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“In the end we will conserve only what we love, we will love only what we understand, and we will understand only what we are taught” –

Baba Dioum.

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Abstract

Understanding the patterns and processes of genetic diversity and differentiation is fundamental for assessing population connectivity to inform the conservation of coastal ecosystems. The tropical West African Atlantic Coast is a good model to address these issues. The influence of oceanographic currents diverging in hotspot ecoregions mediating propagule dispersal along this coastline raises interesting predictions to be tested. In the Gulf of Arguin (Mauritania) nearshore and offshore seagrass populations can be predicted to be highly connected by strong currents driven by tides in this very shallow system. In contrast, in the Bijagós archipelago (Guinea-Bissau), populations in the northern and southern islands could hypothetically be genetically differentiated as predicted based on passive transport by ocean currents. However, they also have a strong relationship of connectivity by migratory species, namely green turtles that travel between feeding and breeding grounds. This study aims to assess these predictions and also to understand seagrass population genetic diversity and genotypic (clonal) diversity, as well as connectivity, along the Western African coast. We used as model the species *Halodule wrightii*, a seagrass with clonal reproduction via rhizome elongation and sexual reproduction via non-buoyant seeds, both traits that do not promote long distance dispersal. We assessed genetic diversity, clonality, and population differentiation with 8 microsatellite markers, using samples from the entire species range along this coastline, from Mauritania to Angola over a distance of ~1,600 km. Generally, populations were genetically homogeneous with low differentiation, while genotypic richness varied among all populations. The results support the hypothesis of high connectivity between populations in the Banc d’Arguin but do not support the isolation hypothesis between the northern and southern regions of the Bijagós. The results also suggest that the Canary Current and Guinea Current do not affect seed dispersal and connectivity through abiotic mechanisms.

Keywords: connectivity, genetic diversity, *Halodule wrightii*, western African coast, seed dispersal, recruitment

Resumo

A compreensão dos processos centrais da diversidade e da diferenciação genética são fundamentais para avaliar a conectividade das populações de pradarias marinhas e prever a conservação dos ecossistemas costeiros tropicais. Contudo, pouco se sabe sobre pradarias marinhas na costa atlântica da África Ocidental, onde grande diversidade ocorre. Para além, também pouco se tem estudado à respeito das influências das correntes de maré que se divergem em 'hotspots' em ecorregiões e mediam a dispersão de sementes ao longo desta linha costeira. O Golfo de Arguin (Mauritânia) exibe populações de pradarias marinhas perto da costa e ao largo, enquanto o arquipélago de Bijagós (Guiné-Bissau) exibe populações de ervas marinhas nas ilhas do Norte e do Sul, que apresentam forte relação com espécies migratórias que se sazonalmente viajam para se alimentam. Este estudo visa compreender a estrutura genética das populações de ervas marinhas e a sua conectividade para prever a dispersão e recrutamento de sementes através de mecanismos abióticos e bióticos ao longo da costa ocidental da África, desde a Mauritânia até Angola, ao longo de uma distância de ~1.600 km. Utilizámos o modelo com a espécie *Halodule wrightii*, uma erva marinha florida com reprodução sexual e clonal através do alongamento do rizoma e sementes não flutuantes. Avaliámos a diversidade genética, clonalidade e diferenciação genética da população com 8 marcadores de microsatelite. Testamos o Isolamento por Distância para traçar a distribuição geográfica espacial correlacionada com a diferenciação em pares para identificar fontes de fluxo gênico. Em geral, apesar de uma zona divergente na zona central (São Tomé e Príncipe) demonstrar diversidade genética, foi identificada uma baixa variabilidade genética, assumindo uma colonização independente, enquanto uma riqueza genotípica significativa variou ao longo de todas populações. Foi encontrada uma diferenciação genética significativa entre a Mauritânia e a Guiné-Bissau, em termos de população aos pares. Os resultados apoiam a hipótese de alta conectividade entre as populações do Banc d'Arguin mas não apoiam a hipótese de isolamento entre as regiões norte e sul dos Bijagós. Os resultados também sugerem que a Corrente Canária e a Corrente da Guiné não afectam a dispersão de sementes e a conectividade através de mecanismos abióticos. Embora o isolamento por distância ocorra, este estudo sugere que a Corrente Canária e a Corrente da Guiné não afectam a dispersão de sementes e a conectividade através de mecanismos abióticos. No entanto, é necessário avaliar, investigar e avaliar áreas maiores e um número mais significativo de amostras ao longo desta costa para uma análise mais detalhada. O estudo tem implicações na utilização da informação genética populacional na conservação, gestão e mitigação dos países em desenvolvimento dependentes da produção costeira.

Palavras-chave: conectividade, diversidade genética, *Halodule wrightii*, costa africana ocidental, dispersão de sementes, recrutamento

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List of Acronyms and Abbreviations

µL:	Microliter
500 LIZ:	Sizing DNA fragments in nucleotides range
Â:	Number of Alleles
AMOVA:	Analysis of Molecular Variance
BC:	Benguela Current
Bp:	Base Pair
CC:	Canary Current
CCU:	Canary Current Upwelling
ETA:	Eastern Tropical Atlantic
FCA:	Factorial Correspondence Analysis
Fis:	Multi-locus Inbreeding coefficient
Fst:	Fixation index
G:	Number of Genets
GC:	Guinea Current
He:	Heterozygosity expected
Hi-Di:	Highly Deionized formamide
Ho:	Heterozygozity Observed
IBD:	Isolation by distance
ITCZ:	Inter-Tropical Convergence Zone
Jost'D:	Allelic Differentiation measure
JVPMNP:	João Vieira and Poilão Marine National Park
K:	Most probable number of populations
LD:	Linkage Disequilibrium
LGM:	Last Glacial Maximum
LME:	Large Marine Ecosystem
MEOW:	Marine Ecoregion of the World
$MgCl_2$:	Magnesium Chloride
MH:	Mid-Holocene
MLG:	Multi-Locus Genotypes
mM:	Millimolar
MPA:	Marine Protected Areas
MPC:	Marine Population Connectivity
N:	Number of samples
NBC:	North Brazil Current
NEC:	North Equatorial Current
PÂ:	Private allele
PCR:	Polymerase chain reaction
PNBA:	Parc National du Banc d'Arguin
R:	Genotypic richness
RAMPAO:	Regional Network of Marine Protected Areas in West Africa
RMUs:	Regional Management Units
SE:	Standard Deviation
SEC:	South Equatorial Currents
***Ta*:**	Temperature annealing
WAT:	West African Transition

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1. Chapter one - General Introduction

The hub processes addressing marine populations in an ecological context refer to their size, structure and the rules determining their assembly. Physiological features and functions, as well as habitat quality and setting, are responsible for the abundance on an individual level. The range of new sites, colonisation, dispersion possibilities and the earth's climate history possibly reinforced the distribution of species on earth in a distinct pattern. Particularly the last one referred to playing a significant role in biogeography and evolution. Biogeographical studies have been of great concern in the history of science since the 18th century.

Consequently, dynamic processes influence several aspects and historical processes, standing in complex relations to an intrinsic beneficial gene configuration, enabling organisms to exist and explaining patterns seen nowadays. However, facing the emerging challenges of climate change, overfishing and misuse of coastal zones affect the occurrences and abundances of these marine populations (Muelbert et al., 2019). Building a solid understanding of this vast biodiversity is vital, particularly in marine science. Therefore, the outcomes across these ecosystems must be assessed, tested, and acknowledged.

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1.1 Marine population connectivity

The term connectivity originates from studies of terrestrial ecosystems and has been discussed in the literature since the 1990s (Taylor et al., 1993; Sheaves, 2009). In an ecological concept, connectivity defines the movement of matter (e.g., nutrients, sediments), energy (e.g., organic matter) or organisms (e.g., migration, dispersion) between habitats over time and space through ecological or physical factors, which may depend on the life history of animals and plants (Sheaves, 2009). With time further research defined marine connectivity; Pineda et al.

(2007) highlight the functional requirements differentiating marine systems among larval, colonisation and post-larval survival, dispersal, transport and connectivity, while Lowe and Allendorf (2010) debate concepts of demographic and genetic connectivity; Pringle (2003) discusses the importance of hydrologic connectivity. Other connectivity terms used in the literature associate the environment where interaction processes occur and facilitate the movement of elements between or within ecosystems. Consequently, another aspect discussed is marine population connectivity (MPC), which characterises the exchange of individuals. The term connectivity originates from studies of terrestrial ecosystems and has been discussed in the literature since the 1990s (Taylor et al., 1993; Sheaves, 2009). In an ecological concept, connectivity defines the movement of matter (e.g., nutrients, sediments), energy (e.g., organic matter) or organisms (e.g., migration, dispersion) between habitats over time and space through ecological or physical factors, which may depend on the life history of animals and plants (Sheaves, 2009). With time further research defined marine connectivity; Pineda et al. (2007) highlight the functional requirements differentiating marine systems among larval, colonisation and post-larval survival, dispersal, transport and connectivity, while Lowe and Allendorf (2010) debate concepts of demographic and genetic connectivity; Pringle (2003) discusses the importance of hydrologic connectivity. Other connectivity terms used in the literature associate the environment where interaction processes occur and facilitate the movement of elements between or within ecosystems.

