relies primarily on clonal propagation throughout the Caribbean region. Seagrasses reproduce both sexually and asexually, and their relative proportions may depend on several factors, including the level of disturbance (Becheler et al., 2010). Specifically, events that destroy seagrass meadows create opportunities for rapid colonization and growth by new shoots or clonal fragments, faster than through seed production (Michot et al., 2002; Byron and Heck, 2006). There are reports of fast establishment of *H. wrightii* after a hurricane (e.g., hurricane Wilma in 2005; van Tussenbroek et al., 2008). Thus, we hypothesize that the low genotypic richness found among Caribbean populations of *H. wrightii* could be due to past disturbances followed by fast colonization (Gallegos et al., 1994) through asexual reproduction of previously established or selected genotypes.

Overall, *H. wrightii* showed a low mean of genotypic richness (R=0.38), lower when compared to the mean genotypic richness found for other Atlantic seagrass species such as *Zostera noltii* (R=0.85; Elso et al., 2017), *Zostera marina* (R=0.61; Alotaibi et al., 2019) or *Thalassia testudinum* (R = 0.55; Van Dijk and van Tussenbroek, 2010), but comparable to *Syringodium filiforme* (R=0.37; Bijak et al., 2018). However, seagrass populations can show a wide range of genotypic richness, from high rates of sexual reproduction (Campanella et al., 2015), to high levels of clonality (Van Dijk and van Tussenbroek, 2010) such as in *Z. noltei* (Jahnke et al., 2016), and also *H. wrightii* (Larkin et al., 2017; Digiantonio et al., 2020).

2.4.4. Concluding Remarks

We found discordance between genetic differentiation and predicted ocean connectivity patterns, suggesting a role for other means of dispersal, such as the hypothesis of grazers mediating transport. This study underlines the importance of taking multi-pronged approaches to understand meta-population dynamics and connectivity. Using genetic data and modelling predictions enabled us to study the dispersal and connectivity patterns at different spatial scales while testing hypotheses of ocean currents mediating connectivity.

Genetic differentiation requires time for evolutionary processes to accumulate population differences. Therefore, although differentiation requires the lack of homogenizing gene flow, the absence of differentiation can be caused by either gene flow or insufficient time for differentiation among recently isolated populations, also designated as shared ancestral polymorphism. We used genetic methods as an empirical indicator of gene flow patterns, and biophysical modeling through propagule dispersion simulations to complement our findings. All these methods, like any other ways of assessing connectivity, have inherent drawbacks that should be highlighted. Genetic differentiation is affected by historical factors, such as founder events, bottlenecks, and variable population sizes. While this offers a significant advantage in discussing such processes, it also poses a limitation in addressing dispersal as the single cause of the patterns, given the simultaneous influence of these other processes. Additionally, the possibility of remaining ancestral polymorphism could create the false impression of contemporary gene flow even where populations are presently isolated. These complex demographic processes like population bottlenecks, founder events, or changes in population size over time can affect the genetic structure of populations in addition to gene flow. In our data interpretation, we therefore considered that there are other ecological factors besides ocean currents, such as habitat continuity and biological dispersal features, that may have an impact on gene flow, raising the hypotheses of influences of large flow of major rivers in breaking habitat continuity, and grazer-mediated transport.

Our results also shed light on the population genetic variability of an important tropical seagrass across its entire range. This new information for *H.*

wrightii can be used to highlight areas where local protection is necessary or where populations could be managed in a metapopulation strategy. It could also be used as a baseline for future comparisons of the genetic diversity and differentiation of this species, motivating further research. The study also comprises a valuable and unique species distribution baseline confirmed by genetic tools, which may be particularly useful in future studies for example of cases where the validity of putative sister taxa to *H. wrightii* may be unclear.

2.5. Methods

2.5.1. Study area and sampling

The present study encompasses the distributional range of *H. wrightii* along the east and west Atlantic coastlines (Figure 1C), which ranges from Mauritania (19.5° N, 16.5° W; Africa) to Angola (9.0° S, 13.0° E; Africa) and from Gulf of Mexico (27.7° N, 97.3° W; USA), including the Caribbean Sea, to Brazil (27.6° S, 48.4° W; Brazil). A variable number of shoots was collected with local permissions at 32 localities (Table S1) according with each country guidelines. Samples collected before 2018 were taken mostly from herbarium collections, resulting in distinct sample sizes. Following 2018, approximately 20 shoots were sampled in shallow and subtidal areas (up to 5 m depth depending on water turbidity) by keeping a minimum distance of approximately 1 m between each sampling unit. At some locations, only small patches were found, resulting in a lower sample size (Table S1). After field collection, all individual plants were dehydrated on silica gel drying crystals. Specimens were identified first by the local teams and later by EAS, voucher samples of the dried plants or their DNA were deposited at the herbarium of the University of Algarve.

2.5.2. DNA extraction and genotyping

Genomic DNA was extracted using the NucleoSpin Plant II Kit (Macherey-Nagel, Duren, Germany) following the protocol from the supplier. Individuals were genotyped for eight microsatellite markers (Table S6) developed for *H. wrightii* (Larkin et al., 2012, 2017). Polymerase chain reactions (PCRs) were performed in a total volume of 15 µl, containing 1x Colorless GoTaq" Flexi Buffer (Promega, Madison, WI, USA), 2 mM MgCl2 (2.5 mM), 10 mM forward and reverse primers, 0.2 mM of dNTP's, 1U GoTaq G2 Flexi DNA Polymerase (Promega, USA) and 5 mL of diluted template DNA (several dilutions for different populations). PCR conditions included an initial denaturation step at 95ºC for 5 min, followed by 30 cycles of 95ºC for 30 s, T_a for 30s, extension at 72°C for 30 s and a final extension at 72°C for 10 min. Amplified fragments were separated on an ABI3130 XL automated DNA sequencer (Applied Biosystems, Waltham, MA, USA, at the CCMAR sequencing facility), with 0.25 mL GeneScanTM 500" LIZ Size Standard (Applied Biosystems, UK) plus 9.75 mL of Hi-Di formamide, after denaturation at 95°C for 5 min. Alleles were manually scored using STRAND (Veterinary Genetics Laboratory, University of California, Davis; http://www.vgl.ucdavis.edu/STRand), and binned using the R package 'MsatAllele' (Alberto, 2009). To minimize all ambiguities, a manual review of microsatellite amplification and scoring was conducted, and the final genotyping alleles for each sample were obtained after a double-check reading process to reduce scoring errors.

2.5.3. DNA statistical analyses

The software Micro-Checker 2.2.3 (van Oosterhout et al., 2004) was used to check for null alleles to avoid bias in estimating genetic parameters. The package 'RClone' (Bailleul et al., 2016) of R software v.3.6.2 (R Development Core Team, 2021) was used to assess clonality for all individuals, because seagrass can proliferate vegetatively, and the number of shoots can represent duplicate genotypes from the same clone. To verify if individuals with the same number of multi-locus genotype (MLG) were true clones, P_{sex} was calculated, i.e., the probability of finding identical MLGs resulting from distinct sexual reproductive events (Arnaud-Haond et al., 2007). When P_{sex} threshold was below 0.01, the identical MLGs were considered the same clone (i.e., genet). Following previous theoretical assumptions (Stoeckel et al., 2021) we estivated population genetic metrics with our dataset spited into 1) a ramet level dataset, that includes genetic information for all genotyped individuals, and 2) a genet level dataset, where only the genets were kept (after P_{sex} estimates). For the second set of data (genet-level, only unique genets per population were considered for genetic diversity analysis. The proportion of genets (G) found among the individuals sampled (N) was used to estimate genotypic diversity in each population, using the clonal richness (*R*) index, $(R = (G - 1)/(N - 1)$), ranging from 0 (one single clone) to 1 (when all sampling units analyzed were from different genets).

Microsatellite genetic diversity was quantified for each population by estimating allele frequencies, mean standardized allelic richness (Â) adjusted for the minimum genet sample size, standardized number of private alleles (PÂ), Nei's gene diversity (H_E), observed heterozygosity (H_O) and inbreeding coefficients (F_{IS}), using GENETIX 4.05 (Belkhir et al., 1996). We have interpreted our results in the context of previous theoretical studies, with a particular focus on the distribution of F_{IS} values among loci and populations for both datasets. By comparing both, we aim to assess whether the clonal rates in populations result in differences in F_{IS} values (Stoeckel and Masson, 2014; Reichel et al., 2016). Also, by examining the distribution of F_{IS} values per locus per site, it may be possible to understand the extent to which asexual and sexual reproduction may be involved at a particular location. A factorial correspondence analysis (FCA) was performed to determine the genetic relationship between populations using GENETIX 4.05. To estimate the distribution of genets among genetic groups that minimize Hardy-Weinberg and linkage disequilibrium, a Bayesian assignment of genotypes to K groups was made using the program STRUCTURE (Falush et al., 2003). The value K was estimated from the mean log-likelihood for each *K* value and the Δ*K* statistic (Earl, 2012) to identify the optimal number of populations groups. This was analyzed for *K* ranging from two to twenty-three with ten replicates per value and a 50,000 burn-in followed by 500,000 MCMC replicates per iteration. All runs were performed in parallel on multiple cores using the R package 'ParallelStructure' (Besnier and Glover, 2013). A discriminant analysis of principal components (DAPC) and a Principal component analysis (PCA) implemented in the R package adegenet 2.1.10 (Jombart et al., 2018) were used to complement structure analysis. Genetic differentiation was estimated between sites (FST).

2.5.4. Biophysical modelling

Biophysical modeling based on simulations of propagule dispersion coupled with network analysis was used to estimate the connectivity potential of *H.*

wrightii. The simulations used daily data of ocean currents assembled from the Hybrid Coordinate Ocean Model (HYCOM), a hindcast of high-resolution three-dimensional ocean velocity fields (regular 1/12-degree horizontal grid with 40 depth layers). This model can resolve key oceanographic processes such as oceanic fronts, eddies, meandering currents, and filaments because it integrates the effect of precipitation, wind stress, wind speed and heat. When hindcast data on ocean current direction and intensity is combined with biological features of pelagic viable time in biophysical modelling, the component of connectivity that is exclusively mediated by ocean currents can be predicted. This modelling approach was previously validated using demographic and genetic data for macroalgae, seagrasses, limpets, mussels, fish, echinoderms, and crustaceans (Assis et al., 2015; Buonomo et al., 2017; Nicastro et al., 2020).

Individual virtual particles were released on a daily basis (matching HYCOM temporal resolution) from source / sink sites, where the species is known to occur, spaced 1 km apart (spatial resolution of the simulation) over the course of a year. These particles simulate *H. wrightii* rafts strictly transported on the ocean surface, and as in other studies at large spatial scales (Assis et al., 2015; Buonomo et al., 2017; Nicastro et al., 2020) no shape or density of rafting fragments is considered. Occurrence records describing the species distribution were compiled from the literature and biodiversity information facilities (OBIS and GBIF). The simulation encompassed locations distributed along the east and west Atlantic coastal shores. On the east Atlantic, the simulation included the west coast of Africa from Angola to Mauritania (~6250 km of coastlines from -10.0° to 21.5° latitude) and on the west Atlantic, from Florida to south Brazil (~ 13000 km of coastlines from -30° to 28° latitude). A high-resolution polygon was used to define landmasses (Haklay and Weber, 2008). Every hour of simulation, the model determined the position of all drifting rafts while fitting a bilinear interpolation estimate over the velocity fields to smooth ongoing trajectories. Rafts were permitted to float for up to 60 days (extreme propagule duration estimate). Rafts that arrived at a coastline or got lost in the open ocean (outside of the model domain) were excluded from the simulation. Trajectories were aggregated to build asymmetric matrices of pairwise probability of connectivity between source / sink sites, by dividing the number of virtual rafts released from site *i* that reached site *j*, by the total number of rafts released from site *i*. For the ten-year period 2008-2017, interannual variability was analysed by running individual simulations per year. The overall approach of the biophysical modelling is documented in (Assis et al., 2021) and the source code is openly available at: http://github.com/jorgeassis/biophysicalModelling.

Graph theory was used to generate networks that allow the visualization of connectivity patterns. The graph nodes (individual source / sink locations) and the strength of edges (probability of connectivity) were structured using a connectivity matrix based on a 10-year average of individual simulations. Stepping-stone probability estimates were determined using the graph, by using a product function over the probabilities of connectivity along the shortest paths between all pairs of sites, as found with Floyd–Warshall's algorithm, which minimises the sum of log-transformed probabilities. This approach aimed to capture all multigenerational potential connectivity across the study region, beyond the time frame considered in the biophysical simulations (Assis et al., 2015; Buonomo et al., 2017; Nicastro et al., 2020; Ntuli et al., 2020). Graph analyses were performed in R (R Development Core Team, 2021), using the package igraph.

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CHAPTER 3

Seagrass Connectivity on the West Coast of Africa Supports the Hypothesis of Grazer- Mediated Seed Dispersal

Chapter 3

Seagrass Connectivity on the West Coast of Africa Supports the Hypothesis of Grazer- Mediated Seed Dispersal

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3.1. Abstract

Population connectivity influences the distribution of genetic diversity and divergence along a species range, as the likelihood of extinction or differentiation increases in isolated populations. However, there is still poor understanding of the processes mediating inter- population dispersal in marine species that are sessile and lack planktonic life stages. One such case is the seagrass species *Halodule wrightii*, which produces basal seeds, although detached plants can drift. Along the tropical western coast of Africa, this species occurs in distant discontinuous habitats, raising the question of how interpopulation dispersal is mediated. The species is a key source of ecosystem functions including feeding large migratory grazers. This study aims to infer whether genetic differentiation of the seagrass *H. wrightii* along the western coast of Africa supports a hypothesis of predominant transportation of rafting seagrass by ocean currents, versus the hypothesis of biotic vectors of dispersal. Additional hypotheses were addressed concerning range-wide clonality and genetic diversity, assessed with microsatellite markers on populations of the west coast of Africa from Mauritania to Angola. Population genetic diversity and structure were compared with predictions from biophysical models of dispersal by oceanographic currents. The genetic data revealed low divergence among most populations, in strong contrast with predictions of very low probability of connectivity mediated by currents along the western African coastline. Moderate to high genotypic diversity showed important seed recruitment, but genetic and genotypic diversities were lower at range edges. Populations north and south of the equator were differentiated, and remarkably, so were neighboring equatorial populations despite their proximity. These results reveal independent sources of colonization of meadows in these islands, which are major habitat for migratory grazing green turtles, also supporting the hypothesis of biotically mediated seed transport. The importance of seagrass for conservation of endangered macrofauna has been widely reported; here we report evidence supporting the reciprocal role, that macrofauna protection can also plays a role in long-term survival and reproductive success of seagrass.

3.2. Introduction

Connectivity among populations plays a fundamental role in their ecology and evolution, mediating population dynamics and gene flow in metapopulation systems, and consequently the resilience of populations to environmental changes (Cowen et al., 2006, 2007; Olds et al., 2016). Low connectivity causes smaller population sizes, increasing the likelihood of local extinctions but also of genetic differentiation and even speciation. In the oceans, the natural connectivity potential of marine populations at evolutionary time scales is often mediated by the oceanic transport of planktonic stages such as propagules, larvae, juveniles and fragments, but also by the active movement of adults (Palumbi, 2003; Cowen and Sponaugle, 2009). However, rafting and anthropogenic activities may also influence the dispersal of organisms over long distances (Thiel and Haye, 2006; Crook et al., 2015). Such dispersal processes are a major determinant of the ecological and evolutionary history of marine species. The marine environment appears to lack obvious obstacles for active or passive migration, suggesting that long distance dispersal is possible for many marine species. Yet, marine gene flow can be restricted by currents, thermal barriers, geographic features, and distance/time relative to the viability of dispersive stages (Goldberg and Lande, 2007). As a result, many species may exhibit genetic variability among populations at several spatial scales, revealing patterns and scales of dispersal that influence population dynamics and distributions (Kinlan and Gaines, 2003; Iacchei et al., 2013).

Dispersal distances in the marine environment may vary by orders of magnitude among or within taxa even for species that occupy similar ecological niches, such as seagrasses. Some seagrass species release their seeds in the sediment with no pelagic life stage while others produce floating propagules (Orth et al., 2006a) and consequently, genetic connectivity can differ greatly among populations (Waycott et al., 2006). There is also insufficient understanding of connectivity processes for species with very limited dispersal capacity, especially when they occupy distant habitats that they are very unlikely to reach without active transportation. Notably, there is almost no empirical evidence to understand the colonization and gene flow of oceanographically discontinuous habitats by seagrass species lacking efficient self-dispersal means, particularly for those that produce basal and negatively buoyant seeds in the genera *Cymodocea* and *Halodule* (McMahon et al., 2014). It can be hypothesized that these can only be transported by remaining attached to drifting detached seagrass shoots or by active transportation by animals, but the relative contribution of these potential dispersal mechanisms is poorly understood.

Seagrasses are marine flowering plants, most of which are distributed along shallow and wave-protected, temperate and tropical coastal waters around the world (Green et al., 2003). They provide important ecosystem services such as nursery and habitat for other organisms (e.g., fish and invertebrates), water quality improvement, sediment stabilization, productivity for food webs and carbon sequestration (Nordlund et al., 2016; Nordlund et al., 2018). However, despite their ecological importance, seagrasses are among some of the most threatened coastal ecosystems around the world (Short et al., 2016; Waycott et al., 2009). The increasing decline of seagrass meadows around the world raises the need to understand their specific reproductive and dispersal strategies that allow the connection between populations, to further implement effective conservation and restoration strategies (Orth et al., 2006b).

Seagrasses have the ability to reproduce both sexually, when genetically distinct individuals (genets) result from seeds, and asexually by clonal growth of plants that produce units capable of independent life (ramets, a plant fragment containing leaves, rhizome and roots, that derive from the breakage of the mother plant into physically independent individuals). Although seagrass species differ in producing seeds with contrasting dispersive properties (negative, neutral or positively buoyant) (Orth et al., 2007), seagrass fragments containing viable shoots can become detached and drift away, transported by currents. These can contribute to recruitment if they eventually reattach in the sediment elsewhere (a process that is more likely in intertidal populations than in subtidal ones), or if the drifting fragments carry fruits and seeds that can disperse over suitable habitat (see Kendrick et al., 2017) for a review). Although seagrass propagules have been reported to be transported by ocean currents, tidal currents, wind, and sediment movement (Ruiz-Montoya et al., 2012, 2015), their subsequent arrival in a suitable habitat and effective recruitment after transportation, although possible (Berković et al., 2014), is insufficiently understood. Another hypothesis for the dispersal of seagrasses is transportation by biotic vectors. Seagrass propagules can be eaten by herbivores such as green sea turtles and dugongs (e.g., Tol et al., 2017) or redhead ducks, which during winter feed exclusively on *Halodule wrightii* (Michot et al., 2008). Seagrass propagules can also attach to aquatic birds (e.g., Figuerola and Green, 2002), which by moving between similar suitable seagrass habitats may increase recruitment rates. Ultimately, such biophysical interactions determine connectivity pathways of seagrass populations across a wide range of spatial scales, depending on the transport mechanisms that are present, and the features of the propagule being dispersed (see McMahon et al., 2014 for a review).

Halodule wrightii Ascherson, 1868, is dioecious (i.e., separate males and females), perennial, and tolerates a wide range of environmental conditions (Bercovich et al., 2019) This species typically occupies areas associated with wave-protected coastal features isolated by unsuitable exposed habitat, across the tropical and subtropical eastern and western Atlantic coastal shores (den Hartog and Kuo, 2007). However, it is unclear whether this distribution reflects the capacity for frequent long-distance dispersal or whether populations are fragmented within their range because their seed biology is not favourable for dispersal *H. wrightii* produces very persistent basal seeds that can remain dormant for years (McMillan, 1981; Orth et al., 2007) and grow at the base of shoots that are neutrally or negatively buoyant, suggesting highly restricted seed dispersal. While several studies have estimated dispersal rates for other seagrasses (Orth et al., 1994; Harwell and Orth, 2002; Verduin et al., 2002; Van Tussenbroek et al., 2009; Berković et al., 2014), to date few studies have focused on *H. wrightii* for estimates of seed (Darnell et al., 2015a) or fragment dispersal (Hall et al., 2006). The reproductive biology of *H. wrightii*, which has very restricted dispersal abilities due to basal and non-buoyant seed production (Darnell et al., 2015a), raises the hypothesis that population genetic isolation may be prevalent and may contribute to the rare occurrence of this seagrass along the coastlines of tropical Atlantic Africa. Among the seagrass species found in the eastern and western tropical Atlantic, *H. wrightii* is the only one that has colonized the eastern Atlantic (western coast of Africa) (Spalding et al., 2003), although there is insufficient phylogenetic data on whether it is indeed the same species on both sides. The absence of other tropical seagrasses of the western Atlantic on the eastern side suggest that they have not been able to disperse across the Atlantic *via* abiotic or biotic means. This shows that despite its apparently non-dispersive seeds, *H. wrightii* has an effective means to achieve long-range dispersal, even if rarely. Possible mechanisms for this could be drifting seagrass fragments carrying attached seeds, resettling fragments without seeds or animal-mediated dispersal.

The west coast of Africa, one of the world's largest coastal systems, is an important center of marine biodiversity and marine food production, ranked among the world's most productive coastal and offshore (Roberts et al., 2002; Ukwe et al., 2003). It comprises very distinct coastal zones influenced by different main currents dominated by major wind-driven ocean gyres, the North Equatorial, the Equatorial, and the South Equatorial gyres (Philander, 2001). A semi-arid coast (Mauritania and Senegal) dominated by the cold equatorward coastal current and the Canary Current off northwest Africa associated with intense coastal upwelling; Guinea-Bissau is the transition to a shallow ocean off a humid tropical coast from Guinea to Gabon dominated by a prevailing offshore current, the Guinea Current, where coastal upwelling occurs seasonally; and a subtropical coast southwards to Angola, where it encounters the temperate Benguela Current from southwest Africa and its important coastal upwelling areas (Philander, 2001; Ukwe et al., 2006; Hutchings et al., 2009).

Despite being a tropical region with high biodiversity, the western coast of Africa is one of the least studied regions for seagrasses in the world (Duarte et al., 2008). To date, there are few reports of populations of *H. wrightii* on the western coast of Africa: it finds its northern limit of distribution at Banc d´Arguin in Mauritania and Senegal (Cunha and Araújo, 2009), was reported in Cape Verde (Creed et al., 2016), São Tomé and Príncipe (Alexandre et al., 2017) and has its southern limit in Angola (Santos, 2007). Other seagrasses that co-occur on this coast have broader distributions reaching colder temperate regions northwards (*Cymodocea nodosa* and *Zostera noltei*) and southwards (*Zostera capensis* in Atlantic South Africa). Although species of *Ruppia* have rarely been reported in Africa (but see Martínez-Garrido et al., 2017) these are expected to occur more widely given their propensity for seed dispersal by migratory aquatic birds (e.g., Figuerola et al., 2002), so the small number of occurrence records might reflect insufficient published observations rather than real absences. The location of seagrasses on coastal ecosystems, and their vulnerability to environmental conditions, exposes seagrasses to different types of human-induced disturbances, frequently leading to habitat loss (Waycott et al., 2009). Quantifying population connectivity within coastal ecosystems is a key component of the management and conservation of seagrass populations and its ecosystem services.

In this study, we aim to estimate the structure of genetic connectivity of the tropical seagrass species *H. wrightii* along its eastern Atlantic distributional range on the western coast of Africa, to assess the support for the hypothesis (i) that hydrodynamic connectivity mirrors genetic structure, as expected if ocean currents are the main dispersal mechanism, versus the alternative hypothesis of biotic seed transportation dependent on large-scale migratory patterns of megaherbivores. Additionally, we also address the hypotheses that (ii) partial clonality occurs throughout this coastline, and that clonal propagation occurs at similar levels among all sampled populations and that (iii) genetic diversity is greater in the center of the species range and lower towards the range edges.

This species model is interesting due to its predicted restricted propensity for self-dispersal, and the west coast of Africa is selected because this species has few rare and distant known occurrences reported, making it important to understand to what extent they may be connected. This study fills in a major gap in the understanding of metapopulation connectivity of seagrasses along the west coast of Africa, a useful resource for conservation and management planning.