Consequently, another aspect discussed is marine population connectivity (MPC), which characterises the exchange of individuals between geographically detached subpopulations (Cowen & Sponaugle, 2009). An example of MPC is the easement of connectivity by water or ocean currents, as is the case of dispersal in larval stages (Selkoe et al., 2016; Fang et al., 2018). When connectivity between populations occurs continuously, in such a way contributing to their maintenance and survival, they are defined as a metapopulation, which encompasses the dispersal phase from reproduction to the completion of the settlement process.

All these connectivity patterns occur over a space-time period that can be affected by organisms' life histories and environmental factors. For example, marine animals connect habitat fragments or ecosystems daily as they move in search of food or shelter; (Berkström et al., 2012). At the same time, they promote connection over a more extended period (e.g., seabirds and sea turtles that migrate annually to breeding and feeding areas) (Hancock et al., 2019; Trierweiler et al., 2014).

Conversely, connectivity can be affected by environmental conditions such as temperature, rainfall, and ocean currents. These factors vary over time, causing local or regional short- or long-term responses. An example is the wind conditions of some regions of the west coast of continents (Abe & Brown, 2020). Additionally, the marine currents can enrich the surface waters by bringing nutrients from the seabed to the surface, stimulating phytoplankton growth, an event called upwelling, where the material can be transported to the coast in a heterogeneous manner (Wieters et al., 2013 Narváez et al., 2004), and events such as precipitation and resurgence can vary in frequency and intensity over the years (Escribano et al., 2004). Therefore, the scale of actions correlation between the environments requires functional and structural connectivity and environmental conditions that vary over space-time.

Ecological and evolutionary interactions perform valuable tools to access MPC (Ellstrand, 2014). Estimating accuracy in MPC is critical for assessing connectivity range, characterising the elements forwarding exchange, and understanding the biogeography, genetic structure, and population dynamics, such as speciation, resilience, and adaptation of marine and coastal species (Cowen et al., 2006). Identifying these tools is fundamental to interpreting demographic and genetic connectivity. Whilst demographic connectivity is the measure of dispersal influenced by survival rates and population growth, genetic connectivity outlines the impacts of gene flow on evolutionary processes within MPC (Lowe & Allendorf, 2010). Additionally, Waples & Gaggiotti (2006) declared, "The ecologically model stands challenging for evaluations using genetic markers alone because the transition from demographic dependence to independence occurs in regions of high migrations, and there is limited power related to genetic methods". Demographic connectivity of marine populations has significant demands in quantifying and interpreting genetic connectivity and evolution within species. Further, demographic and genetic connectivity have distinct conditions but play methods of assessment complementary to each other (Lowe & Allendorf, 2010).

Since MPC relationships contribute to the balance of marine ecosystems and are primarily complex, it becomes a significant challenge to researchers. In addition, the impacts generated by human actions such as overexploitation and the inappropriate use of coastal systems, as well as climate change (Lande & Shannon, 1996) (accelerate the need to understand the factors that promote connectivity (Heller & Zavaleta, 2009). This knowledge is increasingly essential for resource management, creating marine protected areas and evaluating the effects caused by habitat fragmentation (Kondolf et al., 2006; Berkström et al., 2013).

1.2 Connectivity drivers and Biogeography

The range limits of specie's locations define a vast array of historical, physical, and biotic factors (Goldberg & Lande, 2007). Although dispersal processes are vital to marine populations' ecological and evolutionary history, marine species' dispersal demonstrates easy crossing of long distances due to its favourable environment and migration facilities (Tavares et al., 2022). However, gene flow among marine organisms may present restrictions by thermal barriers, geographic features, currents, and relative time feasibility of dispersal phases. Among these factors, local adaptation, competition, and hybridisation are relevant to frame the boundaries between related and ecologically equivalent species (Goldberg & Lande, 2007). Furthermore, several environmental settings define whether marine populations will occur along coastlines. These comprehend biophysical settings that adjust species' physiological function and morphology, such as salinity, currents, depth, temperature, waves, substrate, nutrients, day length, light, wave action, disease, and epiphytes (Coles et al., 2011).

1.2.1 Disturbances

Seagrass meadow plays a considerable function through photosynthesis across the depth scale controlled by light availability. The habitat types for seagrass meadows are divided into coastal, reef, river estuary and deep water. (Carruthers et al., 2002). Most of these habitats are prompted by high disturbance and undergo temporally and spatially variations (Coles et al., 2011). The dynamics played by each habitat present prevailing processes leveraging seagrass growth, survival and community biodiversity. Coastal habitats endure a significant impact on seagrass assemblage. Therefore, they can be found deep below the sea surface (Coles et al., 2009) and in shallow waters. The determination of seagrass species' survival features as exposure, temperature, wave action and associated turbidity (Coles et al., 2011). Tropical seagrasses exhibit differences in their life-history strategies leading into species' assemblages. Some species can demonstrate slow turnover, requiring more time to recover from disturbance, with a low resistance to perturbation (e.g., *Enhalus acoroides*) (Poiner et al., 1987).

On the other hand, wave exposure impacts sedimentation movement, creating an unstable environment for seedlings to settle down or persist. Some genera, such as *Cymodocea* and *Syringodium*, can survive periodic disturbances (storms) at a moderate level. In contrast, other genera, such as *Halodule*, present a faster turnover and high seed set, demonstrating adaptation to high disturbance and grazing rates (Walker et al., 1999). However, these recovery processes depend on the geographical location of the species' survival.

The ideal area for studying and testing disturbance survival hypotheses lies within the large marine ecosystem (LME) region in the Gulf of Guinea, with a critical world marine biological diversity pool, having a shallow coastline that is affected by the Guinea Current (GC) (Ukwe et al., 2003). The area presents distinctive hydrography, productivity, bathymetry and trophodynamics, demonstrating a model changeover in marine resources for flora and fauna. Waves and swell driven by physical disturbance, silt movement and macro-grazers (e.g., sea turtles, manatees) control seagrass growth in those regions (Grech & Coles, 2010). With that, in areas sheltered from wave action or with entrapment of water at low tide (e.g., tide pools), seagrasses have a strong chance of survival, especially in inter-tidal zones, due to the capacity of protection from exposure.

1.2.2 Marine population connectivity in western Africa

The high importance of understanding the patterns of the west coast of Africa can present tools in fields from evolutionary studies to conservation planning (Henderson et al., 2017; Spalding et al., 2007). The West African Transition (WAT) presents a linear coastline with keen environment gradients across near geographical ranges, which has a potential driver of population genetic structure (Tavares et al., 2022). The region is classified as a Marine Ecoregion of the World (MEOW) (Spalding et al., 2007), which covers coastal and shelf waters shallower than 200 m. The WAT is one of the world's most unique coastal systems, with a significant hub of marine food production and biodiversity, classified as the most productive coastline and offshore waters (Ukwe et al., 2003).

The delimitation of WAT starts among the ocean circulations of the tropical Atlantic, with seasonal fluctuations and varying bands on the westward flowing currents in the surface layers (Philander, 2001). WAT has interannual timescales, and net transport of heat from the southern into the northern hemisphere, primarily due to a strong, cross-equatorial coastal current in the Atlantic Ocean, the North Brazil Current (NBC) (Philander, 2001). The widen-driven ocean gyres dominate particular coastal zones shaped by ocean currents, the North Equatorial, the Equatorial, and the South Equatorial (Tavares et al., 2022). These gyres centred on the equator are the result of forces from the equatorial undercurrent situated precisely at that location. The current attains impactful speeds, and the thermocline reaches 100 m depth to the west. The current is present due to westward trade winds, which assist in driving diverging winds to maintain an eastward pressure and bringing upwelling to the west since this occurrence is more intense at the equator. Thus, this pressure force will be the source of thrust for the equatorial undercurrent to the east, which in the downstream direction, will lose strength due to the intense upwelling supporting the surface layers (Philander, 2001)

The elements through the winds parallel to the coast are driven along the coast of Africa, one cold equatorward coastal, the Canary Current upwelling (CCU) off north-west Africa, and the temperate Benguela Current (BC) off south-west Africa. The region's tropical climate is formed through movements of the Inter-Tropical Convergence Zone (ITCZ) (Heileman, 2008). The extreme coastal upwelling associated with these currents and the low sea surface temperatures sustains the westward north and South Equatorial Currents (SEC) (Carton and Huang 1994).

The Canary current upwelling (CCU) influences the semi-arid region in the WET from Mauritania to Senegal, observing the existence of a tropical climate and the presence of a great hydrographic freshwater network (Araujo & Campredon, 2016). These observations are related to the presence of isolated species (e.g., mangrove *Avicennia africana*), living assemblages of the estuarine environment, and similarities with other estuarine areas and tropical coastal lagoons further south in West Africa. Whereas the Guinea current (GC) reaches out from the extreme upwelling area in the north to the northern seasonal boundary of BC in the south, where the northern border of the GC has seasonal fluctuations, and the SEC forms its southern boundary (Binet & Marchal, 1993), featuring the transition to a shallow ocean off a tropically moist coast from Guinea to Gabon. Finally, the influence of the temperate BC founds the subtropical coast southwards to Angola, presenting a significant coastal upwelling zone (Tavares et al., 2022).

The driver structure of MPC along the African Western Coast continues with a lack of succinct studies to obtain a clear understanding of their rich biodiversity (Duarte et al., 2008).

However, there are reported different levels of intraspecific genetic variation of diverse species of marine organisms. These genetic breakage variations are likely considered (Assis et al., 2022), where matching species showed different lineages within neatly drawn regions (Henriques et al., 2014; Reid et al., 2016; Schulze et al., 2020). However, the lifespan must be considered since seagrasses present a specific biologic trait.

1.2.3 Marine ecoregions in western Africa

West Africa is a data-poor region due to a lack of availability of research funding, leaving sites worldwide insufficiently studied (Amano & Sutherland, 2013). As a result, a regional network of marine protected areas (MPAs) was created in 2007 called "Regional Network of Marine Protected Areas in West Africa" – RAMPAO (Patrício et al., 2021) to conserve their biodiversity and protect their ecosystem. Seven western African countries extend the RAMPAO network, starting with Cape Verde, Mauritania, Senegal, Gambia, Guinea-Bissau, Guinea, and Sierra Leone (Selkani, 2022).

Mauritania's coastal waters, located on the Gulf of Arguin, comprise an extensive shallow water area with mudflats interspersed with a set of channels and ditches (Hemminga & Nieuwenhuize, 1991). The National Park of Banc d'Arguin (in French, Parc National du Banc d'Arguin - PNBA) enlarge 12,000 km² and is a crucial area for large- mega-herbivores (turtles), seabirds, elasmobranchs, and fishes (Araujo & Campredon, 2016) PNBA towards one of the most diverse upwelling fishing areas and is considered of great importance regarding their coastal wetland in Western Africa, recognising its ecological and socio-economic value. Furthermore, it has a significant hotspot of blue carbon, carbon stock, and tidal marshes, consisting of the third most scale feeding and breeding grounds for a vast diversity of marine species, including a significant area of seagrass meadows worldwide (Trégarot et al., 2021). Consequently, the biodiversity (e.g., migratory birds, sea turtles) of PNBA has been preserved and the protection of their flora, such as seagrasses banks.

Guinea-Bissau presents the largest coastal archipelago of western Africa, knowns as the Bijagós archipelago, consisting of 88 islands and islets, covering an area of 10,000 km² off the coast of the continental country (Cuq 2001; Leeney & Poncelet, 2015). A key area for biodiversity in the northwest Bijagós archipelago consists of the coastal waters of Unhocomo and Unhocomozinho, a critical feeding ground for juvenile green turtles (Catry et al., 2009).

Another vital area resides in the João Vieira and Poilão Marine National Park (JVPMNP), an MPA of RAMPAO, located in the southwest of the Bijagós archipelago (Catry et al., 2009; Patrício et al., 2019), where comprises four main islands (João Vieira, Cavalos, Meio and Poilão). This area covers 495 km² and has 95% of intertidal areas and shallow waters with less than 30 m of depth (Limoges and Robillard, 1991). The JVPMNP were created to protect one of the six top nesting grounds in the world of the green sea turtle (*Chelonia mydas*) due to the high number of nests each season (ca. 6,000 to 25,000) (SWOT, 2011). Furthermore, the region is a feeding ground for diverse species in the benthic habitats and shelters juvenile turtles, migratory birds, and abundant fish stocks.

Previous research on this area conducted satellite telemetry on green turtles (*Chelonia mydas*) finding that the animals, after post-breeding, travelled around 1000 km north from JVPMNP to PNBA in Mauritania, another MPA of RAMPAO (Godley et al., 2010b). Although only four animals were found in this area, needing further research on the topic demonstrates a marine population connectivity core indicator within this network.

1.3 Understanding connectivity through direct and indirect measurements

Kendrick et al. (2017) propose that the approaches to estimating connectivity between populations' accuracy are crucial to understanding what leads to population adaptation, resilience, speciation, and genetic diversity. Nevertheless, the estrangement in quantifying co-existing and historical connectivity may present challenges in its approaches. Empirically quantification of population connectivity can be investigated through direct and indirect approaches (Tischendorf et al., 2000). The procedures used for the direct techniques (e.g., Geographic Positioning System (GPS) sensor; mark-recapture; Satellite/aerial surveys) (Kool et al., 2013) investigate large-scale individuals and as well migratory species (e.g., megafauna) due to the accurate information on movement over demographic temporal scales. When we investigate over longer scale connectivity to point out evolution, indirect methods are appropriate for quantifying and understanding connectivity between and within populations.

Understanding the functionality of population dynamics and population connectivity is critical if we are to collate our comprehension of genetic connectivity and demographic connectivity in MPC. Despite presenting significant challenges in its approaches, these methodologies are fundamental to the persuasion of proper biodiversity management. Genetic connectivity addresses the gene flow implications for evolutionary patterns within populations (Lowe & Allendorf, 2010), while demographic connectivity is the extent to growth and survival rates influenced by the dispersal of the population (Lowe & Allendorf, 2010) Both genetic and demographic connectivity is essentially distinct conceptual frameworks and demands different but mutually complementary measurement methods. Thus, A thorough comprehension of the prominent roles involved in population connectivity is obtained through integrated and multifaceted efforts, where genetic, demographic, ecological, and hydrodynamic modelling is conducted (Hedgecock et al., 2007; Lowe & Allendorf, 2010).

1.3.1 Genetic connectivity

The vital tools to measure genetic diversity and the long-term survival of populations as genetic variation supplies the basis for adjustment to environmental change through natural selection and confers short-term population-level fitness advantages. Gene flow and population structure, which describe the connectivity and level of genetic differentiation between populations, are essential components and genetic diversity drivers (Tischendorf et al., 2000). Quantifying genetic diversity within and between natural populations increases conservation efficiency, although genetic patterns are challenging to predict, given the complexity of environmental and biological factors that influence population structure and genetic diversity (Birch & Birch, 1984).

Population genetics enables a tool to assess connectivity aggregated across multiple generations while observing restrictions of time scales (Selkoe et al., 2016). In addition, sn genetic markers and the accessibility of new computational developers have grown substantially recently (Jarvis et al., 2012; Arriesgado et al., 2015), assisted in new insights into the genetic MPC patterns (Cowen et al., 2006; Lowe & Allendorf, 2010).

Seagrasses represent an opportunity to investigate genetic diversity's environmental and ecological determinants because they comprise a globally distributed paraphyletic taxon in coastal areas of all continents and belong to nine species assemblages, five of which are exclusively tropical (Les D. et al., 1997). The biological traits of species such as breeding system, pollination mechanisms and dispersal ability strongly influence genetic diversity, as measured by genotypic diversity, genetic copy (or allele) diversity and heterozygosity.

However, Kendrick (2012) suggested that the ability to propagate through horizontal rhizome expansion and propagation by seed led to initial perceptions that seagrasses are predominantly clonal.

The distribution ranges of a single species can differ, from the species distribution limited to a single flower to several widely distributed flowers, such as *Halodule wrightii*, where the process occurs due to different temporal scales (Becheler et al., 2014). Although, the present dispersal of populations is also the result of ongoing extant dynamic processes of colonisation or extinction. The seagrass distribution has been investigated over the years and can be complex and puzzling due to the wide range of species diversity patterns and areas that have not yet been investigated. Genetic diversity data provide perspectives into a range of evolutionary and ecological processes; Thus, these data represent crucial information for conservation management strategies (Duarte et al., 2008).

1.3.2 Modelling dispersal processes

Modelling dispersal processes uses essential hydrodynamic transport facilities whilst embodying physical characteristics of the transported organisms (e.g., vegetive fragments, seeds, pollen, and fruits) (Kendrick et al., 2017) and attribute possible abilities to understand dispersal pathways within coastal and marine systems (Darnell et al., 2015; Cowen & Sponaugle, 2009). Models suggested by recent studies are the biophysical models, investigated on the basis of simulation of the complex two- or three-dimensional time-variance movement patterns of the coastal system, which in return displays propagules transport through current fields. To conduct connectivity analyses using biophysical modelling, we must use functional groups that are environmentally relevant and that have prior insights into their reproductive biology. This connectivity modelling and dispersal method are seen in several pelagic larval phase studies, such as fish and corals (e.g., Chiswell & Rickard, 2011). However, only limited studies focus on macrophytes. Assis et al. (2022) constructed an empirical model to compare the oceanography biophysical model for the Last Glacial Maximum (LGM), the mid-Holocene (MH) and the present to estimate habitats connectivity of the kelp *Laminaria pallida* with microsatellite markers in the southwestern African coast, based on the local BC upwelling system, showing a dispersal barrier consisting of upwelled offshore transport, compared with the results of two different clusters, one in the north and one in the south. Tavares et al. (2022) inferred similar dispersal connectivity by investigating the predictions from biophysical models by oceanographic currents compared with microsatellite markers of the seagrass *Halodule wrightii* along the western African coast. The results showed little probability of connectivity via currents along this coastline, where populations were distinguished in the north and south of the equator, and high contrast in comparison of divergent genetic data among these populations. Thus, research regarding modelling coastal systems addressing ecological, economic and social services highlights the opportunity to understand MPC structures by ocean currents to facilitate conservation plans.

1.4 Taxonomy of seagrasses

Marine macrophytes are the group of aquatic organisms capable of oxygenic photosynthesis large enough to be observed, such as macroalgae, seagrasses and mangroves (Pennesi et al., 2015). Seagrasses have developed from the Alismatidade, the monocotyledon angiosperm group (Kendrick et al., 2012). The term seagrass refers to the grass as the habitat of different species, and according to Arber (1920), to be considered a water plant, there are a set of four properties, following as (i) the plants must be adapted to live in a saline medium; (ii) the plants must be able to grow when fully submerged; (iii) the plants must have a secure anchoring system; and (iv) the plants must have a hydrophilous pollination mechanism (**Fig. 1**).