3.3. Materials and methods

3.3.1. Study area

The present study focused on the distribution range of *H. wrightii* along the east Atlantic coastline, where it occurs at a few scattered locations from Mauritania to Angola, including the Cape Verde and São Tomé and Príncipe Islands. This coastline coincides mostly with the biogeographic province Gulf of Guinea and its 5 eco-regions (*sensu* Spalding et al., 2007), of the Tropical Atlantic biogeographic realm, but also crosses a major biogeographic transition zone located around Guinea-Bissau into the Temperate Northern Atlantic realm at its Lusitanian biogeographic province where the distribution of *H. wrightii* extends into the Saharan upwelling ecoregion (Spalding et al., 2007; Hemingson and Bellwood, 2018). Across this distributional range, we obtained samples from eleven localities (Figure 1B) where, at each location, a variable number of shoots (spatially separated individual plants) were collected (Table 1). All sampled locations were subtidal but very shallow (up to 5 m depth). Samples collected prior to 2018 were mainly obtained from previously collected herbarium samples (Table 1) thereby resulting in distinct sample sizes (i.e., different number of individuals per location). After 2018, directed sampling of ≥ 20 shoots (depending on depth, visibility, cover area) was conducted haphazardly, keeping a minimum distance of approximately 1 m between each sampling unit. All plants were stored individually on silica gel drying crystals in the field or dried in herbarium sheets.

3.3.2. DNA Extraction and Genotyping

Microsatellite development and primer sequences for the eight markers used can be found in (Larkin et al., 2012) for loci Hw180, Hw188, Hw190, Hw196, Hw200 and Hw212; and (Larkin et al., 2017) for loci Hw190b and Hw228. Genomic DNA was extracted using the NucleoSpin Plant II Kit (Macherey-Nagel, Duren, Germany) following the protocol from the supplier. Samples were genotyped for all the variable microsatellite loci divided into two triplex (HW190b; HW196; HW228) and (HW180; HW200; HW188) and one duplex (HW190; HW212) reactions using a GeneAmp ABI2720 Thermal Cycler (Applied Biosystems, Waltham, MA, USA). Final amplification volume was 15 µL, containing 1x Colorless GoTaq Flexi Buffer (Promega, Madison, WI, USA), 2 mM MgCl2 (2.5 mM), 10 µM forward and reverse primers, 0.2 mM dNTP's, 1U GoTaq" G2 Flexi DNA Polymerase (Promega, USA) and 5 µL of template DNA (several dilutions for different populations). PCR conditions included an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, *Ta* for 30s, extension at 72°C for 30 s and a final extension at 72°C for 10 min. Amplified fragments were run on an ABI3130 XL automated DNA sequencer (Applied Biosystems, Waltham, MA, USA) at CCMAR, with 0.25 µL GeneScanTM 500" LIZ Size Standard (Applied Biosystems, UK) plus 9.75 µL of Hi-Di formamide, after denaturation at 95°C for 5 min. Microsatellite alleles were manually scored using STRAND (Veterinary Genetics Laboratory, University of California, Davis; http://www.vgl.ucdavis.edu/STRand), 500 LIZ as size standard (Applied Biosystems) and binned using the R package 'MsatAllele' (Alberto, 2009). A detailed and thorough manual revision of microsatellite amplification and scoring was conducted including positive and negative controls, PCR replications to eliminate all ambiguities, and controls testing allele amplification in more diverse (non-African) samples. The final genotyping scores were obtained for each sample after a double-check reading process, to reduce scoring errors and ensure that the peaks were accurately interpreted.

3.3.3. Data Analysis

To perform the analysis, populations were evaluated according to genetic clusters and close geographical areas to maximize the number of individuals per population due to low sample size in some locations. Because seagrass species can propagate asexually and the number of shoots sampled at each location might represent duplicate genotypes collected from the same clone, the package 'RClone' (Bailleul et al., 2016) on R 3.6.2 was used to assess clonality for all individuals at all sites. Ramets (i.e., sampling units with identical alleles) were assigned as identical multi-locus genotypes (MLGs, or genets). To check if individuals with the same MLG were truly clones, the probability of finding identical MLGs resulting from distinct sexual reproductive events (P_{sex}) was calculated for each population (Arnaud-Haond et al., 2007). When $P_{\text{sex}} > 0.01$, individuals were considered to belong to the distinct clones and included in analyses of genetic diversity. Based on number of individuals (N) and genets (G) sampled, clonal diversity in each population was calculated using the clonal richness (R) index, ($R = (G - 1)/(N - 1)$, ranging from 0 (one single clone) to 1 (when all samples analyzed correspond to a different genet). Only unique genets per population were considered for genetic diversity analysis to avoid biasing allelic frequencies.

The software Micro-checker 2.2.3 was used to check for stuttering and null alleles (van Oosterhout et al., 2004). Microsatellite genetic diversity per sampled site was quantified by estimating standardized mean allelic richness (Â) standardized number of private alleles (PÂ), adjusted for the sample size of 8 samples, Nei's gene diversity (H_E), using GENETIX 4.05 (Belkhir et al., 1996). Levels of population differentiation were calculated using F_{ST} and Jost's D using an analysis of molecular variance (AMOVA) based on allele frequency under 999 permutations, using GENODIVE (Meirmans and van Tienderen, 2004).

To estimate the distribution of genets among genetic groups that minimize Hardy-Weinberg and linkage disequilibrium, a Bayesian assignment of genotypes to K groups was made using the program STRUCTURE (Pritchard et al., 2000). The value K was estimated from the mean log-likelihood for each *K* value and the Δ*K* statistic (Earl, 2012) to identify the optimal number of groups. This was analyzed for *K* ranging from one to eleven with ten replicates per value and a 50,000 burn-in followed by 500,000 MCMC replicates per iteration. All jobs were run in parallel on multiple cores using the R package 'ParallelStructure' (Besnier and Glover, 2013). Genotypic structure was assessed with a factorial correspondence analysis (FCA) implemented with GENETIX 4.05 (Belkhir et al., 1996).

3.3.4. Modelling

Connectivity potential of *H. wrightii* was further inferred with biophysical modelling based on propagule dispersal simulations and network analyses (i.e., graph theory). The model used data from the Hybrid Coordinate Ocean Model (HYCOM), a hindcast of high-resolution three-dimensional ocean velocity fields (regular 1/12-degree horizontal grid with 40 depth layers) able to resolve key oceanographic processes (e.g., filaments, oceanic eddies, fronts and meandering currents) because it integrates effects of precipitation, wind stress, wind speed and heat flux on the final end-products about ocean currents. The hindcast data on the direction and intensity of ocean currents, when integrated with biological traits of planktonic viable time in biophysical modelling, allows to estimate connectivity. This was previously validated with demographic and generic data for macroalgae, seagrasses, limpets, mussels, fish, echinoderms and crustaceans (e.g., Assis et al., 2015; Buonomo et al., 2017; Nicastro et al., 2020).

Individual virtual particles were released on a daily basis (matching HYCOM temporal resolution) throughout a complete year from source/sink sites located 1 km apart (i.e., the spatial resolution of the simulation) where the species is known to occur. The individual particles simulate rafts of *H. wrightii* that strictly float on the ocean surface, and do not consider any particular shape or density of rafting fragments, as in other studies also conducted at large spatial scales (e.g., Assis et al., 2015; Buonomo et al., 2017; Nicastro et al., 2020). Occurrence records describing the distribution of the species were collated from the available literature and from biodiversity information facilities (OBIS and GBIF). The simulation included the west coast of Africa, from Angola to Mauritania (~6250 km of coastlines from -10.0° to 21.5° latitude; model domain of 15,616,841km^2). A high-resolution polygon was used to define landmasses (Haklay and Weber, 2008). The model determined the position of all drifting rafts at every hour of simulation, while fitting a bilinear interpolation estimate over the velocity fields to smooth ongoing trajectories. Rafts were allowed to drift for up to 60 days (extreme propagule duration estimate). Rafts arriving at a shoreline or getting lost in the open ocean (beyond the model domain) were removed from the simulation. Trajectories were aggregated to build asymmetric matrices of pairwise probability of connectivity between source/sink sites, by dividing the number of virtual rafts released from site *i* that reached site *j*, by the total number of rafts released from site *i*. Interannual variability was considered by performing individual simulations per year, for the 10-year period 2008-2017. Over the entire 10-year period, the simulation released 3,653 virtual rafts per site (62,101 rafts in total).

Graph-theory was used to produce networks to visualize connectivity patterns. The connectivity matrix averaging the 10 years of individual simulations structured the graph nodes (individual source/sink sites) and the strength of edges (probability of connectivity). Stepping-stone probability estimates were determined using the graph, by using a product function over the probabilities of connectivity along the shortest paths between all pairs of sites, as found with Floyd–Warshall's algorithm, which minimises the sum of log-transformed probabilities. This approach aimed to capture all multigenerational potential connectivity across the study region, beyond the time frame considered in the

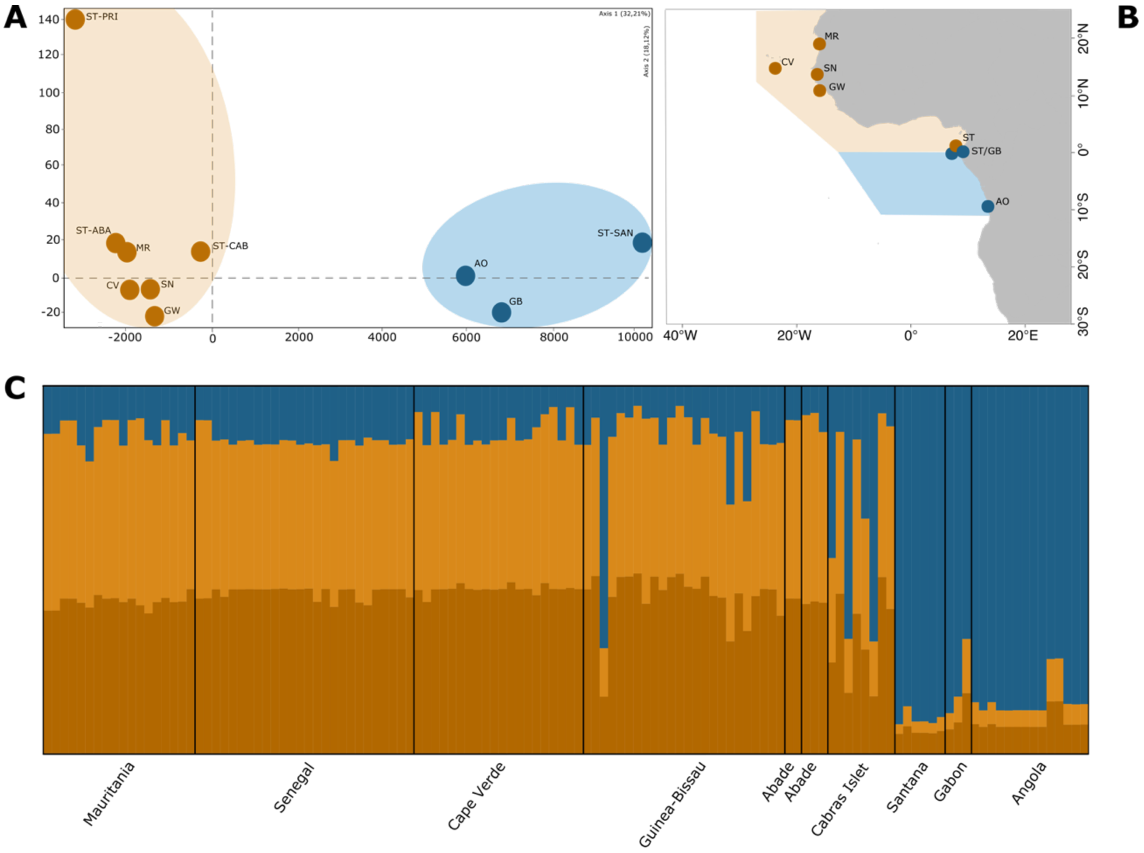


Figure 1 – (**A**): Genetic differentiation of *Halodule wrightii* illustrated by factorial correspondence analysis (FCA); (**B**): Sampling sites of *Halodule wriightii* along West Africa; (**C**): Assignment of individuals to genetic groups that minimize Hardy-Weinberg and linkage disequilibria, estimated by STRUCTURE, K = 3.

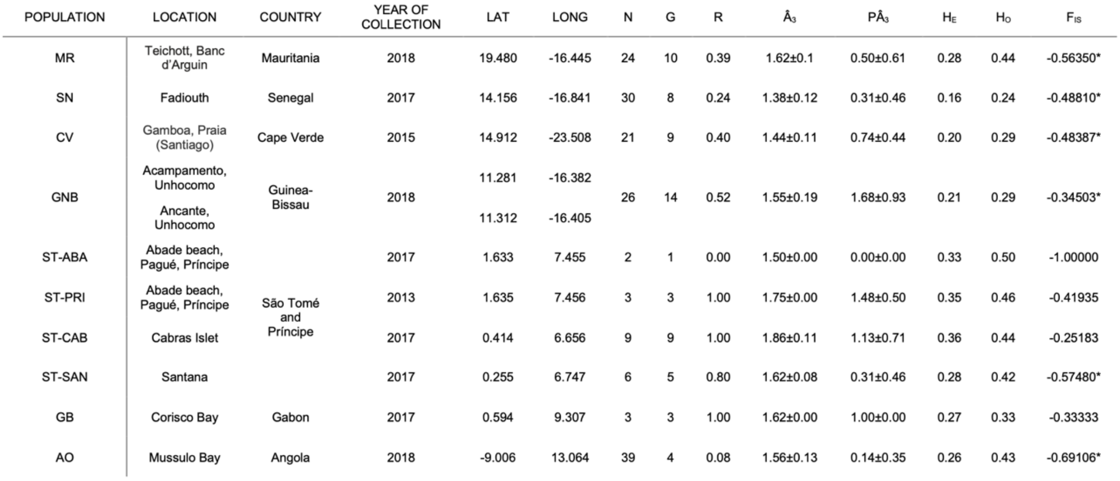
biophysical simulations (e.g., Assis et al., 2015; Buonomo et al., 2017; Nicastro et al., 2020; Ntuli et al., 2020).

Modelling was performed in R (R Development Core Team, 2021), using the packages bigmemory, doParallel, ncdf4, gstat, gdistance and raster for the biophysical modelling and the package igraph for the network analyses.

3.4. Results

A total of 163 sampling units (seagrass shoots) were successfully genotyped with 8 microsatellite loci for 10 populations from the sampled sites along the western coast of Africa. No evidence of null alleles or linkage disequilibrium was found between the eight microsatellite loci. Genetic diversity

Table 1 - Geographic location and genetic diversity indices for sampled sites of *Halodule wrightii.* Population; Location; Country; Year of collection; latitude (Lat) and longitude (Lon) in decimal degrees; sample size (N); number of unique genotypes (G); clonal diversity (R); standardized allelic richness (Â); standardized number of private alleles (PÂ); gene diversity or expected heterozygosity (H_E), observed heterozygosity (H_O); and multi-locus inbreeding coefficient (F_{IS}), * $p < 0.05$.



POPULATION	LOCATION	COUNTRY	YEAR OF COLLECTION	LAT	LONG	N	G	R	$\hat{A}_3$	$P\hat{A}_3$	H_E	H_O	F_{IS}
MR	Teichott, Banc d'Arguin	Mauritania	2018	19.480	-16.445	24	10	0.39	1.62±0.1	0.50±0.61	0.28	0.44	-0.56350*
SN	Fadiouth	Senegal	2017	14.156	-16.841	30	8	0.24	1.38±0.12	0.31±0.46	0.16	0.24	-0.48810*
CV	Gamboa, Praia (Santiago)	Cape Verde	2015	14.912	-23.508	21	9	0.40	1.44±0.11	0.74±0.44	0.20	0.29	-0.48387*
GNB	Acampamento, Unhocomo Ancante, Unhocomo	Guinea-Bissau	2018	11.281 11.312	-16.382 -16.405	26	14	0.52	1.55±0.19	1.68±0.93	0.21	0.29	-0.34503*
ST-ABA	Abade beach, Pagué, Príncipe	São Tomé and Príncipe	2017	1.633	7.455	2	1	0.00	1.50±0.00	0.00±0.00	0.33	0.50	-1.00000
ST-PRI	Abade beach, Pagué, Príncipe		2013	1.635	7.456	3	3	1.00	1.75±0.00	1.48±0.50	0.35	0.46	-0.41935
ST-CAB	Cabras Islet		2017	0.414	6.656	9	9	1.00	1.86±0.11	1.13±0.71	0.36	0.44	-0.25183
ST-SAN	Santana		2017	0.255	6.747	6	5	0.80	1.62±0.08	0.31±0.46	0.28	0.42	-0.57480*
GB	Corisco Bay	Gabon	2017	0.594	9.307	3	3	1.00	1.62±0.00	1.00±0.00	0.27	0.33	-0.33333
AO	Mussulo Bay	Angola	2018	-9.006	13.064	39	4	0.08	1.56±0.13	0.14±0.35	0.26	0.43	-0.69106*

estimates (Table 1) were low overall, but all diversity metrics were higher in the islands of São Tomé and Principe, including standardized allelic richness, gene diversity (mean expected heterozygosity, H_E) and number of private alleles (i.e., endemic genetic diversity represented by alleles that are not present in any other sites sampled along the study region). When P_{sex} values were lower than 0.01, the estimated probabilities of identical MLG were considered derived from independent reproductive events, leading to the detection of a total of 66 individual genets. Clonal richness (R), the proportion of the sampled shoots that were distinct clones arising from sexual reproduction, varied considerably among populations, with an overall value indicating a balanced contribution of seeds and clonal growth (R=0.46). The highest values of clonal richness were found in São Tomé and Príncipe and Gabon (Table 1), indicating that seed propagation is dominating in these regions. The inbreeding coefficient (F_{IS}) was significantly different from zero in six populations (Table 1), and all values were negative (heterozygote excess), suggestive of selection favoring heterozygotes.

All population differentiation analyses revealed a separation between the southern and northern hemisphere sites, with genetically distinct populations in São Tomé and Principe (latitude zero) (Figure 1). The Structure analysis revealed

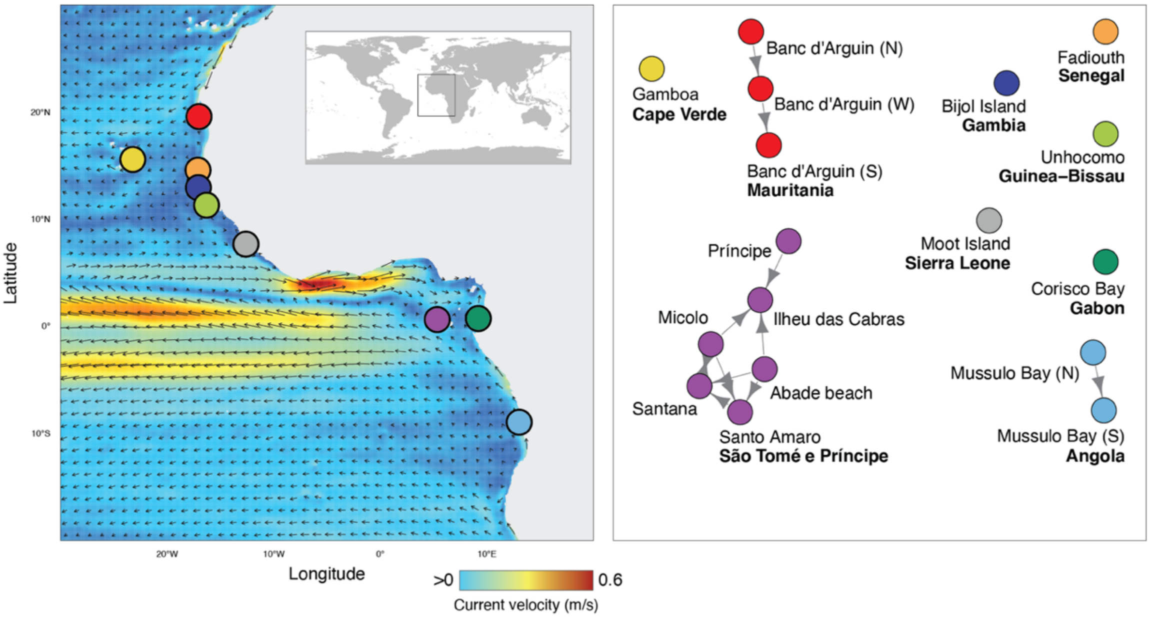


Figure 2 – Potential connectivity of *Halodule wrightii* populations in west Africa, estimated from simulations of transport by ocean currents data (10 years). Circles in the left panel depict all regions where the species is known to occur. Colors of circles in the right panel represent distinct clusters, i.e., sites that are potentially connected by ocean currents. For estimates of probability of connectivity between regions see Supplementary information, Table S3.

three main clusters (K = 3), which were shared (although in distinct proportions) between all populations, suggesting incipient population divergence, which can be caused by either admixture or recent colonization. The proportions of genotype assignments were distinct between the southern (Angola, Gabon and Santana from São Tomé and Príncipe) versus the northern (all others) hemisphere sites (Figure 1C). The factorial correspondence analysis (FCA) also supported this result (Figure 1A) but revealed further differentiation between population sites. Similar patterns were revealed by the values of genetic differentiation indices (FST and Jost's D) among the eleven populations, with their lack of significance likely being strongly influenced by the low sample sizes (Table S1; Supplementary Material).

The data collation from literature (Table S2; Supplementary Material) and biodiversity databases resulted in 29 locations where the species is known to occur. This translated in 17 unique source/sink sites aggregated to 1 km distance (i.e., 12 sites within distances < 1km), which were included in biophysical modelling. The aggregated trajectories produced a network showing potential connectivity only at regional scales (average distance of connectivity events: 29.124 ± 54.539km; maximum: 176.811km) (Figure 2), with low probabilities of connectivity (average probability: 0.037 ± 0.081) resulting from a low degree of connectivity events between paired sites (average number of events: 13.577± 36.628) (see Supplementary Information). In particular, the simulations indicated that the sites within São Tomé and Príncipe, Angola and Mauritania were expected to be connected by oceanographic current transportation (Figure 2). The remaining sites had no probability of connectivity mediated by ocean currents (i.e., there were no events of connectivity from/to these sites, in the 10 years of simulation).

3.5. Discussion

The spatial patterns of genetic structure here found for the seagrass *H. wrightii* in its Tropical Eastern Atlantic range (~6250 km), along the western coast of Africa, did not support our main hypothesis (i) of ocean currents as main dispersal vectors mediating population connectivity. Simulated transportation of drifting seagrass fragments by ocean currents revealed extremely low or null probability of gene flow between all populations along this coast, with a few exceptions for nearby sites. The genetic results, in strong contrast, showed no significant population differentiation besides a broad genetic separation north and south of the equator, while on the equator line (São Tomé and Príncipe) populations were genetically distinct despite close proximity, indicating they originated from independent colonization events by seeds from genetically differentiated sources. This mismatch between the observed genetic structure and the predicted connectivity patterns expected if dispersal was based on ocean currents, suggests that biotic transportation (e.g., seed transport by megaherbivores) may play a larger role in the dispersal and establishment of the species rather than ocean currents, although further studies are needed to investigate this hypothesis.

The moderate or high genotypic (clonal) diversity here discovered across all populations (hypothesis ii) demonstrates successful sexual (seed-based) recruitment, rather than simple clonal propagation, in all populations. However, genotypic (clonal) and genetic (allelic) diversity were not similar along the range (hypothesis iii). Sexual (seed) recruitment and genetic diversity were maximum in the equator (São Tomé and Principe), whereas clonal propagation was more prevalent at both the northern and especially at the southern range of the species, which may reflect either lower seed production and dispersal or lower seed germination and seedling survival, at the range edges. The low genetic (allelic) diversity found across all populations, is compatible with a hypotheses of recent founder events/demographic bottlenecks.

This study also compiled all the distribution records and added new records to fill in the gaps regarding the distribution of *H. wrightii* species in this region and underscores the need to understand the metapopulation dynamics to further implement conservation measures.

3.5.1. Genetic Structure and Oceanographic Connectivity

Population genetic differentiation was low and non-significant across all populations, suggesting high inter-population connectivity, yet *H. wrightii* is thought to have low dispersal capability due to basal non-buoyant seeds. Long-distance migration by this species can be mediated either by floating drifting fragments or by animal-mediated transport (entangled or in the digestive tract). Our results do not support the hypothesis that connectivity is predominantly mediated by oceanographic currents, as genetic and hydrodynamic connectivity show very distinct patterns. Although *H. wrightii* fragments can survive floating in the water column for up to four weeks (Hall et al., 2006), our results indicate that on average, such dispersal by drifting fragments, would not travel far from their source location (Figure 2). Although our oceanographic simulations predict that *H. wrightii* fragments might still be able to disperse successfully over short distances between nearby populations, this was contradicted by the genetic differentiation of nearby populations in the island of São Tomé.