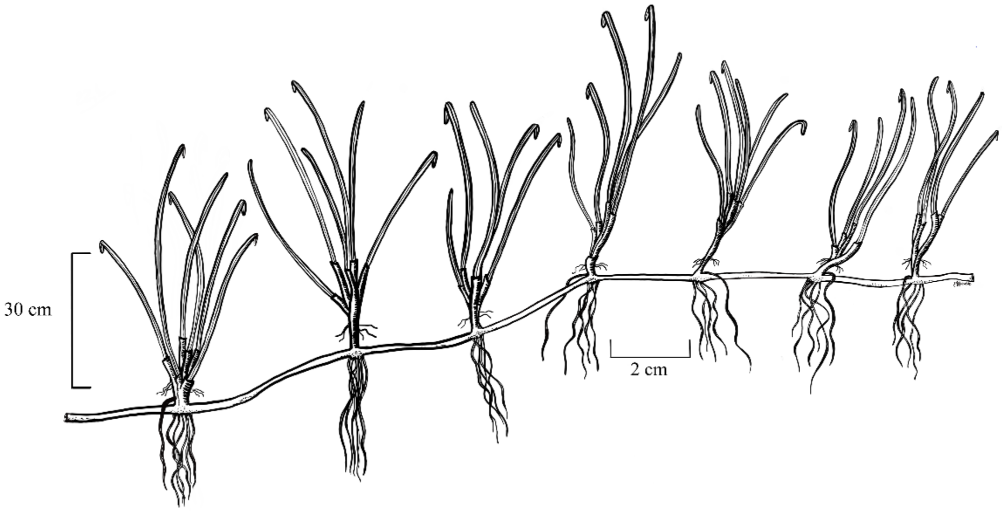

Figure 1. The typical habitat of Halodule wrightii draws from the seagrass meadows in Cabras Islets, São Tomé and Príncipe.

Seagrasses are flowering plants that form large populations in estuarine and protected coastal waters from the equator to high latitudes on all continents except Antarctica (Duarte et al., 2008b). Six seagrass bioregions encompass the world's oceans across tropical and temperate waters (Short et al., 2007). These are now described as 72 seagrass species that spend most of their lives submerged in seawater (Short et al., 2011). The roots of the majority remain in shallow sediment bottoms, and the depth limits are set by sufficient light to support net positive growth, usually 20 meters in depth, which can be deeper in oceanic waters and shallower in turbid estuaries (Heck & Valentine, 2006).

Seagrass form an ecological group where the various families are not closely related. (Larkum et al., 2018). Three of the four families (Zosteraceae, Cymodoceaceae, Posidoniaceae and Hydrocharitaceae) consist exclusively of seagrasses, the *Zosteraceae*, the *Cymodoceaceae*, and the *Posidoniaceae*, having 12 marine genera (Tomlinson, 1982; Dahlgren et al., 1985) altogether. The distribution of seagrasses worldwide has been reported since the first attempt to analyse geographical distribution by Ascherson (1871). Of the 12 genera existing, seven are mainly distributed along tropical coasts between the tropical Atlantic and Indo-Pacific, viz. *Thalassia*, *Halophila*, *Syringodium*, *Halodule*, *Cymodocea*, *Thalassodendron* and *Enhalus*. (Short et al., 2007).

The genus *Halodule*, which belongs to the *Cymodoceaceae* family, has seven species, which can be distinguished by comparing characteristics such as the form of the leaf trip and the width of the leaves (see **Fig. 1**) (Den Hartog, 1970). The first description was made by Du Petit Thouars (1806), describing the genus from Madagascar, and the name *Halodule* was given by Endlicher (1841).

The specie *Halodule wrightii* is widely distributed from Cuba to South America's coast, extending southward along the coast of Brazil (De Oliveira et al., 1983). On the other side of the Atlantic coast, these species can also be found along the Western African coast, e.g., Mauritania, Cape Verde, Senegal, Guinea-Bissau, Gabon, São Tomé & Príncipe, and Angola (Alexandre et al., 2017).

1.4.1 Dispersal and recruitment of marine plants

The life history of marine plants depends on an essential process phase, dispersal, and present crucial dependence to build their connectivity between populations, create resilience to natural, anthropogenic disturbances, and recover from large-scale declines due to limited propagation (Eklf et al., 2009). In most cases, seagrass utilises multiple dispersal mechanisms via both abiotic and biotic factors as written above (**Fig. 2**); however, individual plant species may depend on one species-specific dispersal mechanism such as surface and submarine movement, animal vectors, or via clonal growth (Gulick et al., 2021).

The movement of organisms or their propagules leads to dispersal, determining the scale of interaction between ecosystem dynamics, responding to disturbances, and evolving in the environment, which eventually conducts gene flow (Berković et al., 2014; Kinlan & Gaines, 2003). Consequently, seagrasses movement is limited to specially life-history phases (i.e., seed dispersal).

Thus, mediation of seed dispersal is done through abiotic or biotic factors, and the dispersal mechanism defining the spatial distribution is commonly indicated by seed morphology, plant height, wind speed and direction, and habitat patchiness (**Fig. 2**) (Beckman et al., 2020).

1.4.2 Reproduction via clonal growth

The reproductive strategies of seagrasses expand through vegetative clonal growth by rhizome extension (**Fig. 2**) (Kendrick et al., 2012); thus, individual plants arising from seed present the ability to structure large clones implying disjointed physiological offspring (Waycott et al., 2006). Seagrass genets move by horizontally and vertically growth, where ramification occurs into new ramets onto the existing plant, followed by apical dominance, a process that allows them to move. Thus, clonal growth is independent of biotic factors for dispersal and is directly influenced by an ecological understanding of navigability. Clonal growth proceeds over a lifetime of genet, varying seasonally and in turn to environmental conditions such as light, grazing, and nutrients (Udy & Dennison, 1997; Eklf et al., 2009; Lavery et al., 2009). Genets can grow in nutrient-rich areas and shortcomings in the canopy (Duarte et al., 2006) or from sulphide-rich stress stains (Terrados et al., 1999). Furthermore, the influence of substrate barriers can modify the movement capability, and the direction of movement depends on growth rates and longevity.

The variation of clonal growth depends on the genera, where it is possible to see orders of diverse magnitude in several species and depends on the age of other species (Duartel & Ensen, 1990). Studies related to genet lifespans investigated through genetic data discovered different movement paths in the ages of different species (e.g., 100 000 years for Posidonia) (Arnaud-Haond et al., 2012). Finally, based on clonal sizes and the cultivated distance movement, seagrasses with extreme longevity can move more than 10 km to other areas (Arnaud-Haond et al., 2012). Nevertheless, a more comprehensive study is needed on the seagrass clonal growth to determine their right path and movement.

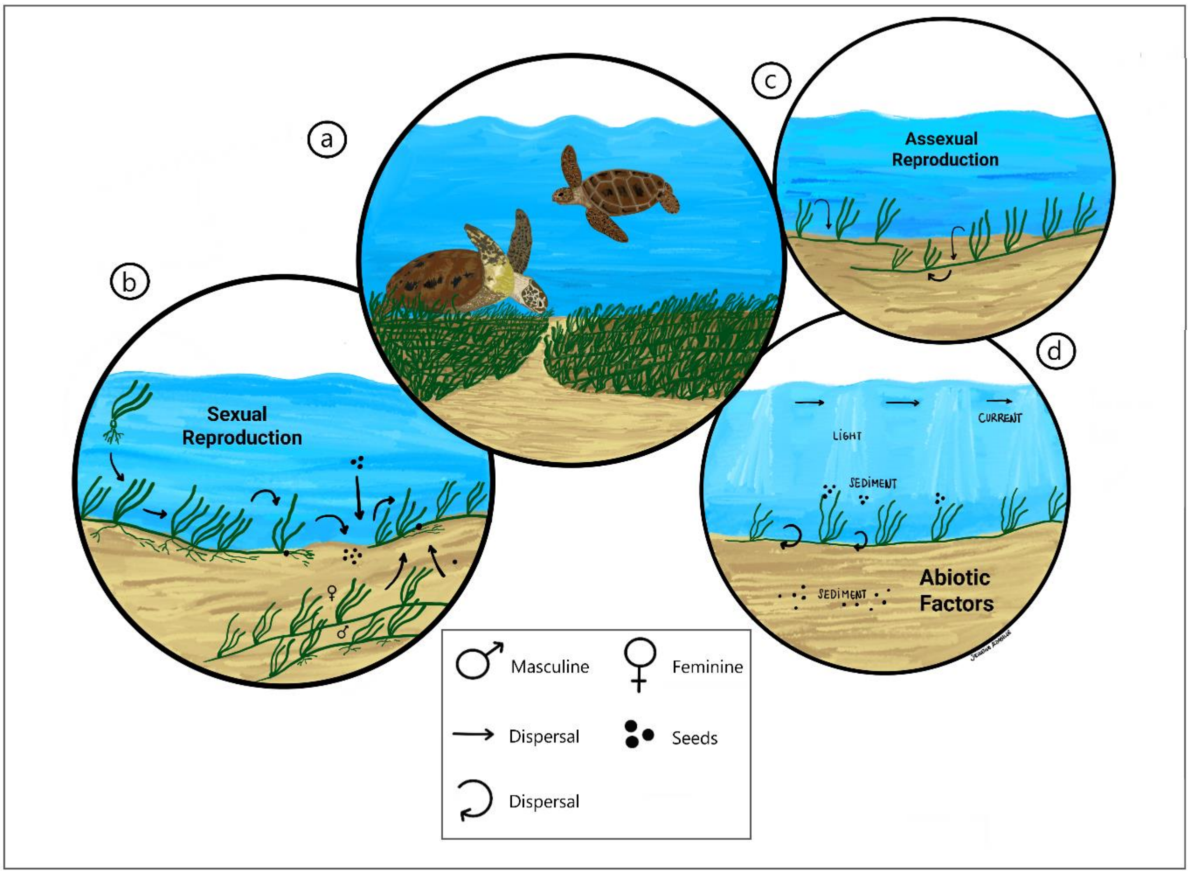

Figure 2. Illustration of different mechanisms on dispersal and recruitment of H. wrightii. (a) Biotic mechanisms. Ingestion of seagrass seeds or propagules by green turtles (b) Sexual reproduction via seed banks and germination (c) Via clonal growth influenced by light, nutrients, sediment, disturbance, and barriers. (d) Abiotic mechanisms (currents, sediment movement, wave, tide). Figure inspired in McMahon et al. (2014).