We cannot rule out the hypothesis of oceanographic transportation playing a role in dispersal given the extremely low variability of the markers in this geographic region, that results in very low power, only capable of detecting genetic differentiation of high magnitude. Besides, stepping-stone connectivity could be higher than predicted if unknown seagrass meadows might exist in between the assessed sites. However, the poor connectivity potential revealed by passive transport simulations among *H. wrightii* populations at such large scales of distribution indicates that additional factors, such as grazer transportation, might be involved in mediating migratory movements in this species. Remarkably, the islands of São Tomé and Principe contain populations located nearby but assigned to distinct northern and southern groups, further suggesting that stochastic rare colonization events might be at the origin of the colonization of these seagrass populations, as these bear little or no correlation with geographical distance or oceanographic distance (i.e., current transport). Multiple independent colonization events on islands were previously reported for coral species: in São Tomé and Principe from Brazil and from the Caribbean (Teschima et al., 2021), and numerous colonization events to the Canary Islands and Cape Verde Islands (de Souza et al., 2017), although such corals have planktonic life stages. The bio-physical modelling considered key ecological traits (e.g., rafting duration; Assis et al., 2021) and oceanographic processes (e.g., eddies and fronts; Manel et al., 2019) to realistically estimate connectivity, but one cannot neglect the effect of additional drivers like distinct raft morphology changing the effect of wind drag (‘windage’) on their exposed surface (Ruiz-Montoya et al., 2015); however, such incorporation into modelling is challenging.

Multiple factors may interact to drive seagrass migrations. Other seagrass species were reported to rely on multiple means of transportation such as water and sediment movements (Kendrick et al., 2012; Darnell et al., 2015b), drifting (Berković et al., 2014) or biotic vectors such as herbivores. Dugongs, manatees, sea turtles, birds or fishes that feed and live among seagrass meadows, can transport seeds through their consumption or transport fragments attached to them (see Heck and Valentine, 2006) for a review). Previous studies have pinpointed the importance of biotic vectors in the dispersal of seagrasses. *Zostera marina* seeds can be dispersed by up to 1.5 km by sea turtles and 19.5 km by birds (Sumoski and Orth, 2012). Other studies have found viable seagrass seeds in megaherbivores feces, suggesting the potential for green sea turtles *Chelonia mydas* (277 – 652 km) and dugongs *Dugong dugon* (173 – 234 km) to act as seed migration agents (Tol et al., 2017). Adding to the potential dispersal distance, it is also important to consider the digestion retention time for these herbivores, with reports for dugongs ranging between 6 – 7 days (Lanyon and Marsh, 1995; Tol et al., 2017) and one to two weeks for green sea turtles (Brand et al., 1999; Mannina N. B., personal communication). Satellite tracking data from post-breeding green sea turtles in our study region indicate that these animals can travel ~ 400 km between *H. wrightii* sites in about 10 days (8 – 11 days, n=4, Patrício A. R. & Catry P., unpublished data). This vector could therefore facilitate the dispersal of viable seeds over such wide distances. Our results support the hypothesis that the broad distribution of *H. wrightii* along the western coast of Africa could be mediated by biotic vectors, although to date, no study has addressed this subject for this species. Overall, animals that feed on seagrass meadows on the west coast of Africa, such as dugongs or green sea turtles (Marshall et al., 2003; Godley et al., 2010; Hancock et al., 2019) seem to be the most likely candidates for seagrass seed dispersal, although wading birds could also play an important role, and indeed might be able to transport seeds rapidly over greater distances. On the western side of the Atlantic, one particular species of duck (*Aythya americana*) actually feeds on *H. wrightii* exclusively for a large part of the year (Michot et al., 2008), but no equivalent trophic behavior has been reported for any bird species on the African coastline. In addition to the hypothesis of biotic transport as main means for seed propagation by *H. wrightii*, we also hypothesize that the very large population of green turtles that migrate along this coast (Catry et al., 2002), one of the largest in the world, might be the main dispersal vectors for *H. wrightii*. Our study raises interesting questions that still require further research to assess the true potential of seagrass seed dispersal *via* animal vectors along the western coast of Africa.

3.5.2. Genotypic Diversity

Clonal reproduction is common among seagrass species, allowing long-term persistence of individual genotypes (e.g., Arnaud-Haond et al., 2012; Arnaud-Haond et al., 2020). In dioecious species like *H. wrightii* maintaining genotypically diverse populations is also important to increase the probability of mating encounters, for successful sexual reproduction to occur. Monoclonal dioecious seagrass populations do exist and may persist for millennia (e.g., Alberto et al., 2008), but evidently only by clonal propagation, as a single male or single female cannot form sexual seeds. The proportion of distinct sexually originated (seed-derived) clones in a population (i.e., genotypic richness) is a measure of the long-term balance between sexual and asexual reproduction. Genotypic richness, in addition to genetic diversity, can also play a major role of seagrass ecosystems resilience to maintain vital functions (Massa et al., 2013). Clonal richness of *H. wrightii* along the western coast of Africa was generally high (mean R=0.46), particularly higher than on the western side of the Atlantic for the same species (mean R=0.34; Larkin et al., 2017), revealing a high contribution of seed recruitment for population maintenance along the west coast of Africa. Our results also revealed that clonal richness was variable among sites, indicating that sexual recruitment from seeds was less relevant and/or successful in some populations, particularly in Angola (R= 0.08, F_{IS}= -0.69), where the lowest R value was observed, and Senegal (R= 0.24, F_{IS} = -0.69), two populations located near the southern and northern range edges of the species. Overall, these results reject our initial hypothesizes of similar observed clonality across the species range, raising the hypothesis of geographical parthenogenesis, i.e., higher clonal propagation in range-edge populations as these occupy marginal habitats (Eckert, 2002; Tilquin and Kokko, 2016). Furthermore, clonal diversity was higher in central populations, showing that sexual reproduction is favored. However, due to the small number of individual samples for some of the central populations, these results should be considered carefully (Table 1). Future studies with more shoot sampling per population could indicate whether sexual reproduction in central populations is indeed higher than in peripheral populations. Seagrasses in general have very variable levels of clonal richness across distinct populations of the same species (e.g., Arnaud-Haond et al., 2007; Alberto et al., 2008; Boyé et al., 2021) and this is therefore not a species-specific trait. However, depending on the levels of genotypic diversity and the number of disturbance events within a population, seagrass recovery can occur through either sexual or asexual reproduction when habitats are disturbed (Becheler et al., 2010; Jarvis et al., 2012). When seed recruitment is frequent, disturbance is predicted to increase and maintain high genotypic variety (Becheler et al., 2010, 2014; Paulo et al., 2019; Arnaud-Haond et al., 2020), because disturbance increases space available thereby reducing competition, which favors seedling recruitment. In contrast, populations with low genotypic diversity are more likely to recover from disturbances through asexual reproduction of established genotypes. This reproductive plasticity may enhance the resistance and resilience of seagrass species to habitat and environmental changes. Future research for *H. wrightii* in West Africa should consider a better sampling design, particularly in central populations such as São Tomé and Principe, to increase the number of sampling units to test the hypothesis of whether clonal or sexual reproduction might vary over different geographical scales and whether asexual reproduction is more prevalent towards to the species range edge.

3.5.3. Genetic diversity

The low genetic diversity found for populations of *H. wrightii* in western Africa (mean Â=1.59) is distinct from *H. wrightii* on the western Atlantic (4.08 in Larkin et al., 2017); 3.26 in (Reynolds et al., 2019), and thus it is not a species-specific trait. This could be caused by diversity loss to population bottlenecks or to founder events, as might be expected if colonization of these regions was recent. Although the overall low allelic richness of *H. wrightii* on the west coast of Africa was comparable to mean levels observed in many other seagrasses around the world (1.44 in Jahnke et al., 2019; 1.56 in Nguyen et al., 2014; 1.62 in Tanaka et al., 2011), these levels rank among the lowest in seagrasses (2.16 in Arriesgado et al., 2015; 4.78 in Becheler et al., 2010; 3.25 in Olsen et al., 2004; 5.60 in van Dijk et al., 2018).This pattern supports the hypothesis of a recent colonization history in the eastern side of the Atlantic with founder effects, a scenario that is supported by the observation that this is the only taxon that occurs on both sides of the tropical Atlantic. Most Atlantic tropical seagrass species occur exclusively on the western side of the Atlantic, suggesting that this oceanic migration route must be a very rare event for seagrasses. The lower allelic richness in near marginal populations such as Senegal or Angola was consistent with our hypothesis of lower genetic diversity towards the range edges, as shown for other species (e.g., Arnaud-Haond et al., 2006; Ridgway et al., 2008; Arriesgado et al., 2015).

The results of this study have important conservation implications, particularly as extremely productive and biologically diverse coastal ecosystems of the west coast of Africa have been under increasing stress due to natural and human-induced pressures (Polidoro et al., 2017). The low genetic variation in *H. wrightii* populations, together with the small number of known populations and their restricted habitats, raises concerns for their conservation and ability to deal with environmental and human mediated impacts, since genetic diversity is the basis for adaptation to future environments (Lande and Shannon, 1996). Seagrasses provide essential habitat for other species and ecosystem services, and their loss is magnified into a loss of multiple ecological functions. Protecting existing seagrass meadows in this region and restoring them where suitable habitats exist, may be vital to prevent loss of existing genetic diversity (Duval-Diop et al., 2014). However, our study suggests that this diversity might also depend on protecting the herbivores that potentially mediate their successful gene flow and colonization of new habitats. The results of this study support the hypothesis that the very large population of green turtles that migrate along this coast might be a main dispersal vector. It is widely accepted that seagrasses must be protected for the survival and persistence of their associated macrofauna; our results suggest that conversely, macrofauna such as green turtles, may also play an important role in long-term seagrass persistence and reproductive success along the tropical western coast of Africa.

3.6. Acknowledgments

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CHAPTER 4

Past and future climate effects on population structure and diversity of North Pacific surfgrasses

Past and future climate effects on population structure and diversity of North Pacific surfgrasses

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4.1. Abstract

Aim: Understanding the impacts of past and future climate change on genetic diversity and structure is a current major research gap. We ask whether past range shifts explain the observed genetic diversity of surfgrass species and if future climate change projections anticipate genetic diversity losses. Our study aims to identify regions of long term climate suitability with higher and unique seagrass genetic diversity and predict future impacts of climate change on them.

Location: Northeast Pacific

Time period: Analyses considered a timeframe from the Last Glacial Maximum (LGM; 20 kybp) until one Representative Concentration Pathway (RCP) scenario of future climate changes (RCP 8.5; 2100).

Major taxa studied: Two seagrass species belonging to the genus *Phyllospadix*.

Methods: We estimated population genetic diversity and structure using eleven polymorphic microsatellite markers. We predicted the distribution of the species for the present, LGM, and near future (RCP 8.5, no climate mitigation) using Species Distribution Models (SDMs).

Results: SDMs revealed southward range shifts during the LGM and potential poleward expansions in the future. Genetic diversity of *P. torreyi* increases from north to south, but in *P. scouleri* the trend is variable. *P. scouleri* displays signals of genome admixture at the southernmost and northernmost edges of its distribution.

Main conclusions: The genetic patterns observed in the present reveal the influence of climate-driven range shifts in the past and suggest further consequences of climate change in the future, with potential loss of unique gene pools. This study also shows that investigating climate links to present genetic information at multiple timescales can establish a historical context for analyses of the future evolutionary history of populations.

4.2. Introduction

Understanding climate effects on genetic diversity and structure is a major goal of population genetics and ecology (Eckert et al., 2008). In the Northern Hemisphere, the extreme climate oscillations of the Quaternary (2.6 MYA to present) impacted the geographical ranges of marine temperate species (Maggs et al., 2008). During glacial periods, populations were pushed to southward habitats of suitable climatic conditions, creating refugial areas of persistence protected from the harsh northern glacial climates. Conversely, during warmer interglacial periods, range expansions might have occurred poleward. These range shifts have shaped population genetic diversity and structure, particularly at marginal populations located near the current edges of species ranges (Hewitt, 1999), where rich distinctive gene pools are often found (Assis et al., 2014; Neiva et al., 2015). Indeed, long-term persistent populations in refugial areas exhibit distinctive genetic signals, such as private alleles that evolved locally and were not involved in subsequent range expansions (Hewitt, 1999; Diekmann and Serrao, 2012; Assis et al., 2016b). However, ongoing climate change is posing threats to such refugia that may contain most of a species gene pool (Assis et al., 2018a, 2018b). The potential loss of uniquely diverse populations may endanger overall genetic diversity and possibly long-term survival and adaptability (Nicastro et al., 2013; Assis et al., 2018a) as reduced genetic diversity can exacerbate the effects of inbreeding and reduce reproductive fitness, thereby raising the danger of extinction (Hoffmann et al., 2017).

Among coastal marine systems, seagrasses are foundation species that form the basis of one of the most productive and important communities (Nordlund et al., 2018) harbouring a diverse range of marine life and providing essential ecosystem services (Cullen-Unsworth and Unsworth, 2013; de los Santos et al., 2020). However, ongoing climatic change has resulted local losses of seagrass habitat (Chefaoui et al., 2018) and associated distributional shifts (Chefaoui et al., 2021). This is especially concerning in temperate locations where some seagrasses have already been impacted near their low-latitude margins (Valle et al., 2014; Hyndes et al., 2017). These shifts may reduce seagrass population area and subsequently their resilience, genetic diversity, species richness, biomass, and productivity (Zimmerman, 2021). Given their global ecological importance, identifying regions of higher and unique seagrass genetic diversity, and projecting the impacts of climate change is of key relevance, particularly among seagrasses where there is little exploration of this important foundation genus (Melo-Merino et al., 2020).

Climate change has had a significant impact on intertidal species distributions, as coastal habitats are more exposed to its effects (Harley et al., 2006; Kunze et al., 2021). *Phyllospadix* is a widely distributed seagrass genus found in the temperate North Pacific region (sensu Short et al., 2007). It comprises five perennial dioecious species (Short et al., 2016), of which *P. scouleri*, *P. torreyi* and *P. serrulatus* are distributed along the Northeast Pacific (NE Pacific; Phillips, 1979; Spalding et al., 2003). In the Northwest Pacific, *P. iwatensis* and *P. japonica* are distributed along the coastlines of Korea and Japan (Spalding et al., 2003). Unlike most seagrass species that grow on soft bottoms, *Phyllospadix* spp. occurs on wave exposed rocky substrates in the low intertidal and shallow subtidal zones (Hemminga and Duarte, 2000). Inhabiting shallow waters (down to 5 m depth), *Phyllospadix* is frequently subjected to sharp environmental fluctuations (Ramírez-García et al., 1998). The genus might thus be particularly susceptible to climate-induced range shifts and their effects on genetic diversity along the temperate North Pacific bioregion, as shown for other marine forest-forming species (Grech et al., 2012; Zhang et al., 2019; Song et al., 2021).

Here, we use surfgrass, the only rocky shore seagrass genus in the world, as a model system to investigate impacts of past and future climatic events on the distribution and genetic diversity of two foundation species. We aim to determine whether past climate-driven range shifts have influenced the present genetic diversity of *Phyllospadix,* if there are genetic hotspots at persistence zones with higher conservation value, and if these are at risk of future extirpations under predicted future climate scenarios. Specifically, we hypothesize that: 1) populations that have persisted during past climatic extremes currently harbor higher endemic genetic diversity; 2) populations in recently available habitats exhibit lower and non-endemic diversity; 3) populations at the lower latitude range limits are threatened by future climate change.

4.3. Methods

4.3.1 Study area and focal species

The present study is focused on the NE Pacific coast, along the distributional ranges of *P. torreyi* and *P. scouleri*, from the Baja California Peninsula to Alaska. Samples of *P. torreyi* were obtained from San Juanico (26.2 N, 112.4 W, Mexico) to Fogarty Creek (44.8 N, 124.1 W, Oregon) and *P. scouleri* was sampled from Government Point (34.4 N, 120.5 W, California) to Vancouver Island (48.6 N, 124.5 W, Canada) (Table S1). To further check possible admixture between east and west Pacific *Phyllospadix* populations, we sampled three populations of *P. iwatensis* in Hokkaido Island (Japan; Table S2). Across sampling areas, approximately 30 individuals were sampled haphazardly at low tide along approximately 30 m, keeping a minimum distance of approximately 1 m between each sampling unit at each location along 23 sites (see tables S1 and S2). All sampled plants were blotted dry and preserved dry in silica gel.

4.3.2 Species distributions models

Species Distribution Modelling (SDMs) were built for *P. torreyi* and *P. scouleri* with two machine learning algorithms, Boosted Regression Trees (BRT) and adaptive boosting (AdaBoost). These are well-suited for modelling complex interactions and non-linear relationships, and have been shown to have high predictive performance, while reducing overfitting through proper hyper-parametrization and forcing of monotonic responses (Elith and Leathwick, 2007; Hofner et al., 2011).

Biologically meaningful predictor variables for seagrasses (Krause-Jensen et al., 2020) were extracted from Bio-ORACLE (Assis et al., 2018c) for present-day conditions, the LGM and the extreme Representative Concentration Pathway (RCP 8.5) future scenario of increased carbon emissions which has no climate mitigation policies (see data availability statement). The predictors chosen reflected essential resources (nutrients as nitrate) as well as factors affecting physiology (salinity and temperature) and perturbation (sea ice cover). Because the focal species are intertidal, predictors were clipped to the regions defined by NE Pacific coastlines. Presence records for the two species were extracted from two biodiversity information facilities, GBIF (https://www.gbif.org/) and OBIS (https://www.obis.org/), and from the fine-tuned dataset of marine forest species (Assis et al., 2020). An equal number of pseudo-absences was randomly generated in locations where no presences were recorded (Assis et al., 2018b). To reduce the potential effect of autocorrelation and sampling bias in the models, we filtered the records based on the minimum distance at which predictors were not significantly correlated. This distance was inferred by building a correlogram per species that identified the variability of Pearson's correlation of predictors with increasing marine distances (Krause-Jensen et al., 2020).

A 6-fold cross-validation approach was implemented, where independent blocks were used to find the optimal combination of hyperparameters, specifically, the number of trees (ranging from 50 to 1000, step 50), tree complexity (ranging from 1 to 6), and learning rate (0.01, 0.005, and 0.001) for BRT (Elith and Leathwick, 2007) and shrinkage (from 0.25 to 1, step 0.25), degrees of freedom (from 1 to 12, step 1) and number of interactions (from 50 to 250, step 50) for AdaBoost (Hofner et al., 2011). The performance of the model was evaluated with the area under the curve (AUC) of the receiver operating characteristic curve and True Skill Statistics (TSS), which is determined by the sum of sensitivity (true presence rate) and specificity (true absence rate) minus one (Allouche et al., 2006). To reduce overfitting, negative monotonic responses were forced for maximum temperature and ice thickness, and positive responses for nitrate, salinity, and minimum temperature (Hofner et al., 2011). The relative importance of each predictor was assessed by measuring the increase in AUC when adding each predictor to the alternative model (Assis et al., 2018b).

To develop parsimonious models per algorithm, with higher transferability potential, a stepwise approach was used, starting with a full model, and iteratively removing predictors from the least to the most contributory until the reduction in AUC was significant. Maps reflecting species' potential distribution for the present-day, the LGM and future conditions were created by averaging (i.e., ensemble modelling; Araújo and New, 2007) the responses of both BRT and AdaBoost parsimonious models. These were then reclassified into binomial maps showing the presence or absence of both species by using a threshold that maximized TSS (Allouche et al., 2006). To assess for past range shifts, we compared the modelled distributions for the LGM and the present and identified refugial areas where the species was predicted to occur in both time periods.

4.3.3 Primer design, microsatellite amplification and genotyping

Genomic DNA for microsatellite development was extracted from 500 mg of dried tissue from a pool of 97 *P. torreyi* individuals and 500 mg from 66 *P. scouleri* individual samples. These samples were used to run 454 sequencing (Biocant, Cantanhede, Portugal). Simple di- to hexanucleotide microsatellite loci motifs were tested for primer design using Primer 3 (http://biotools.umassmed.edu/ bioapps/primer3_www.cgi). A total set of 38 and 27 pairs of primers were designed for *P. torreyi* and *P. scouleri*, respectively, and tested for amplification. To determine the applicability and amplification success of the microsatellites, we tested them on two individual samples from five populations each of *P. torreyi* and *P. scouleri.* From the initial set, a subset of 38 loci for *P. torreyi* and 20 for *P. scouleri*, respectively, were chosen due to their amplification success. These were evaluated for their ease of scoring and polymorphism using primers labeled with fluorophores, and 11 were finally selected due to their number of repeats, similar melting temperature allowing multiplexing and cross-amplification success (primer sequences and microsatellite motifs listed on Table S3, supplementary material).

Genomic DNA was extracted using NucleoSpin96 PlantKit II (Macherey-Nagel, Germany). PCR reactions were performed in a total volume of 15 µl, containing 1x Colorless GoTaq" Flexi Buffer (Promega, Madison, WI, USA), 2 mM $MgCl_2$, 10 μM forward and reverse primers, 0.2 mM of dNTPs, 1U GoTaq" G2 Flexi DNA Polymerase (Promega, USA) and 5 μL of template DNA. Cycling conditions consisted of an initial denaturation step at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, annealing temperature *(T*a, Table S3) for 30s, extension at 72°C for 30 s and a final extension at 72°C for 5 min. Amplified fragments were combined with 0.25 μL GeneScanTM 500" LIZ Size Standard (Applied Biosystems, UK) and 9.75 μL of Hi-Di formamide, followed by 5 min denaturation at 95°C and their analysis were performed on an ABI3130xl automated DNA sequencer (Applied Biosystems, Waltham, MA, USA) .

Although *P. torreyi* and *P. scouleri* were genotyped for 11 microsatellite loci (Table S3), *P. iwatensis* populations were only genotyped for a set of eight loci (Psc07, Pto07, Pto13, Pto15, Pto25, Pto29, Pto31 and Pto35) as amplification failed for the additional three loci.

4.3.4. Microsatellite data analysis

Fragments were scored using STRAND (Veterinary Genetics Laboratory, University of California, Davis; http://www.vgl.ucdavis.edu/STRand) and binned using the R package 'MsatAllele' (Alberto, 2009). Prior to analysis, the final dataset was run in Micro-Checker 2.2.3 software (van Oosterhout et al., 2004) to test for the presence of null alleles and test Hardy–Weinberg equilibrium and run a linkage disequilibrium test. No evidence of null alleles or linkage disequilibrium was found for any of the 11 loci; therefore, they were all considered suitable markers for the analyses of population structure.

Seagrass species occupy space via rhizome growth, a type of clonal growth where genets (i.e., genetic individuals coming from distinct seeds) form ramets (i.e., units capable of independent life if separated, formed by shoots, associated roots, and a piece of rhizome). Therefore, when seagrasses are sampled, duplicate genotypes (clones) may be collected that derive from a single seed. To assess the clonality of each population, the package RClone' (Bailleul et al., 2016) was used to assess the probability that identical multi-locus genotypes (*MLG*s) are the product of the same or separate reproductive events. The probability of finding identical MLGs resulting from distinct sexual reproductive events (P_{sex}) was computed for each population (Arnaud-Haond et al., 2007) to check if individuals with the same MLG were truly clones. When P_{sex} was lower than the threshold value (fixed at 0.01), two identical MLGs were considered to be the same clone. Based on number of individuals (N) and genets (G) sampled, clonal diversity in each population was calculated using the clonal richness index (*R*), ($R = (G - 1)/(N - 1)$, ranging from 0 (one single clone) to 1 (when all samples analysed correspond to a different genet). Only unique genets per population were used for genetic diversity analyses to avoid biasing allelic frequencies, that for the research questions of this study, should represent the population of genets rather than the population of ramets.

4.3.5. Genetic diversity and population genetic structure

To compare population genetic diversity and differentiation, all populations must have the same loci amplified. Two datasets were therefore constructed for genetic analysis as only eight loci amplified for *P. iwatensis* out of the 11 that were used to amplify *P. torreyi* and *P. scouleri*. Dataset 1 includes *P. torreyi* and *P. scouleri* populations (11 loci), and dataset 2 encompasses populations for all three species (eight loci). Additionally, for higher resolution analysis of population differentiation within each species, separate genetic analyses were performed for species in dataset 1 individually (*P. torreyi* and *P. scouleri*). A total of 593 *Phyllospadix* samples were genotyped (304 *P. torreyi* and 289 *P. scouleri* samples) for dataset 1 (11 loci).

Summary statistics of genetic diversity were calculated for each population using the GENETIX 4.05 (Laboratoire Génome, Populations, Interactions, Université de Montpellier II; http:// kimura.univ-montp2.fr/genetix). This included the mean number of alleles per locus (allelic richness), the non-biased expected heterozygosity (H_E), the observed heterozygosity (H_O), the number of private alleles, and the multi-locus inbreeding coefficients (F_{IS}). Sample sizes were also standardized to the smallest in any population using 10^4 randomizations.

Population genetic structure was inferred using STRUCTURE 2.3 (Pritchard Lab, Stanford University; http://pritchardlab.stanford.edu/structure.html) without any prior population assignments. Structure was run for both datasets and for *P. torreyi* and *P. scouleri* individually. The estimated number of genetic clusters, K, was estimated from the mean log likelihood for each *K* value and the Δ*K* statistic (Earl, 2012) to identify the optimal number of groups. This was analysed considering a range of K values from 1 to 23. Ten replicates per K value were used to verify the consistency with a 50,000 burn-in followed by 500,000 MCMC replicates per iteration. All jobs were run in parallel on multiple cores using the R package 'ParallelStructure' (Besnier and Glover, 2013). The best K was estimated using the program Structure Harvester which ranks K's according to Evanno method (Earl, 2012). Structure analyses were complemented with a factorial correspondence analysis (FCA) implemented in GENETIX v.4.05.2 (Belkhir et al., 1996). To quantify pairwise differentiation between populations, we calculated F_{ST} and Jost's D distances using the software GENODIVE (Meirmans and van Tienderen, 2004).