1.4.3 Sexual reproduction

Understanding the patterns of sexual reproductive through seed recruitment is necessary to grasp the full ecosystem dynamics of the site aggregated with such populations. Therefore, the reproductive biology of seagrass must be evaluated at a morphological, functional, and ecological level. With that, the characteristic of their reproductive biology remains unique and has evolved individually across several lineages.

The sexual reproductive seagrasses present a dioecious feature, which requires modifications to assist their survival. Consequently, fluid-dynamic interactions mediate pollination between the pollen and flow rate graduals around flowers, which leads to rotational and repositioning movements in the flow as pollen is carried around the flowers. This biomechanical behavioural response enhances the pollination chance, posing a selective edge for these underwater pollinating plants (**Fig. 3**) (Larkum et al., 2018). Seagrass flowers can set up below the sediment surface (e.g., *Halodule*; *Halophila*) or above or within the canopy (e.g., *Enhalus*) (Kendrick et al., 2012), which indicates that flowers at the sediment are likely to be less hardy than the one on the surface (McMahon et al., 2014).

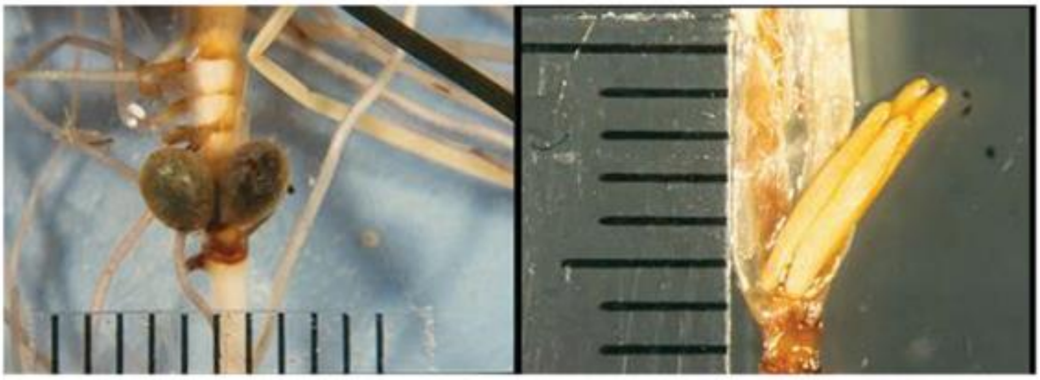

Figure 3. Two fruits of Halodule wrightii are attached to the maternal shoot (left) and the staminate flower of H. wrightii (right). Scale in mm for both images. Image creator's Kowalski, Joseph et al. on "Flowering and seed production in the subtropical seagrass, Halodule wrightii (shoal grass)" Botanica Marina, vol. 59, no. 2-3, 2016, pp. 193-199.

The movement of seeds is based on the reproductive strategy, the reproduction structures' morphology, seedling size and seed, buoyancy and shape, and the individual specie's life history (Darnell et al., 2015; McMahon et al., 2014). An example is the hydrodynamic conditions, where certain species disperse under normal conditions, although other species demand storm conditions to start seed transportation (Ruiz-Montoya et al., 2012). These movements are because the reproductive structures of some species require transport in the water column or on the sediment surface (McMahon et al., 2014; Orth et al., 2006).

There are two stages of seed dispersal, the primary and secondary dispersal, which comprise all seed orientation after the parent plant's release (Darnell et al., 2014). According to Watkinson (1978), primary dispersal encompasses the beginning seed deposition to the substrate, while secondary dispersal entails all other seed orientations until the final seed settlement. Greene and Johnson (1996) provided an example of these two stages, the wind-dispersed done by primary dispersal, which involves the airborne transport of seeds from the parent plant to the ground, and the secondary dispersal involving all movement after the initial establishment. Thus, the source of secondary dispersal can differ from the seed shadow from primary dispersal and may be more prominent than primary dispersal casting plant population demography and structure (Chambers and MacMahon, 1994; Harper, 1977).

On the other hand, seed banks are established when seeds produced by dormant species remain in the sediment, leading to germination when a stimulus occurs, whether genetic or environmental (Steele, 2001). Mainly, species are introduced explicitly for efficient seed dispersal and the construction of a fixed seed bank (Darnell et al., 2014). This sort of distinct period with seed dormancy causes seeds to be dispersed in two ways, either in time or space (Walck et al., 2005).

Studies conducted on the distance seeds travel, investigating primary dispersal of seeds and encapsulated seeds, have revealed a broad long distance travelled (McMillan et al., 1981; Harwell and Orth, 2002; Orth et al., 1994; Ruiz-Montoya et al., 2012; Van Dijk et al., 2018). For example, the genera *Posidonia, Enhalus* and *Thalassia* present enormous seed shadows showing persistence, dispersing hundreds of kilometres from the parent plant in the primary dispersal seeds at large distances. In contrast, these mechanisms are different when discussing the transient genera *Halodule* and *Halophila* due to their tendency to have small seed shadows arising from primary dispersal (Kendrick et al., 2012).

The genera producing negatively buoyant seeds initiate (primary dispersal) settlement to the substrate (McMahon et al., 2014), moving seeds by hydrodynamically mediated transport (McMahon et al., 2014; Orth et al., 2006) throughout the sediment surface (secondary dispersal). These movement results in the dependence on the strength of wave-driven fluctuating fluxes and topographic aspects such as sediment mounds, substrate type, and sand ripples, as such aspects can hamper movement in seed dispersal distance at the sediment surface (Kock et al., 2010; McMahon et al., 2014; Orth et al., 2006).

There is other research regarding secondary dispersal in terrestrial plants. However, little is found in the works related to secondary seed dispersal approaches or the final orientation of marine plants. Although they feature a broad range of reproductive strategies, seagrass species display distinct characteristics regarding conditions (e.g., submarine flowering, pollination, or surface), efforts, timing, and morphological framework (Ackerman, 2006). For example, while some species produce many seeds per fruit (e.g., *Halophila decipiens* 30 seed fruit^{-1}, van Tussenbroek et al., 2010), others produce a single seed within a fruit (e.g., *Syringodium filiforme*, McMillan, 1981). In contrast, some species (e.g., *Posidonia australis*, *Amphibolis griffithii*) reproduce in the fall and winter (Ackerman, 2006), while others (e.g., *Zostera marina*) are reproductive in the spring and summer.

The specie *Halodule wrightii* commonly occurs in mixed beds forming monospecific meadows (Darnell et al., 2014), which tolerates sub-optimal conditions and disturbances. *Halodule wrightii* has a negatively buoyant reproductive stage and has situated primary dispersal and a predominantly small primary seed shadow (Darnel et al., 2014). Its seeds are enclosed by a rigid seed membrane and can remain dormant for four years, setting up a seed reserve in the sediment (**Fig. 4**); therefore, the unit of secondary dispersal for *Halodule wrightii* is a seed (McMillan, 1981). Thus, the round shape and tiny *Halodule wrightii* seeds possibly limit them to shallow speed hydrodynamic requirements adjacent to the substrate (Koch et al., 2006). The small leaf traits of *Halodule wrightii* may also provide an understanding of the force of water that can act over them.

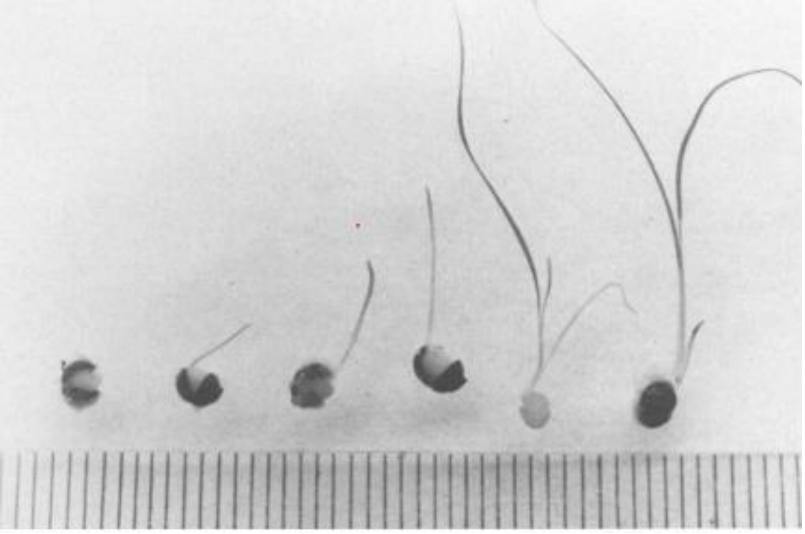

Figure 4. Germination and seedling development of H. wrightii. The seed's opening and the cotyledon's appearance are shown from left to right. The two seedlings on the right, without and with a fruit seed coat, have produced two leaflets beyond the cotyledon and have an adventitious root beginning to appear at the cotyledon node. A millimetre scale is shown at the bottom. Image's creator Calvin McMillan in "Seed reserves and seed germination for two seagrasses, Halodule wrightii and Syringodium filiforme, from the western Atlantic", Aquatic Botany, Volume 11, 1981, Pages 279-296.

Beyond this, another study carried out by Tol et al. (2017) studied the long-distance biotic dispersal of tropical seagrass seed by marine mega-herbivores (dugongs and green turtles). The study used population estimation for green sea turtles (*C. mydas*) by calculating potential seagrass seed dispersal distances in the region of Australia (Great Barrier Reef) and data on digestion time, average daily movement, and numbers of viable seeds of three species of seagrasses (*Zostera muelleri*, *Halodule uninervis* and *Halophila decipiens*) excreted. The dispersal distance confirmed that green sea turtles (*C. mydas*) assist seagrass dispersal and enable a large-scale approach by consuming and passing in the faecal matter for viability on excretion in more considerable distances (maximum 650km), improving connectivity between seagrass meadows.

Consequently, seed dispersal can represent a critical life-history phase for several plant species. Competition between sibling plants decreases when they disperse, allowing seeds to escape from mortality near the parent plant since that location will be of greater competition, aiding in the recolonisation of disturbed habitats and increasing the likelihood that seeds will find a suitable substrate for cultivation (Willson and Traveset, 2000). Furthermore, recent studies show that over grand spatial scales, genotypic diversity for individual species presents high probabilities, implying that (i) the function of sexual reproduction was lastly overlooked and (ii) dispersal encompassed by seagrass beds probably afford to observed genetic diversity (Kendrick et al., 2012). For this reason, studies on seagrass reproduction, seed dispersal and spatial scales are necessary to understand life history dynamics and seagrass propagation fully.

1.5 Understanding the role of green sea turtle habitats

In marine environments, migratory species strongly influence dispersal and connectivity, genetic diversity, recruitment standards, and relations between breeding and feeding grounds (Blumenthal et al., 2009; Godley et al., 2010a). The complex processes regarding dispersal and recruitment often affect species-specific responses for attributes among different marine environments, which influence the coastal and the open ocean. As with most large vertebrates, when plentiful, sea turtles play critical ecological roles in their marine environment as consumers and prey (**Fig. 2**) (Bjorndal 2003). They have a complex life cycle (Heithaus et al., 2014), interacting in different ecosystems, allowing them to migrate between sites.

Mutually beneficial interactions between seagrasses and marine mega-herbivores have undergone evolutionary processes, contributing to the co-evolution of marine environments (Gulick et al., 2021; Godley et al., 2010a). Plant seeds' access through animal intestines drives to a key mutualism leading the animal with food and the plant with seed dispersal improved germination (Jaganathan et al., 2016). Mutualism relationships are well known between terrestrial plants and animals (e.g., birds, reptiles, mammals). However, little is known about marine species. The effects of ingestion and excretion by marine mega-herbivores of seagrass seeds have been studied by Tol et al. (2021) in recent years, discovering that germination of seagrass seeds (*Zostera muelleri*) has success in ingestion by dugongs and green turtles in the Great Barrier Reef (Australia), proving a vital symbiosis relationship and seagrass resilience and connectivity among metapopulations.

The regional management units (RMUs) of sea turtle subpopulations worldwide present 18 subpopulations in Africa (between Indian and the Atlantic Ocean islands) (SWOT Report, 2017). Africa's Atlantic continental shores from Mauritania south to Angola (Northwest and Central West Africa) - including the islands - are the habitat for five species of sea turtles; RMUs networks play a vital key in developing which are the dynamic processes between sea turtles and their environment in those regions. For example, green sea turtles (*Chelonia mydas*) can migrate with a circumglobally distribution throughout tropical and subtropical areas (Catry et al., 2002). Furthermore, green turtles have high site fidelity to feeding grounds (Bjorndal et al., 2003a), which can form resident aggregations that will graze in the same area for years (Hancock et al., 2019). Finally, a critical RMU is in Mauritania's PNBA, where green turtles from the Bijagós Archipelago (Guinea-Bissau) feed in those rich biodiversity hotspot nearshore waters (Belhabib et al., 2012).

Recently studies on the diet composition of green turtles from the oesophagal and cloacal swab presented high dietary richness with the predominance of red algae, seagrass, and diatoms on the Western African coast (Bijagó islands). In addition, benthic macrophyte communities were dominant by *Laurencia dendroidea* and *Halodule wrightii*, with a high frequency of occurrence (Diaz et al. 2022). The assessment of sea turtles' diet combined with feeding ecology research may aid managers in identifying essential habitats that demand long-term conservation and raise understanding of feeding hotspots and MPC (Stokes et al., 2019).

The large-scale changes in ecosystems supplied an understanding of the role of top-down control in forming structure and function in ecosystems, which is essential for the correct management of coastal marine ecosystems (Burkholder et al., 2011; Zarnetske et al., 2012). Among other research priorities, to properly assess and implement successful management strategies and attribute value to the ecosystem services provided by seagrass meadows (Barbier et al., 2011), it has been imperative the knowledge regarding the factors in their distribution as well as the impact (e.g., water quality, fisheries production, climate change, and coastal erosion control) affecting the declining fastness of these ecosystems (Waycott et al., 2009). Furthermore, to tackle these issues, it is essential to conduct science in developing world countries that are resource-rich but highly economically dependent on coastal production.

1.6 Importance of seagrasses

Seagrasses are marine flowering plants that have evolved to live submerged, impacting coastal waters' physical, chemical, and biological environments, acting as ecological engineers (Unsworth et al., 2015), and providing many critical ecological services to the marine environment (Costanza et al. 1997). For example, seagrass stabilises sediments, produces organic carbon, and changes nutrient cycling, water flow, and the structure of food webs (Hemminga and Duarte 2000). In addition, they provide crucial habitats for various animals, including commercially and recreationally important fisheries species, and a significant food supply for mega-herbivores like the green sea turtle, among others (Beck et al. 2001).

Seagrasses have lower species diversity and unique physiological patterns, with a wide-ranging geographically distribution. The evolutionary characteristics of these marine plants include morphological adaptations, including internal gas transport, epidermal chloroplasts, submarine pollination, and marine dispersal (den Hartog 1970; Les D. et al., 1997). These adaptations influence the oxygen needed for their rhizomes and roots, requiring one of the highest light levels of any plant group worldwide (Dennison et al. 1993). The high light requirements lead seagrasses to be responsive to environmental changes. The global distribution and abundance of seagrass have been changed through evolution due to sea-level change, physical modification of coastlines, water temperature and global changes in CO_2 concentration (Muelbert et al., 2019).

1.6.1 Decline and recovery of seagrass ecosystems

The coastal zones are one of the most threatened locations that undergo anthropogenic impacts. As the impact of human activities increases, concern about the decline of biodiversity in habitats around sites that provide a sound basis for an entire ecosystem rises. Seagrass meadows deal with anthropogenic pressures, such as dredging, trawling, eutrophication, coastal development, and climate change (Waycott et al., 2009). Furthermore, seagrass also faces challenges in external influences such as decreased light availability, eutrophication, and sedimentation rate (Erftemeijer and Lewis, 2006; Waycott et al., 2009). Other's habitat degradation can also appear as stressors like the presence of invasive species and diseases. Thus, ecosystems sometimes experience a change of ecological regime resulting from abrupt changes.

Estimates predict that the decline of seagrass meadows has been accelerating in the last decade (Dunic et al., 2021). In addition, the number of losses masked significant differences in rates and directions of change between sites, with sites experiencing long-term stability and others expanding in size. (Dunic et al., 2021). Substantial assessment and monitoring efforts can provide a wealth of additional data that allow us to reassess global patterns of seagrass change in the context of increasing human impacts.

We need to identify the patterns of declines in seagrass meadows. This assessment can be done in correlation studies of genetic analyses and spatial monitoring. Despite the exuberance of seagrasses along the coastlines, our understanding of the status of seagrasses is limited, particularly in bioregions such as the Tropical Atlantic, particularly in the Eastern Tropical Atlantic (Unsworth et al., 2018). Finally, to enable better management at the local scale, we urgently need to increase our understanding of the reasons for the decline of seagrass meadows, as indicated by the variety of measures in their ecological patterns and the attribution of change to multiple variables.