4.4. Results

4.4.1. Species distribution models

The ensemble of both BRT and AdBoost algorithms resulted in high performance predictions, largely matching the known distribution of the two species. Specifically, for *P. scouleri* AUC was 0.99 and TSS 0.93, and for *P. torreyi* AUC was of 0.99 and TSS 0.91. The models considered numerous biologically meaningful predictor variables, from which temperature (maximum and minimum) were key in explaining the distribution of both species (Figures S1 and S2).

The models hindcasted significant equatorward range shifts during the Last Glacial Maximum (LGM). The extreme colder environments might have contracted poleward range margins down to Oregon (*P. scouleri*) and California on the West coast of the USA (*P. torreyi*). Concomitantly, lower latitude range margins might have remained stable or even expanded along Central American latitudes (Figure 1). In contrast, the future scenario of increased carbon emissions (RCP 8.5) projected poleward range expansions with potential habitat losses at the lower latitude range margins of Baja California Sur for both species (Figure 1).

4.4.2. Population genetic and genotypic diversity

Global and endemic genetic diversity generally decreased from north to south for *P. torreyi* but not for *P. scouleri* (Figure 1 and Table S1). Local diversity (private alleles) ranged from high in southern populations of *P. torreyi* to an almost complete lack of endemism in the most northern populations (Figure 1a). The two species differed particularly in their contrasting northernmost range, where *P. torreyi* displayed very low within-population diversities whereas *P. scouleri* showed some of the highest diversity metrics in the north (Table S1).

More than half of the populations from both species had significant heterozygote excess relative to the expectation under random mating (Table S1, significant negative F_{IS}). For dataset 2, *P. iwatensis* revealed lower genetic diversity relative to the two eastern species (e.g., mean A=2.5; $\hat{A}_7$ =2.06 ± 0.1 and P$\hat{A}$= 0.37 ± 0.75; Table S2) and significant heterozygote excess (negative F_{IS}) in all populations (Table S2).

When P_{sex} values were higher than 0.01, the identical MLGs were considered derived from independent sexual reproduction events (i.e., distinct seeds), resulting in a total of 494 unique genotypes for dataset 1 (G= 255 for *P. torreyi* and G= 239 for *P. scouleri;* Table S1). The mean global genotypic richness was relatively high for both species (R=0.88 and R=0.92; Table S1), indicating predominant sexual reproduction. The western species *P. iwatensis* had slightly lower clonal richness, but still a larger proportion of the sampled shoots were derived from sexual reproduction rather than clonal propagation (R=0.74, Table S2).

4.4.3. Genetic differentiation and structure

The structure results for the three species combined (dataset 2), showed differentiation between the three species, the same genetic structure for *P. torreyi* and *P. scouleri* (as in dataset 1) and *P. iwatensis* forming a distinctive cluster. The northernmost population of the eastern Pacific (Vancouver, *P. scouleri*) had individuals with a proportion of the genome assigned to the same group as the western Pacific species, *P. iwatensis*, in addition to the admixture already previously identified with its more southerly sister species, *P. torreyi* (Figure 2). The FCA retrieved similar results supporting the differentiation between the western versus eastern species along the first axis, and among the two eastern species along the second axis (Figure 2).

Structure analysis including all Eastern Pacific samples of both species revealed, at the first hierarchal level, a separation between two species (Figure S3). However, some populations morphologically identified as *P. scouleri* showed genetic admixture from *P. torreyi* (Figure S3 and S4), specifically at the two range extremes of *P. scouleri* (its southernmost and northernmost limits) and at an additional site (San Simeon) in the middle of the distribution of both species.

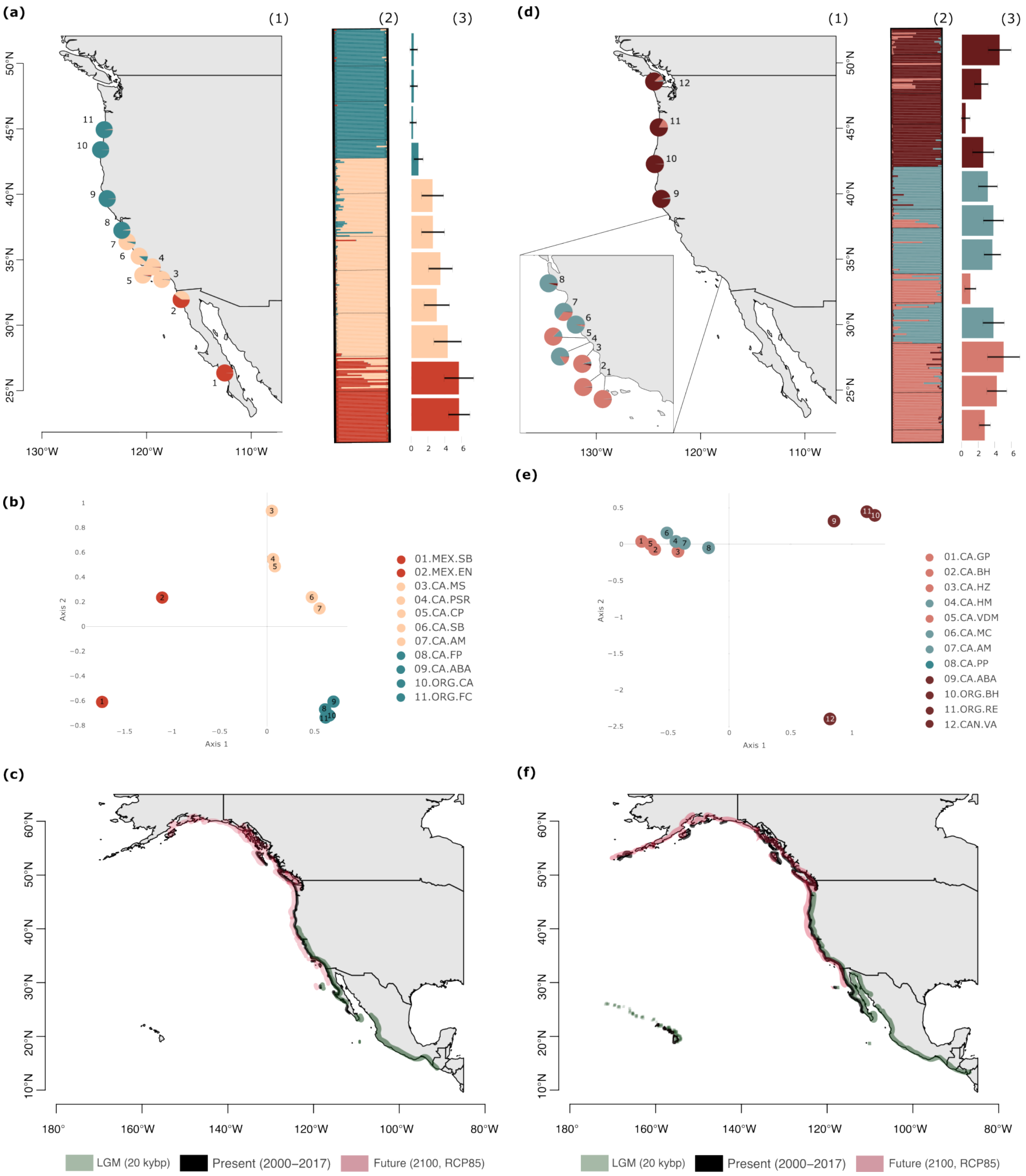

Figure 1: (a) Map of the sampled sites with pie-charts depicting genetic subdivision inferred with STRUCTURE for *P. torreyi* (a_1, a_2); Standardized private diversity for *P. torreyi* (a_3). **(b)** Genetic differentiation of *P. torreyi* illustrated by factorial correspondence analysis. **(c)** Potential occurrence of *P. torreyi* predicted for the Last Glacial Maximum (line in green), Present (line in black) and Future (line in red) using RCP 8.5 greenhouse gas scenario. **(d)** Map of the sampled sites with pie-charts depicting genetic subdivision inferred with STRUCTURE for *P. scouleri* (d_1, d_2); Standardized private diversity for *P. scouleri* (d_3). **(e)** Genetic differentiation of *P. scouleri* illustrated by factorial correspondence analysis. **(f)** Potential occurrence of *P. scouleri* predicted for the Last Glacial Maximum (line in green), Present (line in black) and Future (line in red) using RCP 8.5 greenhouse gas scenario.

The subsequent hierarchical level of genetic structure (K=5) divided south, center, and northern populations for both species (Figure 1a, 1b, 1d, 1e), with some admixture as previously shown at the main split into two species K=2. The same three groups per species were corroborated when structure analyses were run for each species separately (Figure 1a, 1e). *P. torreyi* populations were divided in 1) Mexico group (south cluster); 2) South California group (center cluster) and 3) North California/Oregon group (north cluster) (Figure 1a, 1b). The same number of clusters were found for *P. scouleri*: 1) South California (south cluster); 2) Center California (center cluster) and 3) Oregon/Vancouver (north cluster), with south and center clusters having an intermingled transition region (Figure 1d, 1e).

All populations within each species were significantly differentiated (p < 0.01) for both F_{ST} and Jost'*D* estimates (Figures S5 and S6). The pairwise differentiation values within *P. torreyi* and *P. scouleri* were lower between southern populations, whereas the northern populations against all others had the highest differentiation (Figures S5 and S6).

4.5. Discussion

This study shows how range shifts, triggered by climate change along the NE Pacific coast, have shaped the present genetic diversity and structure of *Phyllospadix* populations and are expected to continue causing further changes and losses. Our empirical genetic data combined with hindcasting predictions from SDM show unexpected differences in the distribution of population genetic variability along the ranges of two sister species, which can be explained by differences in their thermal ranges. Our genetic data show a geographic pattern with a northern, center, and southern clusters in both species but with non-coincident boundaries. We also found genetic evidence for gene flow between species in some regions, leaving introgressed alleles in populations of *P. scouleri*, particularly at edge locations that likely represent recent range expansions for this species.

Findings based on analyses of both independent datasets are discussed below starting with the potential effects of past range shifts (LGM) followed by the observed patterns of genetic diversity and the potential consequences of increased carbon emissions to the future distribution and diversity of *Phyllospadix* populations.

4.5.1. Genetic signatures left by past climatic changes

This study shows that the current genetic variability across the distributional range of surfgrass taxa matches the expectations of effects of past range shifts, inferred by distribution models. During the LGM, the northern part of the NE Pacific coast was affected by cold temperature and ice, apart from some scattered areas where species could have persisted between glacial lobes (Mann and Hamilton, 1995; Jacobs et al., 2004). Thus, populations able to persist in any of these refugial areas should have maintained a larger amount of genetic diversity and genetic distances across populations, in contrast with regions that suffered local extinctions followed by recent settlement.

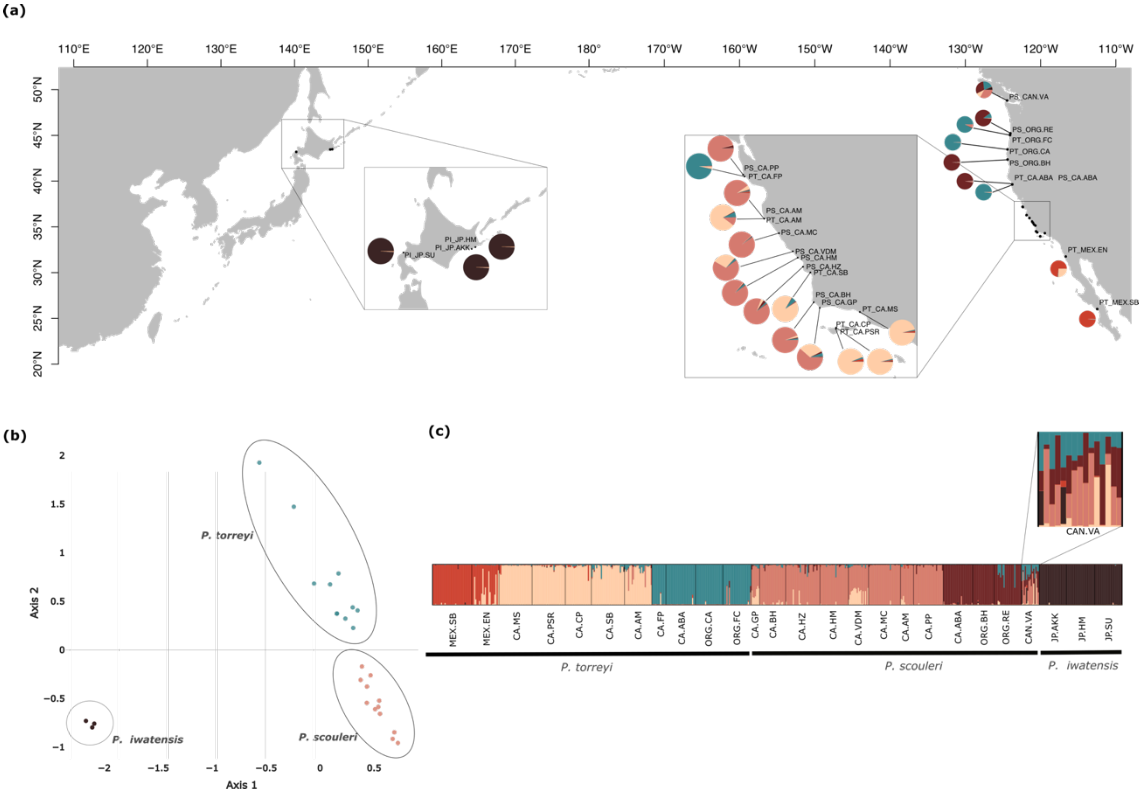

Figure 2: **(a)** Map of the sampled sites along the North Pacific region with pie-charts depicting genetic subdivision inferred with STRUCTURE for *P. torreyi, P. scouleri* and *P. iwatensis*. **(b)** Genetic differentiation between *P. torreyi, P. scouleri* and *P. iwatensis* illustrated by factorial correspondence analysis where each dot is a representative of a population and **(c)** genetic subdivision with STRUCTURE for *P. torreyi*, *P. scouleri* and *P. iwatensis* (k=6) with the southernmost *P. scouleri* population sampled (CAN.VA) highlighted.

In contrast to northern populations, the southern edge populations of *P. torreyi*, located on the Baja California Peninsula in Mexico, were more genetically diverse and differentiated. Higher diversity in more ancient populations at southern range margins is expected in temperate species of the northern hemisphere, as long-term population persistence may have been critical in preserving and accumulating genetic diversity (Hewitt, 1999, 2004; Hampe and Petit, 2005) with no notable effects from drift, local extinctions, or bottlenecks. Population genetic diversity decreasing from lower to higher latitudes corroborates patterns in other intertidal vegetation species (e.g., Neiva et al., 2015; Assis et al., 2018b) and across subtidal marine vegetation as well (e.g., Diekmann and Serrao, 2012; Johansson et al. 2015; Assis et al. 2016, Neiva et al. 2020), all likely to have been affected by the glacial cycles at higher latitudes.

Despite the expectation of lower genetic diversity at higher latitudes (Eckert et al., 2008; Excoffier et al., 2009) due to genetic bottlenecks associated with expansion after colder periods (Marko, 2004), this pattern was not observed in *P. scouleri.* We proposed and analyzed two hypotheses that could explain the high diversity at its northernmost range: 1) admixture of genetically divergent entities such as introgression with western Pacific species of *Phyllospadix*, or 2) long term persistence in this area, which might have remained ice-free up until the late Pleistocene when the glaciers retreated (Mann and Hamilton, 1995; Jacobs et al., 2004). The northernmost population analyzed herein revealed higher private allelic richness but also shared genome assignment with the western Pacific group, suggesting admixture, thereby supporting the hypotheses of hybridization. The alternative hypothesis of incomplete lineage sorting (ILS) is less supported by the evidence because ILS is not expected to affect only the northernmost edge population. This pattern has been found for other NE Pacific species (Jacobs et al., 2004; Carrara et al., 2007).

4.5.2. Future predictions

The Intergovernmental Panel on Climate Change (IPCC) reported that the global temperature is expected to surpass 1.5°C above pre-industrial levels in the next decades, potentially exposing population, and species to detrimental conditions (IPCC, 2022). Accordingly, local population extinctions, shifts, or losses of unique genetic diversity are projected (Chefaoui et al., 2018; Gouvêa et al., 2023). Specifically for our model species, recent observations show that the warming trend of the Baja California peninsula, Mexico, is in line with the RCP 8.5 scenario (Martínez-Austria and Jano-Pérez, 2021). Our projections estimate that both *Phyllospadix* species might have similar trailing edges in the near future as a consequence of range shifts. Due to unsuitable conditions throughout the southern part of the Baja California peninsula, the models project a northward shift of the trailing edge to the Northern more temperate region of Baja California (~30°N) by the end of the century. Baja California Sur, showing high present-day endemic (i.e., private alleles) genetic diversity, lining up with the hypothesis of providing a climatic refugium, is predicted to eventually lose populations there, resulting in a climatic impact of the loss of the global genetic diversity pool of *P. torreyi*. Such loss, dependent on the pace of future range changes, would be irreversible. Moreover, private alleles generally support the potential for species to undergo selection and thereby adapt to future environmental changes, as well as to avoid inbreeding depression (Petit et al., 1998; Hampe and Petit, 2005). Additionally, considering the role of *Phyllospadix* as a foundation species, the regional loss of populations may generate important negative cascading effects to the entire associated tidal community (Shelton, 2010). Therefore, the southern populations of *P. torreyi* have important priority conservation value for hosting the highest and unique genetic diversity found in this study.

On the other hand, the projected northward shifts in the suitable habitats of both *Phyllospadix* species may allow the colonization of new areas, particularly along Canada and Alaska. The projections for the leading-edge differed between both species, with *P. torreyi* potentially experiencing greater poleward expansions along the Gulf of Alaska (to ~57°N) and spreading to new colder and higher-latitude areas with unknown but predictable possible founder effects creating novel mosaics of genetic diversity and structure. In contrast, for *P. scouleri*, the poleward expansion is projected to be less extensive along this coastline, but its colder tolerance suggests that it might hypothetically find opportunities to expand further west into Russian coastlines, although such habitats are not part of the focus of the present study.

Based on projections, neither *Phyllospadix* species is in danger of extinction, despite the previously mentioned negative effects on their genetic diversity levels and localized loss of ecosystem services. However, because only a part of the genetic diversity is expected to shift, the spread into new regions may result in a high frequency of particular genetic variants due to allele surfing (Excoffier and Ray, 2008; Neiva et al. 2010). Species may be able to migrate and follow suitable conditions that better fit their niche if the pace of climate changes allows (Hewitt, 2000; Ackerly, 2003). This process will result in a gradient of increasing genetic diversity toward the leading edge (McInerny et al., 2009). Moreover, it is important to point out that given the short time frame under the RCP 8.5 projection (i.e., until 2100), species that might have limited dispersal traits, such as the *Phyllospadix* genus (Kendrick et al., 2012), will require a larger amount of time for effective gene flow, increasing the likelihood that alleles become extinct before reaching new areas (Knutsen et al., 2013).

4.6. Conclusion

In this study we provide evidence of past climate change effects on genetic diversity and structure. Genetic patterns revealed in this study provide useful information to understand and predict the influence of climate-driven range shifts of the past and future and for population conservation planning and management along the NE Pacific coast. As the region of Baja California peninsula, a well-known biogeographic transition zone, has shown the greatest genetic variability and was forecasted to be most affected by future climate change, this region has important conservation value. We establish an historical context for future studies that further wish to examine geographical patterns and evolutionary history of these two marine foundation species, by investigating climate associations with present genetic information at multiple timescales. This first inference of how past climatic oscillations are reflected in the present genetic variation for these *Phyllospadix* species raises the question of whether this baseline genetic imprint of the past may shape future forecasted responses of populations to climate change while they shift northwards.

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CHAPTER 5

General Discussion

The main goal this thesis was to comprehend how past and present events influence diversity patterns and the current distribution of target habitat forming species. Thus, this thesis brings new data that contributes to our understanding of the impacts of historical and contemporary processes on genetic diversity, connectivity, and current distributional patterns of marine ecosystem founding plants (seagrasses) along tropical and temperate regions.

This final chapter provides a summary of the key findings from the research articles published or submitted in peer-reviewed scientific journals coupled with final remarks and future work that can be done building on the work presented in this thesis.

5.1. *Halodule wrightii* long range gene flow

Limited empirical evidence hinders our understanding of population connectivity in marine biodiversity, especially for species relying on passive dispersal through ocean currents such as the case for the seagrass *H. wrightii*. Despite its broad geographic range, this species has a limited dispersal capacity, prompting questions about the factors influencing its distribution and gene flow. In Chapter 2 and Chapter 3, I examined the genetic structure and connectivity of the seagrass *H. wrightii* across its (almost) entire distributional range (Chapter 2) and focused on the West Africa coast (Chapter 3).

In general, I found that the observed genetic structure did not completely align with the predictions based on particle simulation of ocean currents, suggesting that long-distance dispersal (LLD) might be a rare event (Smith et al., 2018). Indeed, LLD is supposed to be rare as gene flow caused by present day dispersal would potentially play a minor role when compared to past events (Clausen et al., 2002). However, the extent to which present-day dispersal contributes to genetic connectivity and differentiation may vary depending on the species, its life history traits, the characteristics of the dispersal mechanism, and the spatial scale analyzed. Therefore, when studying populations genetic structure and gene flow it is important to consider both historical LDD events and present-day dispersal. However, in both chapters (2 and 3) I only used genetic analysis and biophysical modelling. These analyses may not always effectively captured migration patterns or ancestral polymorphism, considering ongoing gene flow and other factors such as recent historical events and ecological contexts. Additionally, shared ancestral polymorphism, where similar genetic variants are present in different populations due to their common ancestry rather than recent gene flow, may confound the interpretation of contemporary gene flow. Furthermore, ecological factors such as habitat connectivity, dispersal barriers, and species-specific behaviors can also influence gene flow but may not be fully accounted for in genetic structure analyses. Complex demographic processes, such as population bottlenecks, founder events, or changes in population size over time, can also impact the genetic structure of populations and affect the inference of gene flow based solely on genetic structure analysis. Thus, including demographic analysis in both studies perhaps could have improved the overall quality of both chapters, something that can be planned for future studies on this tropical seagrass species.

Nevertheless, based on genetic analysis coupled with the biophysical modelling of particle simulation, I was able to hypothesize that biotic transportation, potentially facilitated by grazers, is a potential mechanism promoting *H. wrightii* populations connectivity. The role of biotic and abiotic factors in genetic diversity and its spatial arrangement brings significant challenges, as they interact at different spatiotemporal scales. However, there is still a lack of information about the role of animals, particularly mega-herbivores, have in seagrass dispersal. Biotic mechanisms have been largely understudied in seagrasses connectivity studies (versus abiotic vectors) and could be an important contribution for long-distance seagrasses dispersal (Moreira-Saporiti et al., 2023). Overall, both chapters highlight the potential role of animal-mediated transportation in enhancing dispersal potential and emphasized the importance of considering multiple factors, including ocean currents, rafting, human activities, and biotic transportation, to better try to understand population dynamics and connectivity.

Additionally, both chapters revealed variation in genetic diversity and genotypic richness among populations, with reduced diversity at the species range edges, indicating the potential impact of recent founder events or demographic bottlenecks. Thus, both studies might serve as a base to understand metapopulation dynamics as a tool for implementing effective conservation strategies for *H. wrightii*, especially for endangered populations with fragmented habitats.

5.2. Climate induced range shifts on surfgrass populations

The paleoclimatic oscillations of the Quaternary have driven important biogeographical shifts in the two *Phyllospadix* species (Chapter 4) and played a significant role in shaping their genetic diversity and structure along the NE Pacific coast. The genetic data and hindcasting predictions indicated differences in genetic variability along the ranges of the two sister species, potentially due to interspecific differences in thermal ranges. Whereas *P. torreyi* revealed a clear poleward decrease in allelic richness and gene diversity, which supported the long-term refugial and the post-glacial colonization of previously glaciated northern latitudes, *P. scouleri* revealed a different distribution of genetic diversity. Since there is no baseline information about the genetic diversity of these populations, it was hypothesized that *P. scouleri* patterns could be attributed to hybridization with west pacific surfgrass species. Yet, these results should be taken with caution as the northernmost population sampled does not represent the northern limit of the distribution of *P. scouleri*. Due to the distribution of the species in remote areas, our research was unable to cover the entire distribution range. As a result, I acknowledge that this brings limitations to Chapter 4 and may affect the generalizability of our findings. Thus, it is important to consider these limitations when interpreting and extrapolating the results of our research. Despite this constraint, this study provides valuable insights into the targeted seagrass species distribution patterns within the accessible regions, contributing to the overall understanding of *P. torreyi* and *P. scouleri* genetic diversity and structure. Future studies able to cover the species full distribution are essential to further provide a full picture on the hypothesis that still lack testing.

The use of SDMs improved the understanding of some of the mechanisms behind the observed genetic patterns among populations. SDM projections suggested northward shifts of the trailing edge of both species, potential colonization of new areas, and a risk of localized loss of genetic diversity at the southern edge. Such losses may compromise the potential for populations to adapt to further less ideal environmental conditions, especially with forthcoming predicted climatic changes.

Other factors might be important to take into consideration for future studies focused on these species such as the prevailing southwards flowing currents in the Baja California area. These currents may pose a potential obstacle for surfgrass populations to disperse towards the north specially taking in consideration the fast pace of the predicted climatic changing conditions. However, local conditions and water circulation patterns differ from the broader current regime (e.g., upwelling zones and eddies) making it difficult (with current available results) to verify how much the intraspecific diversity is an outcome between past climate-driven ranges (Chapter 4) or contemporary ecological condition that mediate populations connectivity. As ocean currents strongly influence populations isolation or connectivity, coupling the results of Chapter 4 with contemporary connectivity analysis could be a major improvement to further infer the imprint extant that both (past and contemporary) drivers have in surfgrass population genetic diversity and structure.

5.3. Final remarks

This thesis combines distinct methodologies, including genetic analysis, biophysical modeling, and species distribution models. Notably, this study was focused in one of the least studied regions for seagrasses in the world, the western coast of Africa. Furthermore, it addresses a significant knowledge gap by examining the influence of climate-induced range shifts on the genetic diversity and distribution of two *Phyllospadix* species that have not been studied in this context.

This thesis significantly advances our understanding on population connectivity in a tropical seagrass species. As the first study to comprehensively gather samples and records spanning the (almost) entire distributional range of *H. wrightii*, the findings on connectivity among its populations serve as a crucial baseline for future investigations focused on resilience, genetic diversity, adaptation, and speciation within this species. These insights hold immense value for the conservation and management of critical coastal habitats, offering practical implications for their preservation and sustainable use.

The work conducted throughout this study holds significant importance, particularly concerning temperate species. With projected increases in global warming, it is highly probable that we will witness unprecedented shifts in species distribution and changes in gene pools. Such changes could lead to substantial transformations in coastal communities' composition. Moreover, the warming trend is expected to interact with other variables such as intensified storms, ocean acidification, and habitat loss, further risking the survival of species. Given these circumstances, the identification of refuge areas where species can persist under changing environment conditions becomes paramount for biodiversity preservation. Therefore, future research that focuses on temperate seagrass species assumes significant importance. Such research will allow us to evaluate how species will respond to climate change and predict the ecological and evolutionary consequences that lie ahead. By gaining insights into these aspects, we can better inform conservation efforts and safeguard the delicate balance of our ecosystems.

In summary, by integrating multiple sources of evidence and conducting research in both tropical and temperate coastal shores, this study makes a substantial and innovative contribution to our understanding of the contemporary, temporal, and evolutionary processes that influence the genetic diversity, connectivity, and distribution of the studied seagrass species. The findings establish a crucial baseline for future research, enabling researchers to investigate into the patterns of connectivity and distributional changes of these species. It offers a valuable framework for exploring the complex interplay among genetic diversity, connectivity, and distribution within seagrass ecosystems. This comprehensive approach enhances the robustness of our knowledge, providing valuable insights into the dynamic nature of seagrass ecosystems. Through these combined efforts, this research paves the way for further research that will undoubtedly refine our knowledge and reveal new dimensions of seagrass ecosystems.

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SUPPLEMENTARY MATERIAL

Supplementary Material

Chapter 2

Table S1 - Geographic location and genetic diversity indices for sampled populations of *Halodule wrightii* (genet-level). Code (#); Population; Location; Country; sample size (N); number of unique genotypes (G); genotypic richness (R); standardized allelic richness (Â); standardized number of private alleles (PÂ) for a common sample size of 2 individuals; gene diversity or expected heterozygosity (H_E); observed heterozygosity (H_O); and multi-locus inbreeding coefficient (F_{IS}) (*=non-significant, p>0.05).

	#	Population	Location	Country	N	G	R	A	Â (2)	PÂ (2)	H_E	H_O	F_{IS}
	1	TE.CC	Corpus Christi	Texas	27	12	0.42	4.63	2.48 ± 0.33	8.44 ± 1.84	0.68	0.67	0.02*
	2	TE.PM	Port Mansfield		28	26	0.93	3.88	1.92 ± 0.21	6.64 ± 1.84	0.46	0.38	0.18*
Mean (Gulf of Mexico)							*0.68*	*4.26*	*2.2±0.27*	*7.54±1.84*	*0.57*	*0.53*	*0.10*
	3	BQ.AQ	Aquarium site	St. Eustatius, Caribbean Netherlands	29	7	0.21	2.13	1.47 ± 0.2	0.57 ± 0.86	0.23	0.32	-0.38
	4	BQ.OB	Oranjested Bay		30	12	0.38	1.75	1.39 ± 0.15	0.06 ± 0.26	0.20	0.28	-0.38
	5	BQ.WW	White Wall		18	3	0.12	1.50	1.29 ± 0.16	0.09 ± 0.31	0.15	0.21	-0.47
	6	CW.BA	Boka Ascension	Curaçao	28	7	0.22	1.50	1.42 ± 0.06	0.14 ± 0.34	0.21	0.33	-0.56
	7	CW.SM	St. Michiel		37	4	0.08	1.38	1.38 ± 0.00	1.00 ± 0.00	0.19	0.35	-0.84
	8	CW.SW	Spanish Water Entrance		27	5	0.15	1.50	1.4 ± 0.07	0.09 ± 0.28	0.20	0.31	-0.56
	9	BR.FN	Fernando de Noronha	Brazil	44	8	0.16	2.00	1.33 ± 0.14	0.56 ± 0.88	0.17	0.22	-0.37
	10	BR.OLI	Olinda		23	17	0.73	2.13	1.69 ± 0.17	0.75 ± 0.63	0.35	0.38	-0.08*
	11	BR.C	Central Brazil		10	8	0.78	2.13	1.65 ± 0.13	1.58 ± 0.80	0.34	0.46	-0.38
	12	BR.LC	Lagoa da Conceição		11	2	0.10	1.37	1.3 ± 0.06	0.41 ± 0.49	0.16	0.28	-0.84
Mean (West Atlantic)							*0.19*	*1.63*	*1.40±0.14*	*0.49±0.42*	*0.20*	*0.32*	*-0.43*
	13	MR	Banc d'Arguin	Mauritania	24	10	0.39	2.13	1.56 ± 0.10	0.3 ± 0.49	0.28	0.44	-0.56
	14	SN	Joal-Fadiouth	Senegal	30	8	0.24	1.63	1.32 ± 0.13	0.22 ± 0.41	0.16	0.24	-0.49
	15	CV	Santiago Island	Cabo Verde	21	9	0.40	1.63	1.39 ± 0.12	0.59 ± 0.49	0.20	0.29	-0.48
	16	GNB	Unhocomo Island	Guiné-Bissau	26	14	0.52	2.50	1.44 ± 0.18	1.06 ± 0.73	0.21	0.29	-0.35
	17	STP	São Tomé e Príncipe	São Tomé e Príncipe	14	12	0.85	2.38	1.69 ± 0.11	0.71 ± 0.73	0.34	0.47	-0.40
	18	GB/STP	Corisco Bay /Santana	Gabon/São Tomé e Principe	9	8	0.88	2.00	1.61 ± 0.14	0.42 ± 0.49	0.32	0.39	-0.25
	19	AO	Mussulo Bay	Angola	39	4	0.08	1.75	1.51 ± 0.13	0.01 ± 0.08	0.26	0.43	-0.69
Mean (West Africa)							*0.40*	*2.00*	*1.51±0.13*	*0.42±0.49*	*0.26*	*0.39*	*-0.46*
Total					*475*	*176*							

Table S2 - Geographic location and genetic diversity indices for sampled populations of *Halodule wrightii* (ramet-level). Code (#); Population; Location; Country; sample size (N); number of unique genotypes (G); genotypic richness (R); standardized allelic richness (Â); standardized number of private alleles (PÂ) for a common sample size of 2 individuals; gene diversity or expected heterozygosity (H_E); observed heterozygosity (H_O); and multi-locus inbreeding coefficient (F_{IS}) (*=non-significant, p>0.05).

	#	Population	Location	Country	N	G	R	A	Â (2)	PÂ (2)	H_E	H_O	F_{IS}
	1	TE.CC	Corpus Christi	Texas	27	12	0.42	4.63	2.43 ± 0.4	8.66 ± 2.20	0.65	0.68	-0.04*
	2	TE.PM	Port Mansfield		28	26	0.93	3.88	1.92 ± 0.22	6.73 ± 1.73	0.46	0.38	0.17*
Mean (Gulf of Mexico)							*0.68*	*4.26*	*2.12 ± 0.31*	*7.70 ± 1.97*	*0.56*	*0.53*	*0.07*
	3	BQ.AQ	Aquarium site	St. Eustatius, Caribbean Netherlands	29	7	0.21	2.13	1.32 ± 0.21	0.44 ± 0.76	0.16	0.22	-0.41
	4	BQ.OB	Oranjested Bay		30	12	0.38	1.75	1.35 ± 0.15	0.17 ± 0.43	0.18	0.25	-0.38
	5	BQ.WW	White Wall		18	3	0.12	1.50	1.2 ± 0.13	0.09 ± 0.33	0.098	0.16	-0.66
	6	CW.BA	Boka Ascension	Curaçao	28	7	0.22	1.50	1.42 ± 0.06	0.03 ± 0.16	0.21	0.34	-0.66
	7	CW.SM	St. Michiel		37	4	0.08	1.38	1.38 ± 0.00	1.00 ± 0.00	0.19	0.36	-0.94
	8	CW.SW	Spanish Water Entrance		27	5	0.15	1.50	1.4 ± 0.05	0.08 ± 0.27	0.20	0.35	-0.78
	9	BR.FN	Fernando de Noronha	Brazil	44	8	0.16	2.00	1.34 ± 0.13	0.54 ± 0.83	0.17	0.24	-0.43
	10	BR.OLI	Olinda		23	17	0.73	2.13	1.68 ± 0.18	0.74 ± 0.66	0.35	0.38	-0.07*
	11	BR.C	Central Brazil		10	8	0.78	2.13	1.65 ± 0.13	1.59 ± 0.81	0.34	0.46	-0.38
	12	BR.LC	Lagoa da Conceição		11	2	0.10	1.38	1.29 ± 0.06	0.35 ± 0.48	0.15	0.28	-0.86
Mean (West Atlantic)							*0.19*	*1,74*	*1.40±0.11*	*0.50 ±0.47*	*0.20*	*0.30*	*-0.56*
	13	MR	Banc d'Arguin	Mauritania	24	10	0.39	2.13	1.55 ± 0.09	0.23 ± 0.44	0.28	0.45	-0.63
	14	SN	Joal-Fadiouth	Senegal	30	8	0.24	1.63	1.32 ± 0.12	0.20 ± 0.4	0.16	0.24	-0.52
	15	CV	Santiago Island	Cabo Verde	21	9	0.40	1.63	1.4 ± 0.12	0.63 ± 0.48	0.20	0.30	-0.49
	16	GNB	Unhocomo Island	Guiné-Bissau	26	14	0.52	2.50	1.42 ± 0.17	1.11 ± 0.71	0.21	0.28	-0.37
	17	STP	São Tomé e Príncipe	São Tomé e Príncipe	14	12	0.85	2.50	1.69 ± 0.12	0.77 ± 0.74	0.35	0.46	-0.32
	18	GB/STP	Corisco Bay /Santana	Gabon/São Tomé e Principe	9	8	0.88	2.00	1.61 ± 0.14	0.42 ± 0.49	0.32	0.39	-0.25
	19	AO	Mussulo Bay	Angola	39	4	0.08	1.75	1.44 ± 0.10	0.00 ± 0.04	0.22	0.40	-0.83
Mean (West Africa)							*0.40*	*2.02*	*1.49±0.12*	*0.48 ± 0.47*	*0.25*	*0.36*	*-0.49*
Total					*475*	*176*							

Table S3 - Inbreeding coefficient (F_{IS}) per population, ramet- level (*=non-significant, p>0.05).

Population	F_{IS}
TE.PM	-0.03886*
TE.PM	0.16640*
BQ.AQ	-0.40541
BQ.OB	-0.37984
BQ.WW	-0.65678
CW.BA	-0.66019
CW.SM	-0.94497
CW.SW	-0.77920
BR.FN	-0.42670
BR.OLI	-0.07052*
BR.C	-0.37888
BR.LC	-0.86047
MR	-0.62617
SN	-0.52355
CV	-0.48810
GNB	-0.36511
STP	-0.32072
GB/STP	-0.25140
AO	-0.83469

Table S3 – Single locus inbreeding coefficient (F_{IS}) values calculated for the ramet-level dataset for each population (*=non-significant, p>0.05).

Locus	Population	F_{IS}
HW 180	TE.CC	-0.31955
	TE.PM	-0.08829*
	BQ.AQ	-0.04186*
	BQ.OB	-0.03571*
	BQ.WW	0.00000*
	BR.FN	-0.17808*
	BR.OLI	-0.10000*
	BR.C	0.04255*
	MR	-0.05143*
	CV	-0.21212*
	GNB	-0.02041*
	STP	-0.00000*
	AO	-0.01333*
HW 188	TE.CC	0.31579*
	TE.PM	0.30591*
	BQ.AQ	-0.16667*
	BQ.OB	-0.09434*
	BQ.WW	-0.03030*
	CW.BA	-0.85224
	CW.SM	-0.88906
	CW.SW	-0.84659
	BR.FN	0.24138*
	BR.OLI	0.00000*
	BR.C	-0.05263*
	MR	0.00000*
	STP	-0.12069*
	GB/STP	0.58974*
	AO	-1.00000
HW 190	TE.CC	0.22139*
	TE.PM	0.08280*
	BQ.AQ	-1.00000
	BQ.OB	-1.00000
	BQ.WW	-1.00000
	CW.BA	-1.00000
	CW.SM	-0.94595
	CW.SW	-1.00000
	BR.FN	-0.95455
	BR.OLI	-0.60127
	BR.C	-1.00000
	BR.LC	-1.00000
	MR	-1.00000
	SN	-0.93333
	CV	-0.81818
	GNB	-1.00000
	STP	-1.00000

	GB/STP	-1.00000
	AO	-1.00000
HW 196	TE.CC	-0.62500
	TE.PM	0.00000*
	BQ.AQ	0.00000*
	BQ.OB	-0.09434*
	BQ.WW	-0.03030*
	BR.FN	-0.06679*
	BR.OLI	-0.46667
	BR.C	0.00000*
	MR	-0.32374
	SN	-0.01754*
	CV	-0.08108*
	GNB	0.00568*
	STP	-0.02463*
	GB/STP	-0.06667*
HW 200	TE.PM	0.24492*
	TE.PM	0.29493*
	BR.C	-0.05882*
HW 212	TE.PM	-0.15789*
	TE.PM	0.31646*
	BQ.OB	-0.00578*
	CW.BA	-0.24008
	CW.SM	-1.00000
	CW.SW	-0.52163
	BR.FN	-0.12602*
	BR.OLI	0.49425*
	BR.C	1.00000*
	BR.LC	-1.00000
	MR	0.00000*
	SN	-0.03571*
	GNB	-0.32743
	STP	0.25180*
	GB/STP	-0.47368*
	AO	-0.90000
HW 190b	TE.PM	0.13028*
	TE.PM	0.15515*
	BR.OLI	0.08626*
	BR.C	-0.39806*
	MR	-0.76923
	SN	-0.09434*
	CV	-0.05263*
	GNB	0.48718*
	STP	-0.36842*
	GB/STP	-0.14286*
	AO	-0.04110*
HW 228	TE.PM	-0.24121
	TE.PM	0.00000*

	BQ.AQ	0.32121*
	BQ.OB	-0.01754*
	BR.C	-1.00000
	MR	-0.70370
	SN	-0.41463
	CV	-0.60000
	GNB	-0.06383*
	STP	-0.53763
	GB/STP	-0.33333*
	AO	-0.04110*

Table S4 - Geographic location and genetic diversity indices for the first hierarchal level of Structure subdivision (K=3) for *Halodule wrightii.* Cluster; sample size (N); number of unique genotypes (G); genotypic richness (R); standardized allelic richness (Â); standardized number of private alleles (PÂ) for a minimum common sample size of 38 individuals; gene diversity or expected heterozygosity (H_E); observed heterozygosity (H_O); and multi-locus inbreeding coefficient (F_{IS}).

Cluster	**N**	**G**	**R**	**A**	**Â** $_{(38)}$	**PÂ** $_{(38)}$	H_E	H_O	F_{IS}
Gulf of Mexico	55	38	0.69	5.38	5.38±0.00	34.02±0.64	0.58	0.47	0.19
West Atalntic	257	61	0.23	5.13	4.08±0.27	19.11±1.79	0.50	0.31	0.38
West Africa	163	52	0.31	3.62	2.92±0.20	9.72±1.46	0.27	0.34	-0.27

Table S5 – *Halodule wrightii* data from literature, our samples and biodiversity database.

LAT_WGS84_DEG	LON_WGS84_DEG	Local name	Country	Source/Reference
19.739766	-16.457939	Banc d'Arguin	Mauritania	Wolff. W. J.. et al. "Biomass of macrobenthic tidal flat fauna of the Banc d'Arguin. Mauritania."Hydrobiologia, 258.1-3 (1993): 151-163.
20.228214	-16.225236	Banc d'Arguin	Mauritania	Cardona. Luis. A. Aguilar. and L. Pazos. "Delayed ontogenic dietary shift and high levels of omnivory in green turtles (Chelonia mydas) from the NW coast of Africa."Marine Biology, 156.7 (2009): 1487-1495.
19.48	-16.445	Teichott, Banc d'Arguin	Mauritania	Biogeographical Ecology and Evolution Group, CCMAR
14.27166667	-16.90472222	Joal Fadiouth	Senegal	Cunha. A. H.. & Araújo. A. (2009). New distribution limits of seagrass beds in West Africa. Journal of Biogeography. 36(8). 1621-1622. doi:10.1111/j.1365-2699.2009.02135.x
14.15222222	-16.83416667	Bamboung-Sourou area	Senegal	Cunha. A. H.. & Araújo. A. (2009).New distribution limits of seagrass beds in West Africa. Journal of Biogeography. 36(8). 1621-1622.doi:10.1111/j.1365-2699.2009.02135.x
14.156	-16.841	Fadiouth	Senegal	Biogeographical Ecology and Evolution Group, CCMAR
13.384509	-16.811234	Bijol Islands	Gambia	https://resiliensea.org/2020/01/27/participant-diary-from-the-national-seagrass-species-identification-mapping-and-monitoring-training-in-the-gambia/
14.90888889	-23.51027778	Santiago Island	Cabo Verde	Creed. Joel C.. et al. "First record of seagrass in Cape Verde. eastern Atlantic."Marine Biodiversity Records, 9.1 (2016): 57.
14.912	-23.508	Gamboa, Praia (Santiago)	Cabo Verde	Biogeographical Ecology and Evolution Group, CCMAR
11.281	-16.382	Acampamento, Unhocomo	Guinea-Bissau	Biogeographical Ecology and Evolution Group, CCMAR
11.312	-16.405	Ancante, Unhocomo	Guinea-Bissau	Biogeographical Ecology and Evolution Group, CCMAR
7.641079	-13.05448	Moot Island	Sierra Leone	https://news.grida.no/finding-treasure-in-the-turtle-islands
7.660010	-13.029801	Mania	Sierra Leone	ResilienSEA porject
7.660056	-13.029837	Mania	Sierra Leone	ResilienSEA porject
7.660111	-13.029945	Mania	Sierra Leone	ResilienSEA porject
7.660138	-13.030000	Mania	Sierra Leone	ResilienSEA porject
7.660129	-13.030000	Mania	Sierra Leone	ResilienSEA porject
7.660111	-13.030045	Mania	Sierra Leone	ResilienSEA porject
7.660093	-13.030045	Mania	Sierra Leone	ResilienSEA porject
7.660808	-13.030160	Mania	Sierra Leone	ResilienSEA porject
7.659867	-13.030173	Mania	Sierra Leone	ResilienSEA porject

7.659858	-13.030182	Mania	Sierra Leone	ResilienSEA porject
7.659707	-13.030763	Mania	Sierra Leone	ResilienSEA porject
7.659471	-13.030374	Mania	Sierra Leone	ResilienSEA porject
7.659403	-13.031480	Mania	Sierra Leone	ResilienSEA porject
0.414204	6.68026	Cabras islet	São Tomé e Príncipe	Alexandre. Ana. et al. "First description of seagrass distribution and abundance in São Tomé and Príncipe."Aquatic Botany, 142 (2017): 48-52.
0.395462	6.730666	Cabras islet	São Tomé e Príncipe	Alexandre. Ana. et al. "First description of seagrass distribution and abundance in São Tomé and Príncipe."Aquatic Botany, 142 (2017): 48-52.
0.246614	6.7465	Santana Bay	São Tomé e Príncipe	Alexandre. Ana. et al. "First description of seagrass distribution and abundance in São Tomé and Príncipe."Aquatic Botany, 142 (2017): 48-52.
1.632689	7.454281	Abade beach (island of Pri?ncipe)	São Tomé e Príncipe	Alexandre. Ana. et al. "First description of seagrass distribution and abundance in São Tomé and Príncipe."Aquatic Botany, 142 (2017): 48-52.
0.57277778	6.86166667	Ilheu das Cabras	São Tomé e Príncipe	Hancock. Joana M.. et al. "Stable isotopes reveal dietary differences and site fidelity in juvenile green turtles foraging around São Tomé Island. West Central Africa."Marine Ecology Progress Series, 600 (2018): 165-177.
1.633	7.455	Abade beach, Pagué, Príncipe	São Tomé e Príncipe	Biogeographical Ecology and Evolution Group, CCMAR
1.635	7.456	Abade beach, Pagué, Príncipe	São Tomé e Príncipe	Biogeographical Ecology and Evolution Group, CCMAR
0.414	6.656	Cabras Islet	São Tomé e Príncipe	Biogeographical Ecology and Evolution Group, CCMAR
0.255	6.747	Santana	São Tomé e Príncipe	Biogeographical Ecology and Evolution Group, CCMAR
0.892721	9.349402	Corisco Bay	Gabon	http://www.yaqupacha.org/fileadmin/user_upload/pdf/seagrass_magazine_46_2012.pdf
0.594	9.307	Corisco Bay	Gabon	Biogeographical Ecology and Evolution Group, CCMAR
-9.006	13.064	Mussulo Bay	Angola	Biogeographical Ecology and Evolution Group, CCMAR
-8.95653	13.108091	Ba¡a do Mussulo	Angola	Santos. Carmen I. "Comunidades de macroinvertebrados e peixes associadas … pradaria marinha de Halodule wrightii (Ascherson. 1868) na Laguna do Mussulo. Angola." (2007).
27.718	-97.324	Corpus Christi, Texas	United States	Biogeographical Ecology and Evolution Group, CCMAR
26.319	-97.223	Port Mansfield, Texas	United States	Biogeographical Ecology and Evolution Group, CCMAR
34.71666667	-76.66666667	Beaufort, North carolina	United States	Biber. Patrick D.. W. Judson Kenworthy. and Hans W. Paerl. "Experimental analysis of the response and recovery of Zostera marina (L.) and Halodule wrightii (Ascher.) to repeated light-limitation stress."Journal of Experimental Marine Biology and Ecology369.2 (2009): 110-117.
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		of Sebastian River, Florida		
30.38333333	-87.4	Big Lagoon. Perdido Bay, Florida	United States	Heck Jr. K. L.. et al. "Effects of nutrient enrichment and grazing on shoalgrass Halodule wrightii and its epiphytes: results of a field experiment."Marine Ecology Progress Series326 (2006): 145-156.
28.34527778	-80.70361111	Indian River Lagoon , Florida	United States	Taplin. K. A.. Irlandi. E. A.. & Raves. R. (2005).Interference between the macroalga Caulerpa prolifera and the seagrass Halodule wrightii. Aquatic Botany. 83(3). 175-186.doi:10.1016/j.aquabot.2005.06.003
28.10833333	-82.78611111	Indian Bluff Island, Florida	United States	Dawes. C. J.. & Lawrence. J. M. (1980).Seasonal changes in the proximate constituents of the seagrasses Thalassia testudinum. Halodule wrightii. and Syringodium filiforme. Aquatic Botany. 8. 371-380.doi:10.1016/0304-3770(80)90066-2
30.3	-87.5	Perdido Bay, Alabama	United States	Shafer. Deborah J. "The effects of dock shading on the seagrass Halodule wrightii in Perdido Bay. Alabama."Estuaries22.4 (1999): 936-943.
27.35	-97.36666667	Laguna Madre, Texas	United States	Burd. Adrian B.. and K. H. Dunton. "Field verification of a light-driven model of biomass changes in the seagrass Halodule wrightii."Marine Ecology Progress Series209 (2001): 85-98.
26.15	-97.23416667	Gulf Intra-coastal Waterway, Texas	United States	Kowalski. Joseph L.. et al. "Productivity estimation in Halodule wrightii Aschers: comparison of leaf-clipping and leaf-marking techniques and the importance of clip height."Marine Ecology Progress Series220 (2001): 131-136.
26.06666667	-97.15	Isla Blanca Park. South Padre Island, Texas	United States	Kowalski. Joseph L.. and Hudson R. DeYoe. "Flowering and seed production in the subtropical seagrass. Halodule wrightii (shoal grass)."Botanica marina59.2-3 (2016): 193-199.
18.620293	-91.787836	Laguna de Términos	Mexico	Coria-Monter. Erik. and Elizabeth Dur n-Campos. "Análisis proximal de los pastos marinos de la Laguna de Términos, México."Hidrobiológica, 25.2 (2015): 249-255.
24.50416667	-97.74055556	Punta Carbajal	Mexico	Rodr¡guez-Almaraz. Gabino A.. and Víctor M. Ortega-Vidales. "Primer registro de Caprella scaura y Caprella penantis (Crustacea: Peracarida: Amphipoda) en la laguna Madre. Tamaulipas. México."Revista mexicana de biodiversidad84.3 (2013): 989-993.
19.589034	-96.381382	Laguna de la Mancha	Mexico	del Pilar Reyes-Barrag n. Ma. and Sergio I. Salazar-Vallejo. "Bentos asociado al pastizal de Halodule (Potamogetonaceae) en Laguna de la Mancha. Veracruz. México."Revista de Biolog¡a Tropical(1990): 167-173.
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12.01416667	-69.83305556	El Supí	Venezuela	Mariño, Joany, María Daniela Mendoza, and Beatriz López-Sánchez. "Composition and abundance of decapod crustaceans in mixed seagrass meadows in the Paraguaná Peninsula, Venezuela." Iheringia. Série Zoologia 108 (2018).
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17.7575	-64.59638889	St. Croix, Virgin Islands	United States	Feser. Kelsey M.Utilizing the Subfossil Record of Seagrass-Associated Mollusks to Reveal Recent Changes in Coastal Marine Environments. Diss. University of Cincinnati. 2015.
12.273369	-69.052366	Ascension	Curaçao	Stuij. T. M.Distinct Microbiomes in three Tropical seagrasses around the island of Curaçao: Halophila stipulacea. Halodule wrightii and Thalassia testudinum. MS thesis. 2018.
12.204255	-69.052609	Jankok	Curaçao	Stuij. T. M.Distinct Microbiomes in three Tropical seagrasses around the island of Curaçao: Halophila stipulacea. Halodule wrightii and Thalassia testudinum. MS thesis. 2018.