2. Aims of study

The main aim of the current study is to assess if genetic connectivity among regional populations of seagrass meadows contrasts oceanographic influences by divergent ocean currents that are strongly connected by tidal currents in two grazing turtle habitats and marine biodiversity hotspots. The hypothesis is tested using the familiar and essential habitat-forming shoalgrass *Halodule wrightii* on the Western African coast. The hypotheses tested in this project were:

(i) Investigate the genetic differentiation and structure of seven groups subdivided into 20 populations of *H. wrightii* along the distribution on the western coast of Africa.

(ii) Assess genetic differentiation and genotypic richness along the distribution of populations in two regions: Banc D'Arguin (Mauritania), comparing nearshore and offshore distribution; and the Bijagos Archipelago (Guinea-Bissau), comparing populations between the north and south. The hypothesis is to test if the genetic diversity diverges among the limit of the two ocean currents (CC and GB) occurring in those regions.

(iii) Estimate genotypic richness to compare through this coastline if clonal growth occurs at similar levels betweens all 20 populations.

(iv) Test isolation by distance (IBD) within populations in the core groups of Banc D’Arguin, and Bijagós Archipelago to suggest long-distance dispersal.

Genetic analyses of 20 populations from northern Mauritania to Northern Angola, including the islands of Cape Verde and São Tomé and Príncipe, were conducted using eight microsatellite markers. Furthermore, we compared the results obtained with published reports for population connectivity from the distribution centre (Western and Southern African coast). The findings discuss how to improve seagrass management on the Western African coast to sustain healthy seagrass populations with high resilience to environmental change.

Genetic connectivity and diversity of the seagrass *Halodule wrightii* in contrasting habitats of the Western African coast

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Abstract

Understanding the patterns and processes of genetic diversity and differentiation is fundamental for assessing population connectivity to inform the conservation of coastal ecosystems. The tropical West African Atlantic Coast is a good model to address these issues. The influence of oceanographic currents diverging in hotspot ecoregions mediating propagule dispersal along this coastline raises interesting predictions to be tested. In the Gulf of Arguin (Mauritania), nearshore and offshore seagrass populations can be predicted to be highly connected by strong currents driven by tides in this very shallow system. In contrast, in the Bijagós archipelago (Guinea-Bissau), populations in the northern and southern islands could hypothetically be genetically differentiated as predicted based on passive transport by ocean currents. However, they also have a strong relationship of connectivity by migratory species, namely green turtles that travel between feeding and breeding grounds. This study aims to assess these predictions and understand seagrass population genetic diversity, genotypic (clonal) diversity, and connectivity along the Western African coast. We used as a model the species *Halodule wrightii*, a seagrass with clonal reproduction via rhizome elongation and sexual reproduction via non-buoyant seeds, both traits that do not promote long-distance dispersal. We assessed genetic diversity, clonality, and population differentiation with 8 microsatellite markers, using samples from the entire species range along this coastline, from Mauritania to Angola, over a distance of ~1,600 km. Generally, populations were genetically homogeneous with low differentiation, while genotypic richness varied among all populations. The results support the hypothesis of high connectivity between populations in the Banc d'Arguin but do not support the isolation hypothesis between the northern and southern regions of the Bijagós. The results also suggest that the Canary Current and Guinea Current do not affect seed dispersal and connectivity through abiotic mechanisms.

Keywords: connectivity, genetic diversity, *Halodule wrightii*, western African coast, seed dispersal, recruitment

3. Chapter two - Introduction

Understanding population connectivity, long-distance dispersal, gene flow, and patterns in ecosystem dynamics contribute to conservation plans in spatially heterogeneous coastal seascapes (Kendrick et al., 2016). Marine population connectivity (MPC) – defined here as the movement of elements to quantify rates of exchange among or within marine ecosystems to regulate mechanistic understanding of the abundance and distribution of marine populations - plays a vital role in the ecology and evolution of populations and species. The transport and exchange of individuals between geographical areas contribute to their maintenance and survival, creating metapopulations (Cowen and Sponaugle 2009). In addition, these movements contribute to preserving and combining genetic diversity, a crucial mechanism for organisms' adaptation and resilience to create capacity for survival in different habitats.

Several mechanisms promote MPC, such as the passive transport through the ocean currents of planktonic stages (i.e., propagules, larvae, juvenile and fragments) or active adult movements (Cowen and Sponaugle 2009). Marine species display a remarkable range in dispersal ability, resulting in near panmixia to highly structured metapopulations. Sessile organisms (i.e., invertebrates with direct development, macroalgae, and marine plants) lacking pelagic larval stages are poor dispersers and thus tend to exhibit substantial genetic population structure across their ranges (Assis et al., 2022). Several mechanisms promote MPC, such as the passive transport through the ocean currents of planktonic stages (i.e., propagules, larvae, juvenile and fragments) or active adult movements. Marine species display a remarkable range in dispersal ability, resulting in near panmixia to highly structured metapopulations. Sessile organisms (i.e., invertebrates with direct development, macroalgae, and marine plants) lacking pelagic larval stages are poor dispersers and thus tend to exhibit substantial genetic population structure across their ranges.

Genetic variability at distinct spatial scales can display different patterns among many populations, impacting their dynamics and distribution dispersal (Tavares et al., 2022). Therefore, comprehending this complex system is crucial for the management, restoration, and conservation of natural resources, in particular nowadays with rapid global change. For example, marine gene flow may be constrained by thermal barriers, geographic traits, currents, and related separation to the feasibility of dispersive phases (Goldberg and Lande

2007). In addition, a population's size is associated with dispersal abilities, whereby little connectivity can lead to smaller populations (Palumbi 2003). As a result, the margins of the geographical distribution of species, where populations tend to be smaller, may be affected by climate variations, causing significant consequences of divergent genetic differentiation and allopatric speciation (Petit et al., 2003; Vucetich and Waite, 2003). For the management of coastal zones, genetic data enables access to unsuspected resources in conservation aspects to mitigate degradation. Then, population genetics can provide a valuable asset for comprehending the resources needed for small and large-scale coastal management. Genetic variability at distinct spatial scales can display different patterns among many populations, impacting their dynamics and distribution dispersal. Therefore, comprehending this complex system is crucial for the management, restoration, and conservation of natural resources, in particular nowadays with rapid global change. For example, marine gene flow may be constrained by thermal barriers, geographic traits, currents, and related separation to the feasibility of dispersive phases. In addition, a population's size is associated with dispersal abilities, whereby little connectivity can lead to smaller populations. As a result, the margins of the geographical distribution of species, where populations tend to be smaller, may be affected by climate variations, causing significant consequences of divergent genetic differentiation and allopatric speciation (Petit et al., 2003; Vucetich and Waite, 2003). For the management of coastal zones, genetic data enables access to unsuspected resources in conservation aspects to mitigate degradation. Then, population genetics can provide a valuable asset for comprehending the resources needed for small and large-scale coastal management.

Seagrasses are excellent models for investigating limited dispersal ability's demographic and genetic effects. They lack larval stages and are often composed of populations distributed discontinuously in distant habitats, so they are expected to be highly structured in space. Direct tracing of movements of propagules or drifting individuals is nevertheless impossible to quantify over large spatial scales, particularly in marine environments. Indicators of population recruitment, such as genetic diversity and genotypic richness, are likely to raise implications throughout the distribution ranges. Seagrass fragments consisting of viable shoots can be drifted away, while seeds can have different dispersive characteristics (sinking seeds banks or floating units) (Larkum, Orth, and Duarte, 2006). These traits contribute to seed recruitment, transporting fragments (propagules or fruits) elsewhere, drifting away to suitable areas (Kendrick et al., 2017), or contributing to reattachment in the sediment-forming seed banks. The recruitment process is likely to vary between intertidal and subtidal populations, which can differ the recruitment and seed persistence.

Furthermore, many seagrass species produce seeds that remain dormant for long periods (i.e., *Cymodocea*, *Halodule*) that potentially allow recolonization and population resilience in the long term (Baskin and Baskin, 2014). Genetic data may thus be used as a proxy to infer broad-scale patterns of historical colonisation and modern connectivity. Consequently, understanding the paths for such colonization and, thus, their gene flow in oceanographical habitats is essential to assess the ways of their seed dispersal and recruitment by drifting way (McMahon et al., 2014; Tavares et al., 2022).

Seagrass relies on the growth of surviving plants, the establishment, germination and survival of sexual propagules via biotic and abiotic mechanisms (Evans et al., 2021). Oceanic currents can be a key mechanism for fragments, influencing population genetic structure and the retention or weakening of the signatures of past events (Assis et al., 2018; Buonomo et al., 2016; Lourenço et al., 2017). Therefore, short-distance dispersal is likely to be affected by oceanographical recurring features. At the same time, other oceanographic processes can also affect dispersal (e.g.., upwelling), disrupting gene flow and long-distance connectivity for organisms with distinct physiological traits (Assis et al., 2022). Thus, it is crucial to understand which processes and traits affect dispersal. - due to the capacity of transportation versus negatively buoyant seeds, which disperse through drifting released seagrass shoots attached to the leaf (Tavares et al., 2022).

Active transportation by marine animals represents another mechanism for long-distance dispersal (Begon et al., 1990). Migratory species have complex life cycles (Van Buskirk and Crowder, 1994), moving between different areas and ecosystems. Plant seeds consumed and excreted by marine animals on the move can survive and contribute to dispersal and connectivity among populations and to the colonization of new habitats (Tol et al., 2021). Marine animals create connectivity routes along a broad range of spatial scales., These complex interactions, however, remain largely understudied.