12.066101	-68.853585	Spanish Water	Curaçao	Stuij. T. M.Distinct Microbiomes in three Tropical seagrasses around the island of Curaçao: Halophila stipulacea. Halodule wrightii and Thalassia testudinum. MS thesis. 2018.
12.272	-69.055	Boka ascension	Curaçao	Biogeographical Ecology and Evolution Group, CCMAR
12.148	-68.998	St. Michiel	Curaçao	Biogeographical Ecology and Evolution Group, CCMAR
12.066	-68.854	Spanish water entrance	Curaçao	Biogeographical Ecology and Evolution Group, CCMAR
17.513	-63.000	Aquarium site, St. Eustatius Island	Netherlands	Biogeographical Ecology and Evolution Group, CCMAR
17.483	-62.988	Oranjested Bay, St. Eustatius Island	Netherlands	Biogeographical Ecology and Evolution Group, CCMAR
17.465	-62.956	White Wall, St. Eustatius Island	Netherlands	Biogeographical Ecology and Evolution Group, CCMAR
10.690153	-61.746430	Chacachacare Island, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.687663	-61.743271	Chacachacare Island, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.684581	-61.740295	Chacachacare Island, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.687684	-61.687504	Monos Island, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.686772	-61.685994	Monos Island, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.697099	-61.679489	Monos Island, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.668788	-61.646508	Gaspar Grande Island, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.662527	-61.597124	Caledonia Island,Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).

10.701635	-61.665601	Scotland Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.697588	-61.664677	Scotland Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.690281	-61.602919	Carenage Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.683852	-61.593131	Carenage Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.682546	-61.584118	Carenage Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.336678	-61.461383	Claxton Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
10.141755	-61.001047	Guayaguayare Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
11.167222	-60.826915	Bon Accord Lagoon, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
11.141501	-60.804047	Canoe Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
11.266350	-60.546648	King’s Bay, Trindade e Tobago	Republic of Trinidad and Tobago	Juman, Rahanna A., and Karlene James Alexander. "An Inventory of Seagrass Communities around Trinidad and Tobago." (2006).
-22.95138889	-43.90777778	Itacurucá Island	Brazil	Filho. G. M. A.. Creed. J. C.. Andrade. L. R.. & Pfeiffer. W. C. (2004).Metal accumulation by Halodule wrightii populations. Aquatic Botany. 80(4). 241-251.doi:10.1016/j.aquabot.2004.07.011
-23.054443	-43.926944	Jaguanum Island	Brazil	Filho. G. M. A.. Creed. J. C.. Andrade. L. R.. & Pfeiffer. W. C. (2004).Metal accumulation by Halodule wrightii populations. Aquatic Botany. 80(4). 241-251.doi:10.1016/j.aquabot.2004.07.011
-22.98333	-44.434444	Saco de Piraquara	Brazil	Filho, G. M. A., Creed, J. C., Andrade, L. R., & Pfeiffer, W. C. (2004).Metal accumulation by Halodule wrightii populations. Aquatic Botany, 80(4), 241-251.doi:10.1016/j.aquabot.2004.07.011
-22.883052	-42.011112	Araruama Lagoon	Brazil	Filho, G. M. A., Creed, J. C., Andrade, L. R., & Pfeiffer, W. C. (2004).Metal accumulation by Halodule wrightii populations. Aquatic Botany, 80(4), 241-251.doi:10.1016/j.aquabot.2004.07.011
-5.104827	-36.321424	Guamare	Brazil	Silva, Juliana, et al. "Biological activities of the sulfated polysaccharide from the vascular plant Halodule wrightii."Revista Brasileira de Farmacognosia22.1 (2012): 94-101.
-17.96666667	-38.7175	Bahia (65 km off the southern coast of Bahia state)	Brazil	de Paula, Alline Figueira, MA de O. Figueiredo, and Joel Christopher Creed. "Structure of the macroalgal community associated with the seagrass Halodule wrightii Ascherson in the Abrolhos Marine National Park, Brazil."Botanica Marina46.5 (2003): 413-424.

-3.69194444	-38.58027778	Goiabeiras Beach	Brazil	Barros, Kcrishna VS, and CRISTINA A. ROCHA-BARREIRA. "Responses of the molluscan fauna to environmental variations in a Halodule wrightii Ascherson ecosystem from Northeastern Brazil."Anais da Academia Brasileira de Ciˆncias85.4 (2013): 1397-1410.
-12.83333333	-38.63333333	Todos os Santos Bay, Bahia	Brazil	Brito, G. B., de Souza, T. L., do N. Costa, F., Moura, C. W. N., & Korn, M. G. A. (2016).Baseline trace elements in the seagrass Halodule wrightii Aschers (Cymodoceaceae) from Todos os Santos Bay, Bahia, Brazil. Marine Pollution Bulletin, 104(1-2), 335-342.doi:10.1016/j.marpolbul.2016.01.044
-7.72944444	-34.82555556	Jaguaribe, Itamaracá Island	Brazil	PITANGA, MARIA ELISA, et al. "Quantification and classification of the main environmental impacts on a Halodule wrightii seagrass meadow on a tropical island in northeastern Brazil."An Acad Bras Cienc84.1 (2012).
-7.75333333	-34.82361111	Pilar, Itamaracá Island	Brazil	PITANGA, MARIA ELISA, et al. "Quantification and classification of the main environmental impacts on a Halodule wrightii seagrass meadow on a tropical island in northeastern Brazil."An Acad Bras Cienc84.1 (2012).
-2.90194444	-41.40944444	Barra Grande Beach, in Piauí State	Brazil	Cavalcante, L. L., Barroso, C. X., Carneiro, P. B. de M., & Matthews-Cascon, H. (2019).Spatiotemporal dynamics of the molluscan community associated with seagrass on the western equatorial Atlantic. Journal of the Marine Biological Association of the United Kingdom, 1-10.doi:10.1017/s0025315419000183
-17.97722222	-38.71666667	Abrolhos archipelago	Brazil	Creed, Joel C., and Gilberto M. Amado Filho. "Disturbance and recovery of the macroflora of a seagrass (Halodule wrightii Ascherson) meadow in the Abrolhos Marine National Park, Brazil: an experimental evaluation of anchor damage."Journal of experimental marine biology and ecology235.2 (1999): 285-306.
-23.5	-45.11666667	Saco da Ribeira, São Paulo	Brazil	Corbisier, Thais Navajas. "Macrozoobentos da Praia do Cod¢ (Ubatuba, SP) e a presen‡a de Halodule wrightii Ascherson."Boletim do Instituto Oceanogr fico42.1-2 (1994): 99-111.
-22.769548	-41.884128	Buzios, Rio de Janeiro	Brazil	Omena, Elianne, and Joel C. Creed. "Polychaete fauna of seagrass beds (Halodule wrightii Ascherson) along the coast of Rio de Janeiro (Southeast Brazil)."Marine Ecology25.4 (2004): 273-288.
-22.882278	-42.005635	Cabo Frio, Rio do Janeiro	Brazil	Omena, Elianne, and Joel C. Creed. "Polychaete fauna of seagrass beds (Halodule wrightii Ascherson) along the coast of Rio de Janeiro (Southeast Brazil)."Marine Ecology25.4 (2004): 273-288.
-22.996072	-43.917471	Jaguanum Island	Brazil	Omena, Elianne, and Joel C. Creed. "Polychaete fauna of seagrass beds (Halodule wrightii Ascherson) along the coast of Rio de Janeiro (Southeast Brazil)."Marine Ecology25.4 (2004): 273-288.
-22.948193	-43.908769	Itacuruçá Island	Brazil	Omena, Elianne, and Joel C. Creed. "Polychaete fauna of seagrass beds (Halodule wrightii Ascherson) along the coast of Rio de Janeiro (Southeast Brazil)."Marine Ecology25.4 (2004): 273-288.
-23.016326	-44.226415	Monsuaba	Brazil	Omena, Elianne, and Joel C. Creed. "Polychaete fauna of seagrass beds (Halodule wrightii Ascherson) along the coast of Rio de Janeiro (Southeast Brazil)."Marine Ecology25.4 (2004): 273-288.
-23.215295	-44.619266	Parati	Brazil	Omena, Elianne, and Joel C. Creed. "Polychaete fauna of seagrass beds (Halodule wrightii Ascherson) along the coast of Rio de Janeiro (Southeast Brazil)."Marine Ecology25.4 (2004): 273-288.
-16.40777778	-38.99305556	Parque Municipal Marinho do Recife de Fora	Brazil	Schneider, Geniane. " Efeitos do aumento de CO^2 na fisiologia, anatomia e ultraestrutura de Halodule wrightii Ascherson" (2014).
-25.45238	-48.443468	Baixio do Perigo, Paran	Brazil	Leis, Mirella de Oliveira. "Interação entre o ambiente sedimentar e pradarias de Halodule wrightii do Complexo Estuarino de Paranaguá-PR."
-25.539571	-48.308123	Saco do Limoeiro, Paran	Brazil	Leis, Mirella de Oliveira. "Interação entre o ambiente sedimentar e pradarias de Halodule wrightii do Complexo Estuarino de Paranaguá-PR."
-3.692549	-38.584539	Goiabeiras beach, Fortaleza	Brazil	Barros, Kcrishna VS, et al. "Seasonal variation of the crustacean fauna in the belowground and aboveground strata in a Halodule wrightii meadow of northeastern Brazil." Iheringia. Série Zoologia 107 (2017).

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-3.867	-32.424	Fernando Noronha	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-7.981	-34.834	Olinda	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-19.911950	-40.097230	Praia Mar Azul, Aracruz	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-19.953790	-40.141317	Praia de Santa Cruz II, Aracruz	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-20.037988	-40.167251	Praia Grande, Fundão	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-20.096035	-40.173115	Praia de Costa Bela, Serra	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-20.119948	-40.175779	Praia de Jacaraípe III, Serra	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-20.200215	-40.192379	Praia de Manguinhos III, Serra	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-19.972685	-40.137331	Praia do Mamão, Aracruz	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-20.301384	-40.285798	Ilha do Frade, Vitória	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-20.324801	-40.271780	Praia do Ribeiro, Vila Velha	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-20.309373	-40.283671	lha do Boi, Vitória	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-27.581	-48.446	Lagoa da Conceição	Brazil	Biogeographical Ecology and Evolution Group, CCMAR
-2.966595	-39.749809	Almofala	Brazil	https://research.unl.pt/ws/portalfiles/portal/3337967/Livro_Braspor_2015_SFRH_BPD_70384_2010_UID_ELT_00657_2013_.pdf
-2.966633	-39.751758	Almofala	Brazil	https://research.unl.pt/ws/portalfiles/portal/3337967/Livro_Braspor_2015_SFRH_BPD_70384_2010_UID_ELT_00657_2013_.pdf
-2.953404	-39.765741	Almofala	Brazil	https://research.unl.pt/ws/portalfiles/portal/3337967/Livro_Braspor_2015_SFRH_BPD_70384_2010_UID_ELT_00657_2013_.pdf
-25.530833	-48.396389	Ilha Rasa da Cotinga	Brazil	Guebert, Flávia Maria. "Ecologia alimentar e consumo de material inorgânico por tartarugas-verdes, Chelonia mydas, no litoral do Estado do Paraná." Master's thesis. Universidade Federal do Paraná, Curitiba, PR, Brazil (2008).
-25.493611	-48.340000	Ilha do Mel	Brazil	Guebert, Flávia Maria. "Ecologia alimentar e consumo de material inorgânico por tartarugas-verdes, Chelonia mydas, no litoral do Estado do Paraná." Master's thesis. Universidade Federal do Paraná, Curitiba, PR, Brazil (2008).
-25.471944	-48.429722	Ilha das Cobras	Brazil	Guebert, Flávia Maria. "Ecologia alimentar e consumo de material inorgânico por tartarugas-verdes, Chelonia mydas, no litoral do Estado do Paraná." Master's thesis. Universidade Federal do Paraná, Curitiba, PR, Brazil (2008).

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19.5063	-16.42793333			iNaturalist

Table S6 – Eight loci used for microsatellite amplification (Larkin et al., 2012; 2017)

Locus	**GenBank accession number**	**Primer sequence (5'-3')**	**Fluorescent dyes**
Hw180	JN614999	F: GTGGAGGCCGAACTGTATCT	FAM
		R: CGACCTTCATCCTAATCATCG	
Hw188	JN615000	F: ACCTTCATAAATGGCAACTTG	ATTO550
		R: CAACTTGGTTCTGGTAGTCATC	
Hw190	JN615001	F: ATGACGAATCCCGAGGTAT	HEX
		R: CCAGGTATTGTCGCTTTCA	
Hw190b	KT002048	F: ATGACGAATCCCGAGGTAT	HEX
		R: GTTTCACCCACGTTAAAGCACAAT	
Hw196	JN615002	F: ACAACCTAGATCATCCTCACAC	HEX
		R: AGCAGGAAGTCAAGAGATAGG	
Hw200	JN615003	F: TTATGGGATCTATTTGTGGTCT	ATTO565
		R: TTTTGCTTTGTAGTCTTGGTG	
Hw212	JN615005	F: ATGGATGTTCATTGAGTTTGAC	FAM
		R: CAAGGCTAAGGTAGTGGACC	
Hw228	KT002050	F: AAGACGGCATTGGAAAATAAG	ATTO550
		R: GTTTGGTATCATCGGAAGCACTGT	

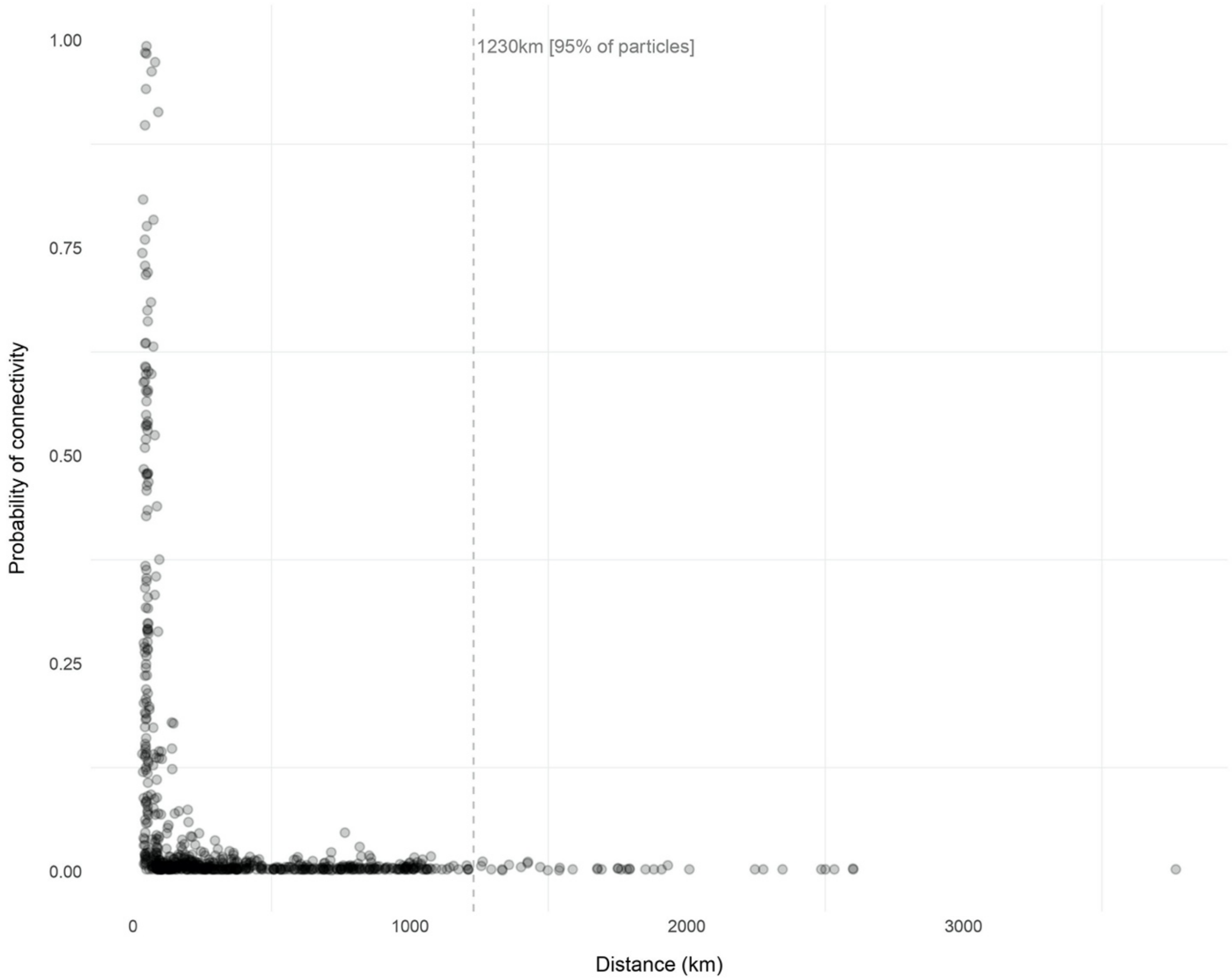


Figure S1 – Probability of connectivity of the dispersal units according to distance (km).

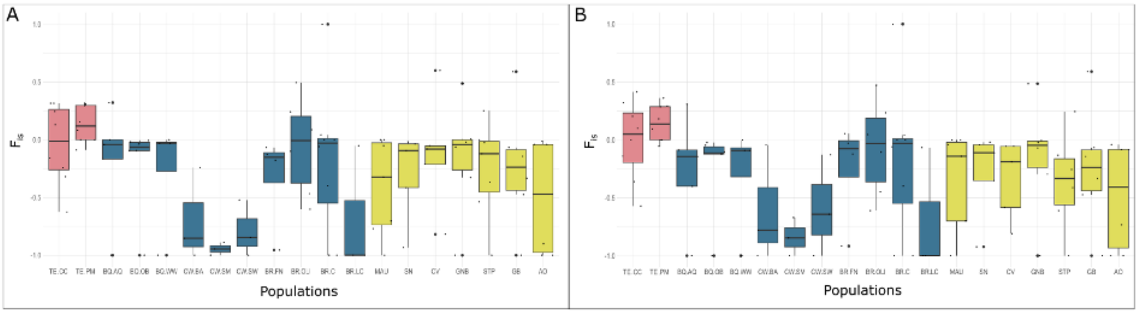
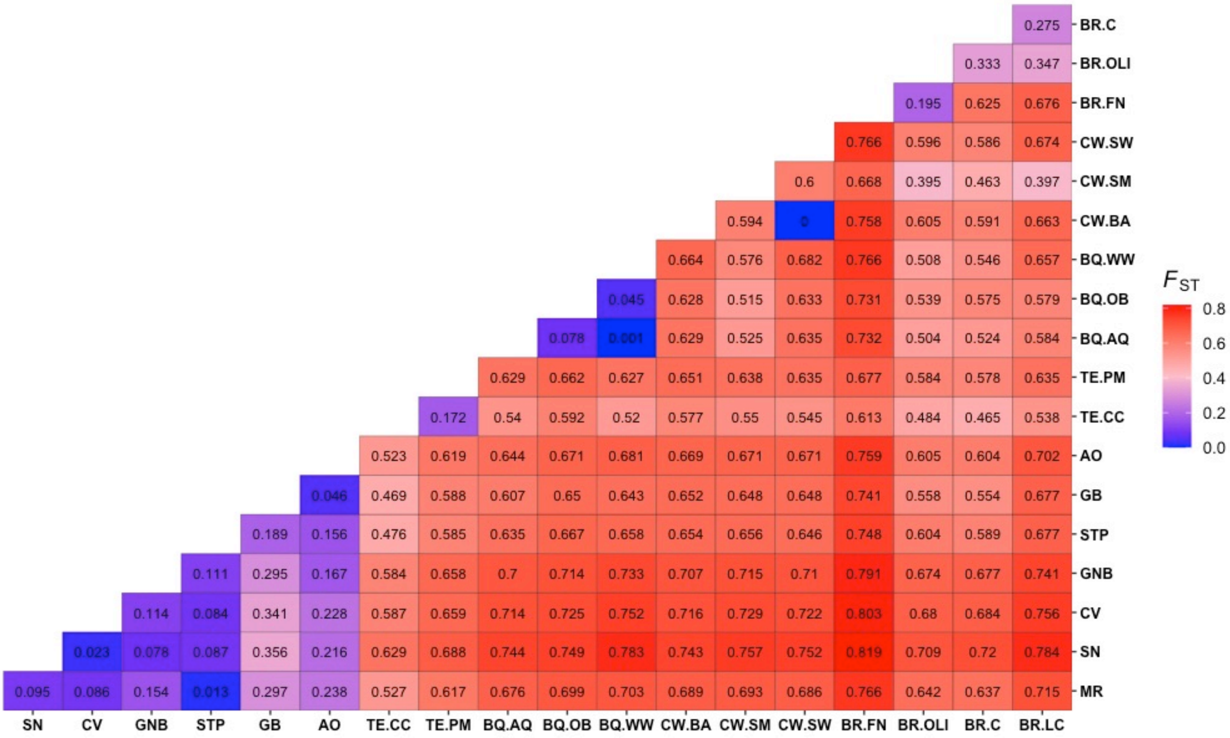


Figure S2 – Inbreeding coefficient (F_{IS}) by location, a) calculated for the ramet-level data set and b) calculated for the genet-level data set. Each point represents the F_{IS} value for one locus per location.



	SN	CV	GNB	STP	GB	AO	TE.CC	TE.PM	BQ.AQ	BQ.OB	BQ.WW	CW.BA	CW.SM	CW.SW	BR.FN	BR.OLI	BR.C	BR.LC
BR.C																		0.275
BR.OLI																	0.333	0.347
BR.FN																0.195	0.625	0.676
CW.SW															0.766	0.596	0.586	0.674
CW.SM														0.6	0.668	0.395	0.463	0.397
CW.BA													0.594	0	0.758	0.605	0.591	0.663
BQ.WW												0.664	0.576	0.682	0.766	0.508	0.546	0.657
BQ.OB											0.045	0.628	0.515	0.633	0.731	0.539	0.575	0.579
BQ.AQ										0.078	0.001	0.629	0.525	0.635	0.732	0.504	0.524	0.584
TE.PM									0.629	0.662	0.627	0.651	0.638	0.635	0.677	0.584	0.578	0.635
TE.CC								0.172	0.54	0.592	0.52	0.577	0.55	0.545	0.613	0.484	0.465	0.538
AO							0.523	0.619	0.644	0.671	0.681	0.669	0.671	0.671	0.759	0.605	0.604	0.702
GB						0.046	0.469	0.588	0.607	0.65	0.643	0.652	0.648	0.648	0.741	0.558	0.554	0.677
STP					0.189	0.156	0.476	0.585	0.635	0.667	0.658	0.654	0.656	0.646	0.748	0.604	0.589	0.677
GNB				0.111	0.295	0.167	0.584	0.658	0.7	0.714	0.733	0.707	0.715	0.71	0.791	0.674	0.677	0.741
CV			0.114	0.084	0.341	0.228	0.587	0.659	0.714	0.725	0.752	0.716	0.729	0.722	0.803	0.68	0.684	0.756
SN		0.023	0.078	0.087	0.356	0.216	0.629	0.688	0.744	0.749	0.783	0.743	0.757	0.752	0.819	0.709	0.72	0.784
MR	0.095	0.086	0.154	0.013	0.297	0.238	0.527	0.617	0.676	0.699	0.703	0.689	0.693	0.686	0.766	0.642	0.637	0.715

Figure S3 – Population pairwise differentiation FST between *Halodule wrightii* populations.

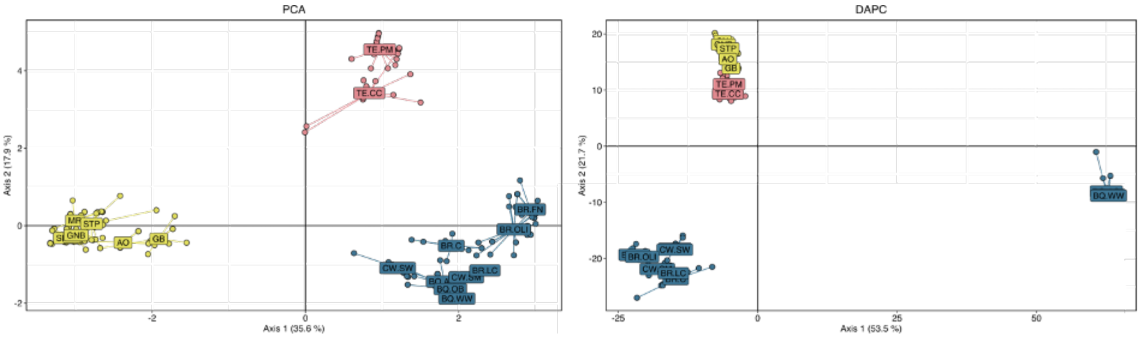


Figure S4 – Principal Components Analysis (PCA) and discriminant Analysis of Principal Components (DAPC) among *Halodule wrightii* populations.

Supplementary Material

Chapter 3

Table S1 - Pairwise population F_{ST} estimates (below diagonal) and Jost's D values (above diagonal); * Not significant, $p > 0.05$.

	MR	SN	CV	GNB	ST-ABA	ST-PRI	ST-CAB	ST-SAN	GB	AO
MR	--	0.028	0.029	0.058	-0.005*	0.039	0.008*	0.222	0.157	0.115
SN	0.095	--	0.005	0.019	0.057	0.054	0.022	0.199	0.112	0.067
CV	0.086	0.023	--	0.033	0.055	0.055	0.026	0.214	0.128	0.083
GNB	0.154	0.078	0.114	--	0.086	0.068	0.029	0.205	0.079	0.060
ST-ABA	0.000*	0.000*	0.000*	0.000*	--	0.042*	0.003*	0.250	0.188*	0.158
ST-PRI	0.093	0.202	0.172	0.187	0.000*	--	0.022*	0.184	0.139*	0.103
ST-CAB	0.021	0.078	0.077	0.080	0.000*	0.035*	--	0.157	0.078	0.061
ST-SAN	0.369	0.465	0.440	0.410	0.000*	0.318	0.250	--	0.139	0.052
GB	0.299	0.352	0.337	0.222	0.000*	0.257*	0.134	0.288	--	0.052
AO	0.238	0.216	0.228	0.167	0.000*	0.225	0.130	0.131	0.142	--

Table S2 - Literature records of *Halodule wrightii* on west Africa coast

ID	Lat	Long	Local name	Country	Reference
1	19.739766	-16.457939	Banc d'Arguin	Mauritania	Wolff, W.J., Duiven, A.G., Duiven, P. et al. Biomass of macrobenthic tidal flat fauna of the Banc d'Arguin, Mauritania. Hydrobiologia 258, 151–163 (1993). https://doi.org/10.1007/BF00006193
2	20.228214	-16.225236	Banc d'Arguin		Cardona, L., Aguilar, A. & Pazos, L. Delayed ontogenic dietary shift and high levels of omnivory in green turtles (*Chelonia mydas*) from the NW coast of Africa. Mar Biol 156, 1487–1495 (2009). https://doi.org/10.1007/s00227-009-1188-z
3	20.007245	-16.300842	Kiaone		Chefaoui, R. M., Duarte, C. M., Tavares, A. I., Frade, D. G., Cheikh, M. S., Ba, M. A., & Serrao, E. A. (2021). Predicted regime shift in the seagrass ecosystem of the Gulf of Arguin driven by climate change. Global Ecology and Conservation, e01890.
4	19.893792	-16.500052	Arel		
5	19.735806	-16.493225	Kiji		
6	19.37591	-16.46436	Mamghar		
7	14.27166667	-16.90472222	Joal Fadiouth	Senegal	Cunha, A. H. & Araújo, A. (2009). New distribution limits of seagrass beds in West Africa. Journal of biogeography, 36(8), 1621-1622.
8	14.15222222	-16.83416667	Bamboung-Sourou area		
9	14.90888889	-23.51027778	Santiago Island	Cape Verde	Creed, J.C., Engelen, A.H., D´Oliveira, E.C. et al. First record of seagrass in Cape Verde, eastern Atlantic. Mar Biodivers Rec 9, 57 (2016). https://doi.org/10.1186/s41200-016-0067-9
10	13.384509	-16.811234	Bijol Islands	Gambia	https://resiliensea.org/2020/01/27/participant-diary-from-the-national-seagrass-species-identification-mapping-and-monitoring-training-in-the-gambia/
11	7.641079	-13.054480	Moot Island	Sierra Leone	https://news.grida.no/finding-treasure-in-the-turtle-islands
12	0.892721	9.349402	Corisco Bay	Gabon	http://www.yaqupacha.org/fileadmin/user_upload/pdf/seagrass_magazine_46_2012.pdf
13	0.414204	6.68026	Cabras Islet	São Tomé and Príncipe	Alexandre, A., Silva, J., Ferreira, R., Paulo, D., Serrão, E. A., & Santos, R. (2017). First description of seagrass distribution and abundance in São Tomé and Príncipe. Aquatic Botany, 142, 48-52.
14	0.395462	6.730666	Cabras Islet		
15	0.246614	6.7465	Santana Bay		
16	1.632689	7.454281	Abade beach (Príncipe)		
17	0.57277778	6.86166667	Cabras Islet		Hancock JM, Vieira S, Jimenez V, Carvalho Rio J, Rebelo R (2018) Stable isotopes reveal dietary differences and site fidelity in juvenile green turtles foraging around São Tomé Island, West Central Africa. Mar Ecol Prog Ser 600:165-177. https://doi.org/10.3354/meps12633
18	-8.95653	13.108091	Mussulo Bay	Angola	Santos, C. I. (2007). Comunidades de macroinvertebrados e peixes associadas à pradaria marinha de *Halodule wrightii* (Ascherson, 1868) na Laguna do Mussulo, Angola.

Table S3 - Estimates of probability of connectivity between regions.

Pair from	Pair to	Probability (mean)	Probability (sd)	Probability (max)	Time (mean)	Time (sd)	Time (max)	Events (mean)	Events (sd)	Events (max)	Distance
Mussulo Bay (S)	Mussulo Bay (S)	0,027671233	0,004736549	0,035616438	1,411948977	0,625477493	2,12962963	10,1	1,728840331	13	0
Mussulo Bay (N)	Mussulo Bay (S)	0,005088063	0,00292889	0,010958904	4,75	3,324380338	11,875	1,857142857	1,069044968	4	7,401572135
Mussulo Bay (N)	Mussulo Bay (N)	0,005479452	0,002450485	0,008219178	0,166666667	0	0,166666667	2	0,894427191	3	0
Santana	Santana	0,007762557	0,003641535	0,01369863	1,049074074	0,400314948	1,75	2,833333333	1,329160136	5	0
Santana	Micolo	0,003835616	0,00150061	0,005479452	1,158333333	0,452999264	1,958333333	1,4	0,547722558	2	9,276426873
Abade beach	Santana	0,003424658	0,001369863	0,005479452	2,270833333	0,48769583	3	1,25	0,5	2	5,981486174
Abade beach	Santo Amaro	0,002739726	0	0,002739726	1,916666667	0	1,916666667	1	0	1	26,15040811
Abade beach	Ilheu das Cabras	0,002739726	0	0,002739726	28,66666667	0	28,66666667	1	0	1	172,9506784
Santo Amaro	Santana	0,002739726	0	0,002739726	1	0	1	1	0	1	26,15040811
Santo Amaro	Santo Amaro	0,047945205	0,017097393	0,084931507	0,271802315	0,090754638	0,44047619	17,5	6,240548409	31	0
Príncipe	Ilheu das Cabras	0,002739726	0	0,002739726	14,125	0	18,66666667	1	0	1	146,8019365
Micolo	Santana	0,018630137	0,008740446	0,032876712	0,554500661	0,296360141	1,166666667	6,8	3,190262964	12	9,276426873
Micolo	Santo Amaro	0,002739726	0	0,002739726	29	0	29	1	0	1	35,42683498
Micolo	Micolo	0,010350076	0,006955044	0,024657534	0,4375	0,291666667	1	3,777777778	2,538591035	9	0
Micolo	Ilheu das Cabras	0,002739726	0	0,002739726	14,66666667	0	14,66666667	1	0	1	176,8107469
Ilheu das Cabras	Ilheu das Cabras	0,209041096	0,036553645	0,257534247	0,266843578	0,025147439	0,293154762	76,3	13,34208046	94	0
Unhocomo	Unhocomo	0,002739726	0	0,002739726	0,166666667	0	0,166666667	1	0	1	0
Gamboa	Gamboa	0,119178082	0,020349114	0,145205479	1,222401747	0,233285825	1,74829932	43,5	7,427426652	53	0

Fadiouth	Fadiouth	0,350958904	0,062052762	0,419178082	0,260435767	0,040803572	0,356481481	128,1	22,64925802	153	0
Banc d'Arguin (N)	Banc d'Arguin (N)	0,010958904	0,006549198	0,019178082	1,261904762	0,377535699	2	4	2,390457219	7	0
Banc d'Arguin (N)	Banc d'Arguin (W)	0,002739726	0	0,002739726	10,63888889	0	11	1	0	1	74,97551338
Banc d'Arguin (W)	Banc d'Arguin (W)	0,01260274	0,007552903	0,02739726	1,647063492	0,874740804	3,80952381	4,6	2,75680975	10	0
Banc d'Arguin (W)	Banc d'Arguin (S)	0,002739726	0	0,002739726	7	0	12	1	0	1	36,89979671
Banc d'Arguin (S)	Banc d'Arguin (S)	0,069589041	0,031109264	0,115068493	1,064527917	0,084818157	1,19047619	25,4	11,35488148	42	0

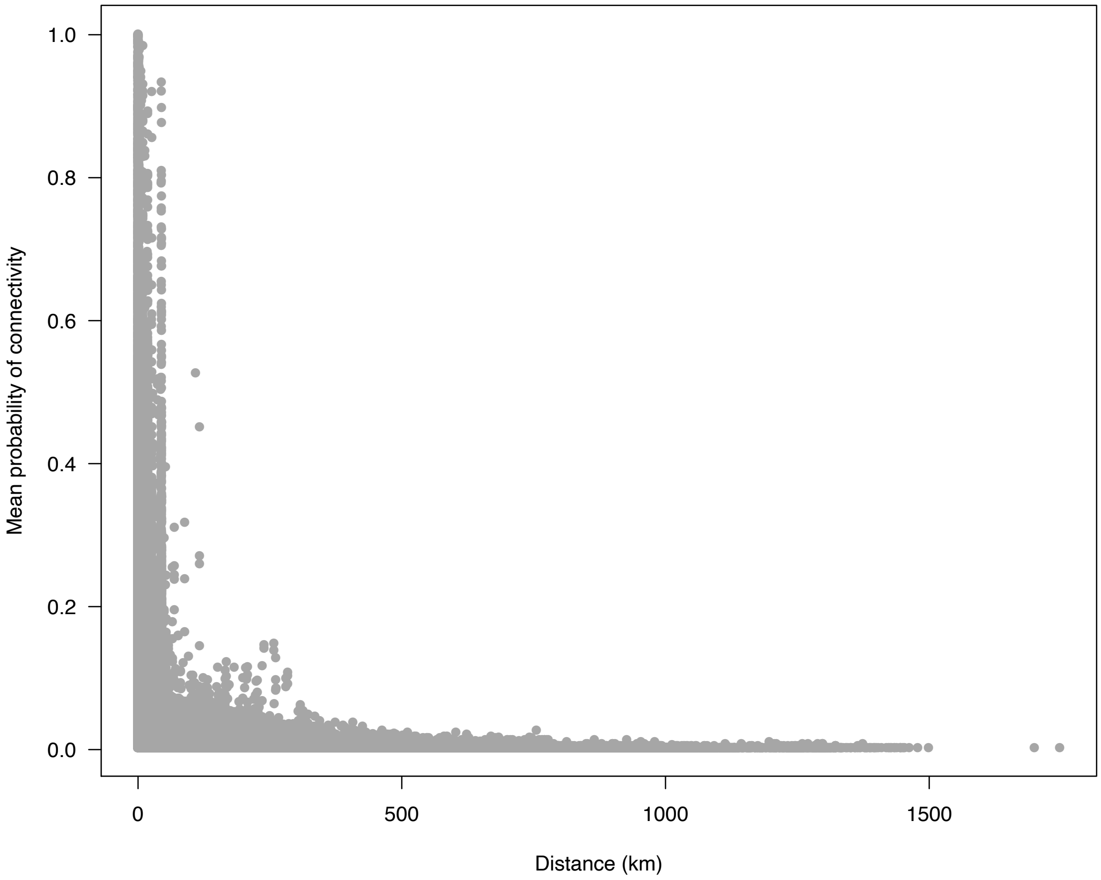


Figure S1 - Relationship between probability of connectivity and distance traveled by all particles for a simulation of 60 days.

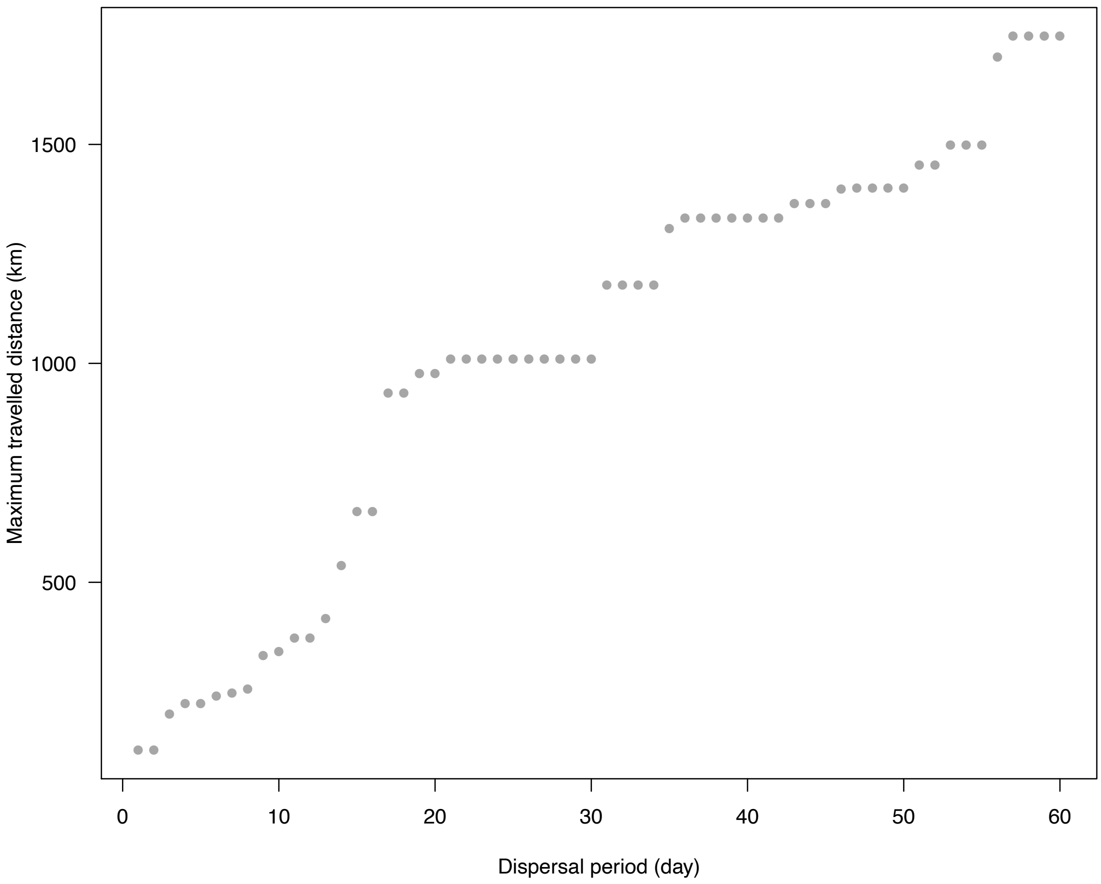


Figure S2 - Relationship between maximum travelled distance and increasing propagule duration.

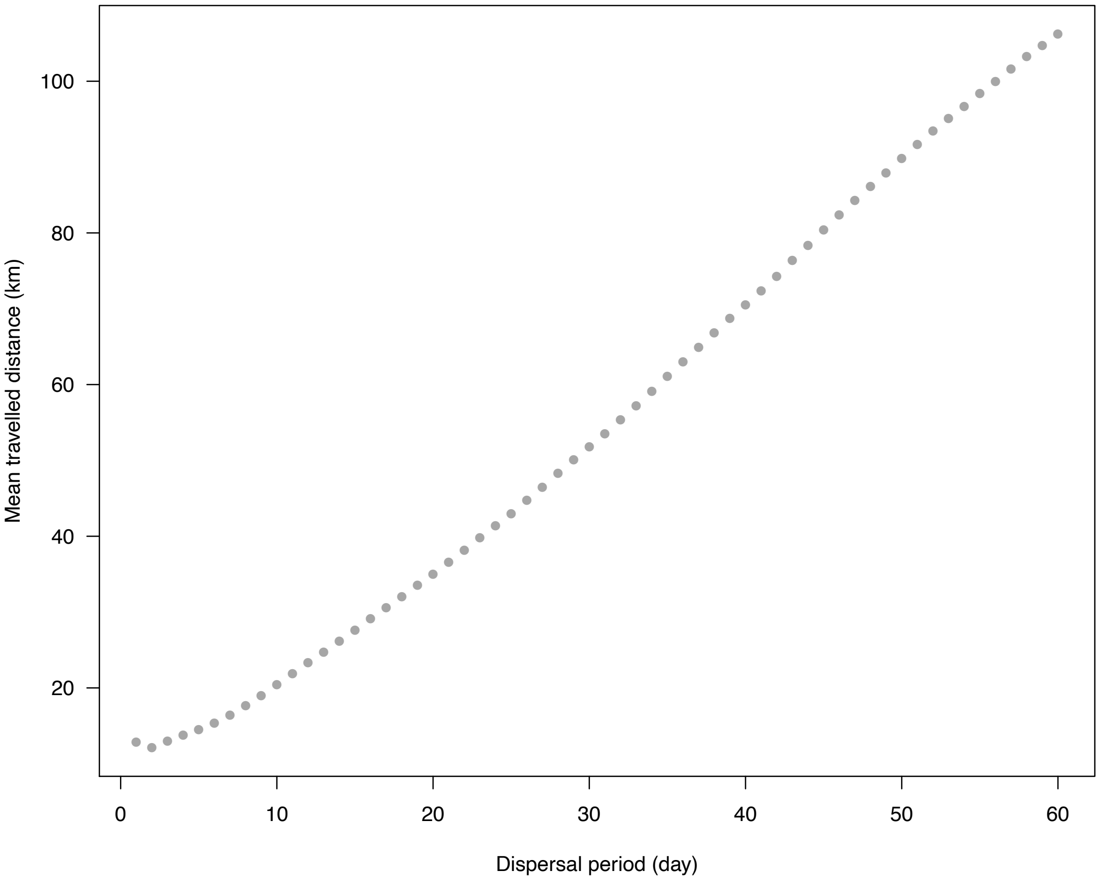


Figure S3 - Relationship between mean travelled distance and increasing propagule duration.

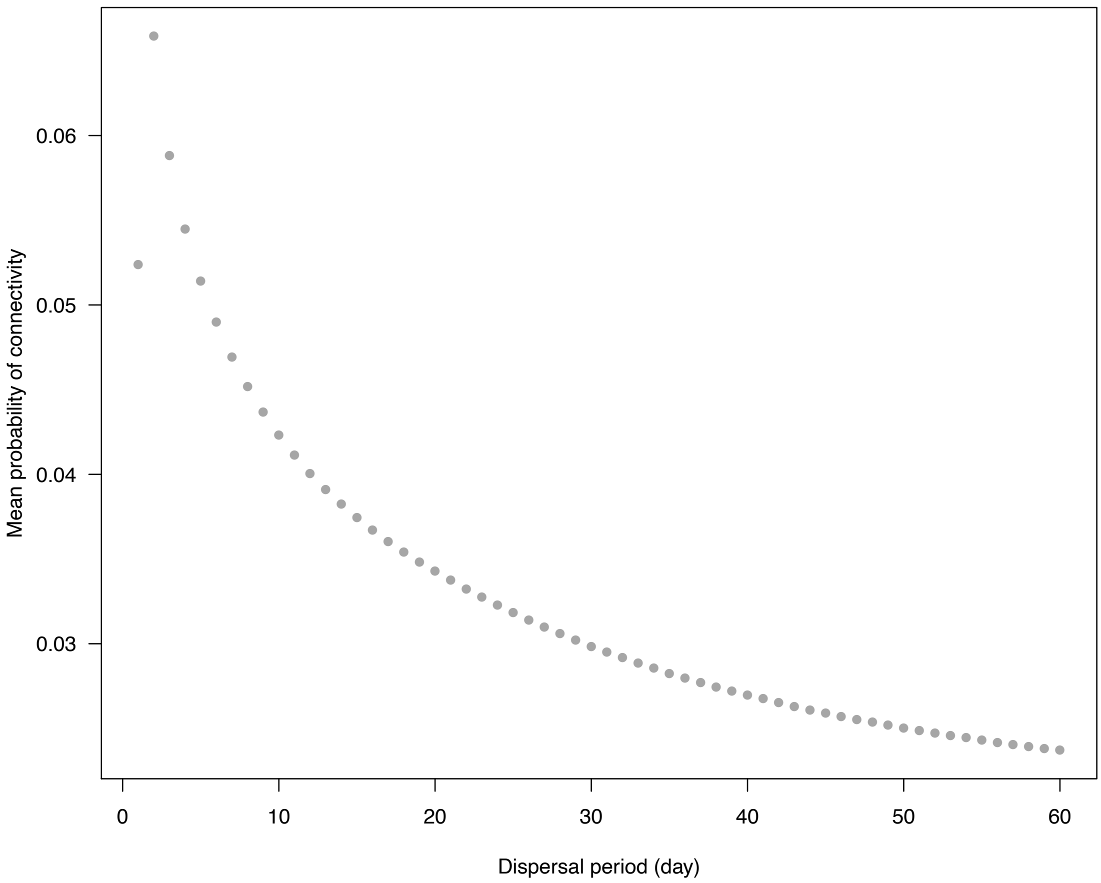


Figure S4 - Relationship between mean probability of connectivity and increasing propagule duration.

Supplementary Material

Chapter 4

Table S1: Geographic location and genetic diversity indices for *P. torreyi* and *P. scouleri*. Population (sample code); Location; Country; latitude (Lat) and longitude (Long) in decimal degrees; sample size (N); number of unique genotypes (G); clonal diversity (R); allelic richness (A); allelic richness standardized for the highest common G value of 7 ($\hat{A}_7$); standardized number of private alleles (P$\hat{A}$); expected heterozygosity (H_E), observed heterozygosity (H_O); and multi-locus inbreeding coefficient (F_{IS}), * $p < 0.05$.

	Population	**Location**	**Country**	**Lat**	**Long**	**N**	**G**	**R**	**A**	**$\hat{A}_7$**	**P$\hat{A}$**	**H_E**	**HO**	**F_{IS}**
P. torreyi	MEX.SB	San Juanico, Scorpion Bay	Mexico	26.250	-112.476	33	33	1.00	4.18	2.73 ± 0.17	5.73 ± 1.29	0.38	0.34	0.09
	MEX.EN	Ensenada	Mexico	31.862	-116.665	26	23	0.88	5.27	3.8 ± 0.29	5.71 ± 1.75	0.62	0.46	0.27
	CA.MS	Mussel Shoals	California	34.355	-119.443	29	28	0.96	5.91	4.01 ± 0.31	4.36 ± 1.65	0.65	0.62	0.05*
	CA.PSR	Santa Rosa Island	California	34.009	-120.049	28	28	1.00	5.55	3.80 ± 0.24	3.05 ± 1.53	0.57	0.50	0.12
	CA.CP	Carrington Point (Santa Rosa Island)	California	34.033	-120.045	24	22	0.91	5.91	4.37 ± 0.38	3.50 ± 1.43	0.67	0.73	-0.09*
	CA.SB	Shell Beach	California	35.169	-120.697	28	28	1.00	5.36	3.55 ± 0.23	2.59 ± 1.38	0.59	0.50	0.15
	CA.AM	Andrew Molera	California	36.281	-121.863	28	23	0.81	5.09	3.61 ± 0.42	2.55 ± 1.32	0.56	0.55	0.02*
	CA.FP	Franklin Point	California	37.149	-122.361	29	11	0.36	2.27	2.13 ± 0.12	0.86 ± 0.55	0.33	0.41	-0.26*
	CA.ABA	Abalobadiah	California	39.569	-123.772	29	19	0.64	2.27	1.83 ± 0.14	0.21 ± 0.41	0.22	0.21	0.06*
	ORG.CA	Cape Arago	Oregon	43.309	-124.401	23	19	0.82	1.91	1.65 ± 0.09	0.30 ± 0.46	0.22	0.21	0.06*
	ORG.FC	Fogarty Creek	Oregon	44.837	-124.058	27	21	0.77	3.00	2.33 ± 0.42	0.29 ± 0.46	0.34	0.36	-0.07*
						304	*255*	*0.88*	*5.09*	*3.55 ± 0.24*	*2.59 ± 1.32*	*0.56*	*0.46*	*0.04*
P. scouleri	CA.GP	Government Point	California	34.442	-120.456	9	7	0.75	3.55	3.55 ± 0.00	2.93 ± 0.72	0.64	0.77	-0.22*
	CA.BH	Boathouse	California	34.555	-120.611	26	22	0.84	5.64	4.08 ± 0.37	4.47 ± 1.26	0.58	0.60	-0.03*
	CA.HZ	Hazards	California	35.289	-120.883	29	29	1.00	7.36	4.25 ± 0.55	5.34 ± 2.07	0.61	0.55	0.10
	CA.HM	Harmony	California	35.475	-121.017	25	24	0.96	6.00	3.91 ± 0.33	4.05 ± 1.37	0.59	0.55	0.07
	CA.VDM	Vista Del Mar (San Simeon)	California	35.604	-121.142	27	17	0.62	5.27	4.33 ± 0.26	1.09 ± 0.73	0.72	0.80	-0.11*
	CA.MC	Mill Creek	California	35.979	-121.490	28	27	0.96	4.73	3.45 ± 0.28	3.90 ± 1.08	0.56	0.52	0.06*
	CA.AM	Andrew Molera	California	36.281	-121.863	28	11	0.37	4.27	3.92 ± 0.15	4.04 ± 1.30	0.60	0.63	-0.05*
	CA.PP	Pigeon Point	California	37.185	-122.397	27	25	0.92	3.91	2.98 ± 0.21	3.32 ± 1.23	0.49	0.51	-0.04*
	CA.ABA	Abalobadiah	California	39.569	-123.772	25	25	1.00	3.27	2.28 ± 0.18	2.74 ± 1.39	0.29	0.29	-0.01*
	ORG.BH	Burnt Hill	Oregon	42.228	-124.388	25	15	0.58	2.45	2.12 ± 0.14	0.49 ± 0.57	0.32	0.32	0.01*
	ORG.RE	Roads End	Oregon	45.026	-124.013	24	22	0.91	3.18	2.47 ± 0.29	2.49 ± 0.88	0.41	0.30	0.27
	CAN.VA	Botanical Bay (Vancouver island)	Canada	48.529	-124.452	16	15	0.93	4.09	3.29 ± 0.37	4.83 ± 1.47	0.56	0.36	0.36
						289	*239*	*0.92*	*4.18*	*3.50 ± 0.27*	*3.61 ± 1.25*	*0.57*	*0.53*	*0.03*
						593	*494*							

Table S2: Geographic location and genetic diversity indices for *P. torreyi*, *P. scouleri* and *P. iwatensis*. Population (sample code); Location; Country; latitude (Lat) and longitude (Long) in decimal degrees; sample size (N); number of unique genotypes (G); clonal diversity (R); allelic richness (A); allelic richness standardized for the highest common G value of 7 ($\hat{A}_7$); standardized number of private alleles (PÂ); expected heterozygosity (H_E), observed heterozygosity (H_O); and multi-locus inbreeding coefficient (F_{IS}), * $p < 0.05$.

	Population	**Location**	**Country**	**Lat**	**Long**	**N**	**G**	**R**	**A**	**$\hat{A}_7$**	**PÂ**	**H_E**	**H_O**	**F_{IS}**
P. torreyi	MEX.SB	San Juanico. Scorpion Bay	Mexico	26.250	-112.476	33	33	1.00	4.63	2.93 ± 0.21	4.47 ± 1.23	0.38	0.30	0.20
	MEX.EN	Ensenada	Mexico	31.862	-116.665	26	22	0.84	5.88	4.14 ± 0.38	5.50 ± 1.70	0.64	0.50	0.22
	CA.MS	Mussel Shoals	California	34.355	-119.443	29	26	0.89	6.88	4.53 ± 0.39	3.85 ± 1.57	0.71	0.69	0.03
	CA.PSR	Santa Rosa Island	California	34.009	-120.049	28	28	1.00	6.13	4.16 ± 0.29	2.13 ± 1.16	0.63	0.56	0.11
	CA.CP	Carrington Point (Santa Rosa Island)	California	34.033	-120.045	24	22	0.91	6.88	5.11 ± 0.44	2.53 ± 1.35	0.78	0.82	-0.05
	CA.SB	Shell Beach	California	35.169	-120.697	28	28	1.00	6.00	4.05 ± 0.29	1.89 ± 1.12	0.64	0.60	0.07
	CA.AM	Andrew Molera	California	36.281	-121.863	28	23	0.81	5.25	3.64 ± 0.39	1.74 ± 1.25	0.55	0.60	-0.08*
	CA.FP	Franklin Point	California	37.149	-122.361	29	11	0.36	2.38	2.18 ± 0.15	0.87 ± 0.53	0.33	0.40	-0.21*
	CA.ABA	Abalobadiah	California	39.569	-123.772	29	17	0.57	2.38	1.99 ± 0.17	0.00 ± 0.00	0.28	0.27	0.01
	ORG.CA	Cape Arago	Oregon	43.309	-124.401	23	17	0.73	2.13	1.79 ± 0.11	0.21 ± 0.41	0.27	0.28	-0.03
	ORG.FC	Fogarty Creek	Oregon	44.837	-124.058	27	21	0.77	3.13	2.53 ± 0.39	0.00 ± 0.00	0.40	0.46	-0.15*
							248	*0.84*	*5.25*	*3.64 ± 0.29*	*1.89 ± 1.16*	*0.55*	*0.50*	*0.01*
P. scouleri	CA.GP	Government Point	California	34.442	-120.456	9	7	0.75	3.50	3.50 ± 0.00	2.34 ± 0.48	0.63	0.77	-0.25*
	CA.BH	Boathouse	California	34.555	-120.611	26	20	0.76	5.88	4.25 ± 0.43	3.75 ± 1.21	0.60	0.62	-0.02
	CA.HZ	Hazards	California	35.289	-120.883	29	29	1.00	6.63	3.80 ± 0.61	3.15 ± 1.52	0.55	0.50	0.11
	CA.HM	Harmony	California	35.475	-121.017	25	24	0.96	5.13	3.28 ± 0.36	0.80 ± 0.80	0.52	0.48	0.07
	CA.VDM	Vista Del Mar (San Simeon)	California	35.604	-121.142	27	15	0.54	4.88	4.08 ± 0.24	1.04 ± 0.69	0.70	0.82	-0.17*
	CA.MC	Mill Creek	California	35.979	-121.490	28	27	0.96	3.88	2.91 ± 0.27	1.39 ± 0.58	0.50	0.50	0.02
	CA.AM	Andrew Molera	California	36.281	-121.863	28	11	0.37	4.25	3.88 ± 0.17	2.37 ±0.87	0.64	0.68	-0.08
	CA.PP	Pigeon Point	California	37.185	-122.397	27	24	0.88	3.38	2.65 ± 0.22	1.48 ± 0.9	0.44	0.45	-0.03
	CA.ABA	Abalobadiah	California	39.569	-123.772	25	24	0.96	3.00	2.06 ± 0.18	0.57 ± 0.81	0.26	0.27	-0.04
	ORG.BH	Burnt Hill	Oregon	42.228	-124.388	25	15	0.58	2.63	2.17 ± 0.19	0.48 ± 0.57	0.30	0.29	0.02
	ORG.RE	Roads End	Oregon	45.026	-124.013	24	20	0.83	3.13	2.50 ± 0.31	2.39 ± 0.84	0.42	0.30	0.30
	CAN.VA	Botanical Bay (Vancouver island)	Canada	48.529	-124.452	16	15	0.93	4.25	3.48 ± 0.45	0.84 ± 0.78	0.59	0.35	0.42
							231	*0,86*	*4.06*	*3.38 ± 0.26*	*1.44 ± 0.81*	*0.54*	*0.49*	*0.03*
P. iwatensis	JP.AKK	Akkeshi, Hokkaido	Japan	43.023	144.838	24	17	0.70	2.25	2.04 ± 0.10	0.34 ± 0.75	0.32	0.43	-0.36*
	JP.HM	Hamanaka	Japan	43.423	145.267	24	18	0.74	2.63	2.06 ± 0.19	0.75 ± 0.99	0.30	0.39	-0.31*

	JP.SU	Suttsu	Japan	42.781	140.307	24	21	0.87	2.50	2.20 ± 0.15	0.37 ± 0.56	0.38	0.49	-0.33*
						72	*56*	*0.74*	*2.50*	*2.06 ± 0.15*	*0.37 ± 0.75*	*0.32*	*0.43*	*-0.34*
						665	*535*							

Table S3: Characterization of 11 polymorphic microsatellite markers in the seagrass *Phyllospadix scouleri* (Psc) and *Phyllospadix torreyi* (Pto).

Locus name	Primer sequences (5′-3′)	Repeat motif	Ta (°C)	Size range (bp)
Psc7	F: AGGTTACTTTCCCAGTCTTTTCCT	$(TA)_{12}$	56	210-250
	R: GCTGCTATTTTGGACGGTATGT			
Psc10	F: TGAAACGAAGCAAAGAGTGAGA	$(TC)_{20}$	55	310-350
	R: AGCGCGGACGACTTACTTATAC			
Psc21	F: CGCTTTGGTTCTTTCACTTCTT	$(GCA)_{8}$	55	340-380
	R: AAACCTTCTGCTGTTACCTGTGA			
Pto07	F: TGGCGAGAGTTGTAATGGAGTA	$(GA)_{18}$	55	200-250
	R: TACGATGACGTGGCAGTTTTAT			
Pto11	F: CTCGTCGCCCATAAGAGAAC	$(TA)_{9}$	55	200-250
	R: GCCAAGGAAATCAGAAGAAGAA			
Pto13	F: GATCAGAAACCCTAGATGTGGC	$(GAA)_{10}$	54	100-140
	R: GTTCGCACTTCCACTCCACT			
Pto15	F: AACACCGAAGCTGTAGACATCA	$(AGA)_{9}$	54	210-230
	R: TTCAAACCAAGCACCTGACAT			
Pto25	F: GCACATATCACCCTCCATCTTT	$(CA)_{9}$	55	110-130
	R: TACGAACGGGAATAACCGAATA			
Pto29	F: CGACTTCCAGTATCTTGGTTTTC	$(TA)_{10}$	56	180-250
	R: ATCAGGGTTCCACTTCAGGAT			
Pto31	F: GTCGGATGTGGATTTTGTTGTT	$(TC)_{12}$	55	140-160
	R: TTCCTCCAAGAGTTCCCTATCA			
Pto35	F: CTCAGACGACATGGCTGG	$(TCT)_{8}$	55	100-120
	R: TCCATAAATAGGCACACATTGG			

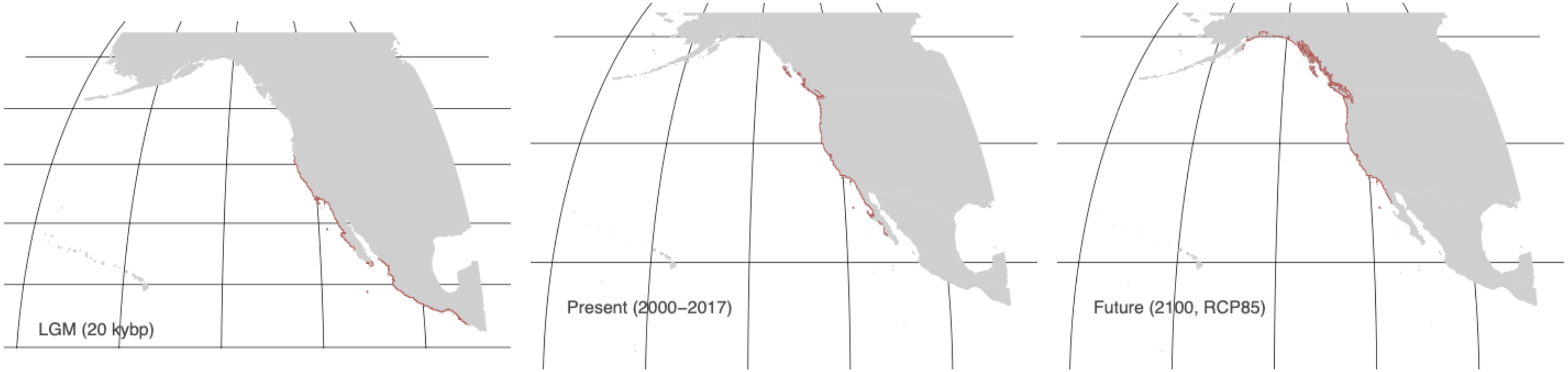


Figure S1: Predicted distribution of *P. torreyi* for the last glacial maximum (left figure), present (middle figure) and future emission scenario, RCP 8.5 by 2100 (right figure).

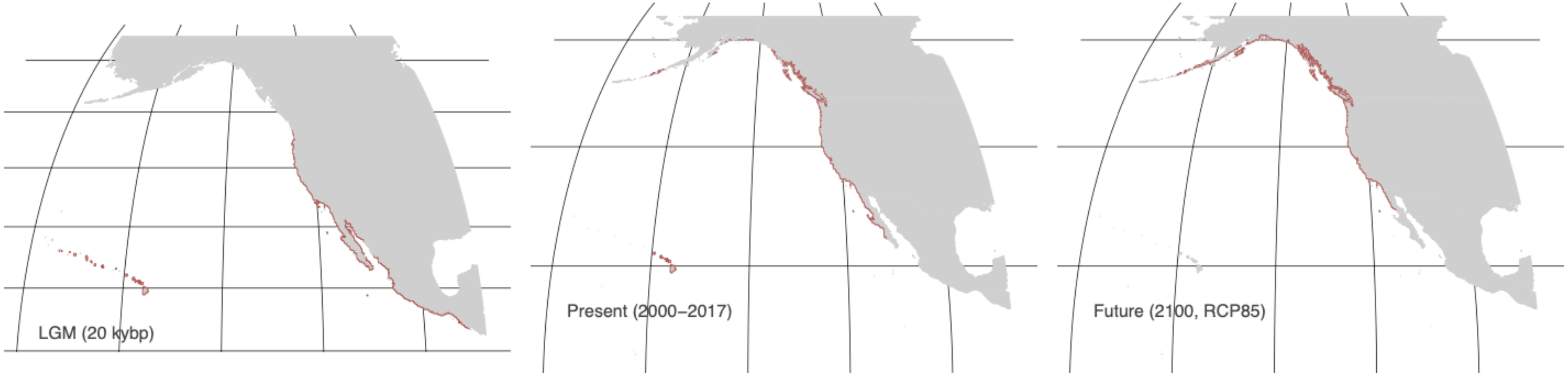


Figure S2: Predicted distribution of *P. scouleri* for the last glacial maximum (left figure), present (middle figure) and future emission scenario, RCP 8.5 by 2100 (right figure).

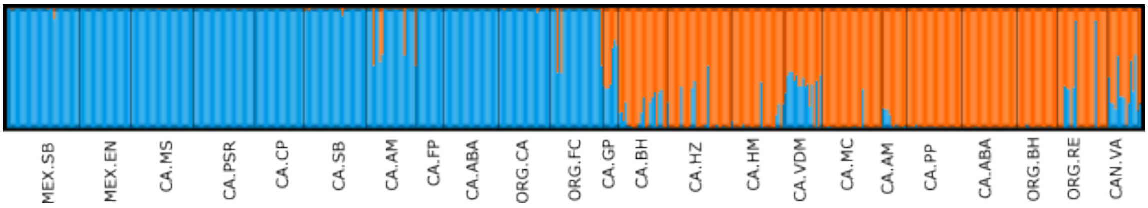


Figure S3: First hierarchal level of structure (k=2) for *P. torreyi* (in blue) and *P. scouleri* (in orange).

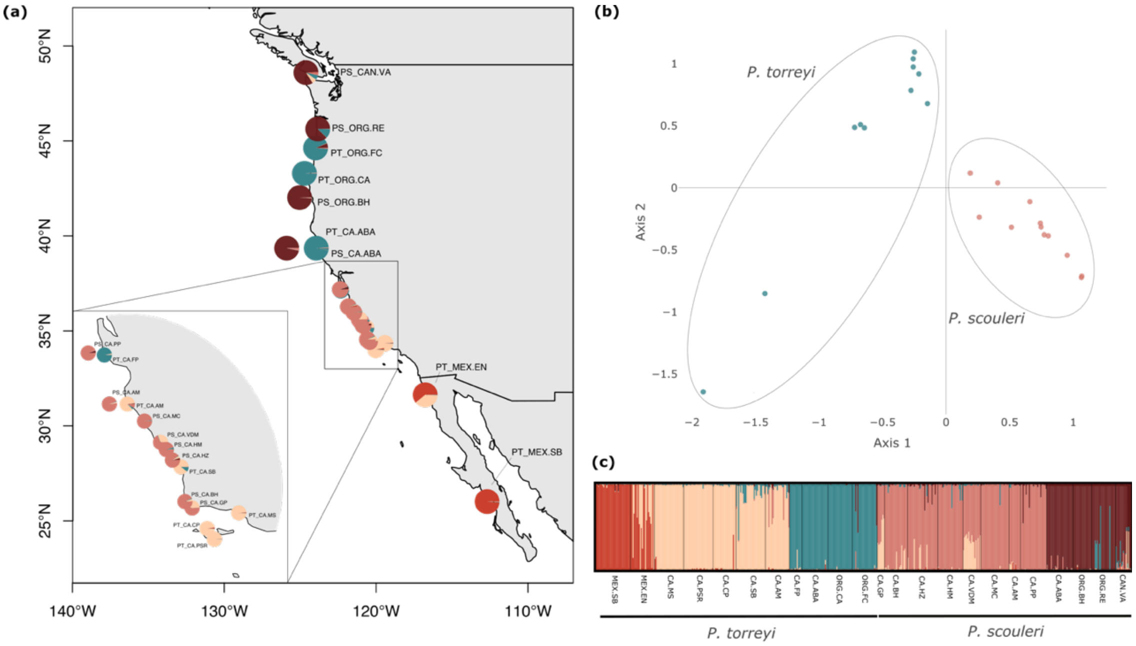


Figure S4: (**a**) Map of the sampled sites with pie-charts depicting genetic subdivision inferred with STRUCTURE for *P. torreyi and P. scouleri*; **(b)** Genetic differentiation between *P. torreyi* and *P. scouleri* illustrated by factorial correspondence analysis and **(c)** genetic subdivision with STRUCTURE for *P. torreyi* and *P. scouleri* (k=5).

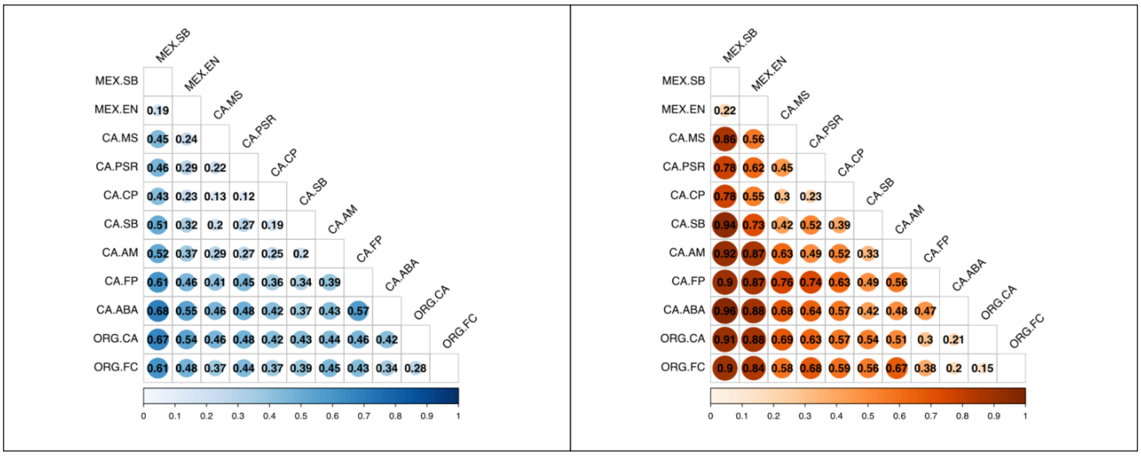


Figure S5: Pairwise population differentiation in *P. torreyi*, estimated by F_{ST} and Jost'D. All values are significantly different from zero at $p<0.05$.

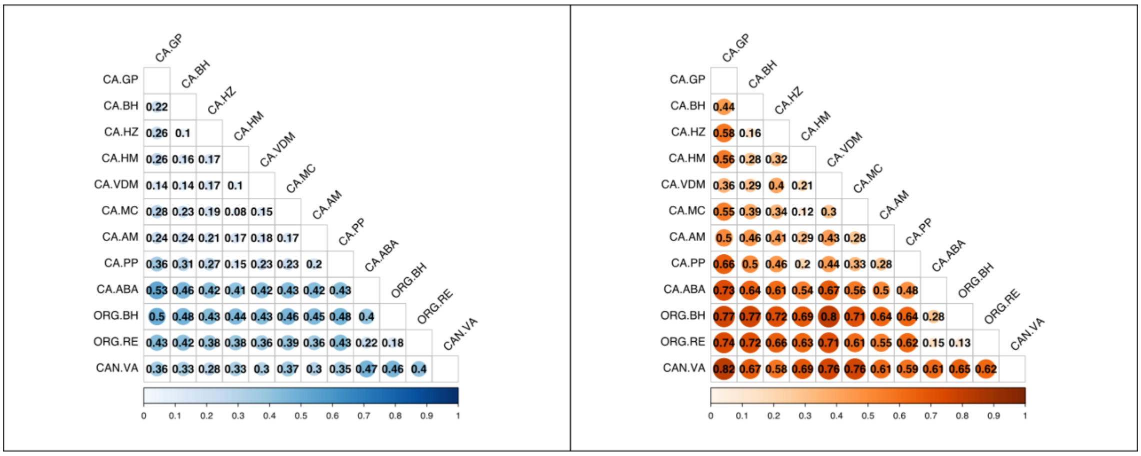


Figure S6: Pairwise population differentiation in *P. scouleri*, estimated by F_{ST} and Jost'D. All values are significantly different from zero at $p<0.05$.