Seagrasses are submerged marine angiosperms influencing coastal waters' physical, chemical, and biological environments. They act as ecological engineers (Wright and Jones, 2006) and provide many critical ecological services to the marine environment and human settlements

(Costanza et al., 1997). For example, seagrasses stabilize sediments, produce organic carbon, and change nutrient cycling, water flow, and the structure of food webs (Hemminga and Duarte 2000). In addition, they provide crucial habitats for various animals, including commercially and recreationally important fisheries species, as well as a significant food supply for megaherbivores (Beck et al., 2001). In addition, the evolutionary traits of these marine plants include morphological adaptations, including internal gas transport, epidermal chloroplasts, submarine pollination, and marine dispersal (Les, Cleland, and Waycott 1997; den Hartog 1970).

Halodule wrightii (shoal grass) (Ascherson) (family, phylum) is a perennial dioecious seagrass. It produces hard coat seeds on the bottom of the canopy that are neutral or non-fluctuating forming seed banks that can remain dormant for an extended period (McMillan 1981). Its distribution is highly fragmented, as populations only develop in specific disconnected habitats. Consequently, it is predicted to exhibit highly structured populations and strong population structures (Larkum et al. 2006; Tavares et al., 2022). This species, therefore, can contribute to understanding genetic patterns and structure along the western coast of Africa. Studies in this coastal region report the presence of *H. wrightii* in the region of the Gulf of Arguin (Mauritania) and Senegal, on the coast of Santiago island (Cape Verde), on the Bijagós Archipelago (Guinea-Bissau), in the Gulf of Guinea (São Tomé and Príncipe), and the southwest African coast (Gabon and Angola) (Alexandra H. Cunha and Araújo 2009; Alexandre et al. 2017; Creed et al. 2016; Díaz-Abad et al. 2022; Santos 2007; Tavares et al. 2022). Despite being one of the most common seagrass species in the Atlantic Ocean, with a wide distribution along tropical and subtropical areas, studies focusing on *H. wrightii* biogeography in the eastern and western Atlantic are still scarce. Studies of this central species have focused on regional genetic diversity; thus, it remains unclear whether genetic links exist in both locations (Tavares et al., 2022). Although this tropical region presents vast marine biodiversity, there are gaps related to genetic connectivity studies on seagrass meadows needing further research to be tested.

There are two crucial hotspot areas (Gulf of Arguin and Bijagos Archipelago) with a strong presence of the green turtle (*Chelonia mydas*) transiting seasonally between areas of the coastal region to feed and breed (Hancock et al., 2017). Thus, an exciting question to explore is whether the connectivity of *H. wrightii populations* displays genetic similarities between regions corresponding to the dispersal of its fragments via ocean currents between the divergences; or whether this connectivity displays similarity through the movements of the grazing green turtle (*Chelonia mydas*) when migrates contributing to seed dispersal.

Seagrasses are capable of reproduction in two different modes – via sexual reproduction when individuals reproduce by hydrophilous pollination to produce seeds with paternal and maternal genetic contribution (genets); and via asexual clonal growth by subsurface rhizome elongation and production of new shoots (ramets), which may eventually detach and have an independent life (Tavares et al., 2022). For species with both sexual and clonal reproductive strategies, a biogeographical hypothesis is associated with the predominance of clonal reproduction patterns in geographically isolated populations (Eckert 2001); yet species with these patterns are more likely to be influenced by environmental gradients, in particular by physical stress at the margins of their distributional range (Araújo and Rozenfeld 2014; Diekmann and Serrão 2012). Thus, regions with geographic transitions and central patterns of edge distribution confront genotype richness, a parameter with the significance of sexual and clonal reproduction on population dynamics (Alberto et al. 2008). Nevertheless, the distributional patterns of genotypic richness for marine clonal organisms with both sexual and asexual reproductive traits need more evaluation (Arriesgado et al. 2015; J. a Coyer et al. 2004; Olsen et al. 2004). The distributional patterns of genotypic and genetic diversities for marine clonal organisms are still scarce, even more in tropical seagrass species (Alberto et al., 2006; Olsen et al., 2004). Furthermore, range edge populations decrease sexual reproductive activity in some seagrass species (Billingham et al. 2003; Cabaço, Ferreira, and Santos 2010; Eckert 2001); however, little data is reported for the tropical seagrass *H. wrightii* on the west coast of Africa (Alexandre et al., 2017; Chefaoui et al., 2021; Creed et al., 2016; Cunha & Araújo, 2009; Tavares et al., 2022; Santos, 2007).

This study explores the importance of the Atlantic transition zone on the northwest and southwest African coast as an oceanographic barrier to current gene flow, using as our model the geographic range populations of the tropical seagrass *Halodule wrightii*. We used eight microsatellite markers. Our specific aims were to:

(i) Investigate the genetic differentiation of seven groups subdivided into 20 populations of *H. wrightii* along the distribution on the western coast of Africa.

(ii) Assess genetic differentiation along the distribution of populations in two regions: Banc D'Arguin (Mauritania), comparing nearshore and offshore distribution, and the

Bijagos Archipelago (Guinea-Bissau), comparing populations between the north and south. The hypothesis is to test if the genetic diversity diverges among the limit of the two ocean currents (CC and GB) occurring in those regions.

(iii) Estimate genotypic richness to compare through this coastline if clonal growth occurs at similar levels between all 20 populations.

(iv) Test isolation by distance (IBD) between populations and within the population in the core groups of Banc D'Arguin, Senegal and Bijagós Archipelago suggests long-distance.

This project is of essential relevance for understanding patterns of genetic diversity and resilience of *H. wrightii* along its distributional range to infer their conservation value in the context of a rapid-change environment. Furthermore, to tackle these issues, it is essential to conduct science in developing world countries that are resource-rich but highly economically dependent on coastal production.

4. Materials and Methods

4.1 Study region

The west coast of Africa, a biogeographic eco-region of the Atlantic Ocean, with rich biodiversity along its shorelines (Duarte et al. 2008), presents a great coastal system to explore the processes driving population genetic structure, mainly because of its continuous coastline with steep environmental contrasts along geographical distances (Spalding et al. 2007). Surface currents in the Tropical Atlantic region are driven by main currents predominantly powered by wind ocean gyres, with a wide range of space-time variability (**Fig. 5**). The Inter-Tropical Convergence Zone (ITCZ) presents a complex area of zonal currents composed of westward and eastward countercurrents (Heileman, 2008). Two broad currents feature the Tropical Atlantic region, the North and South Equatorial current (NEC and SEC, respectively, see **Fig. 5**), divided into north, central, and south currents (Carton and Huang 1994; Vilela et al. 2018). The cold equatorward coastal current features upper north (Mauritania and Senegal), the semi-arid coast region and the Canary Current off northwest Africa, related to the solid coastal upwelling among the NEC and SEC (**Fig. 5**) (Heileman 2009). In the central (Guinea-Bissau) there is a transition zone with a solid offshore current to a shallow ocean off a humid tropical coast, from Guinea to Gabon, featured by a seasonal coastal upwelling, the Guinea Current (Philander 2001).

Further south, a subtropical coast encounters the temperate Benguela Current southwards (Angola), from southwest Africa featuring a critical coastal upwelling zone (Hutchings et al. 2009; Ukwe et al. 2003). The west coast of Africa, a biogeographic eco-region of the Atlantic Ocean, with rich biodiversity along its shorelines (Duarte et al. 2008), presents a great coastal system to explore the processes driving population genetic structure, mainly because of its continuous coastline with steep environmental contrasts along geographical distances (Spalding et al. 2007). Surface currents in the Tropical Atlantic region are driven by main currents predominantly powered by wind ocean gyres, with a wide range of space-time variability (**Fig. 5**). The Inter-Tropical Convergence Zone (ITCZ) presents a complex area of zonal currents composed of westward and eastward countercurrents (Arnault & Kesternare, 2004). Two broad currents feature the Tropical Atlantic region, the North and South Equatorial current (NEC and SEC, respectively, see **Fig. 5**), divided into north, central, and south currents (Carton and Huang 1994; Vilela et al. 2018). The cold equatorward coastal current features upper north (Mauritania and Senegal), the semi-arid coast region and the Canary Current off northwest Africa, related to the solid coastal upwelling among the NEC and SEC (**Fig. 5**) (Heileman 2009). Finally, in the central (Guinea-Bissau) there is a transition zone with a solid offshore current to a shallow ocean off a humid tropical coast, from Guinea to Gabon, featured by a seasonal coastal upwelling, the Guinea Current (Philander 2001). Further south, a subtropical coast encounters the temperate Benguela Current southwards (Angola), from southwest Africa featuring a critical coastal upwelling zone (Hutchings et al. 2009; Ukwe et al. 2003).

The present study covers the entire distributional range of *H. wrightii*.in Western African, from Banc d'Arguin National Park (Mauritania) southwards to Angola, including Cape Verde and São Tomé & Príncipe archipelagos. This coastline is very heterogeneous in terms of biodiversity, climate and pattern of currents. It encompasses the province Gulf of Guinea and its five eco-regions of the Tropical Atlantic biogeographic realm (sensu, Spalding et al. 2007). Also, it has a major biogeographic transition (WAT) zone surrounding Guinea-Bissau into the Temperate Northern Atlantic realm at its Lusitanian biogeographic province, where the geographical range of *H. wrightii* extends into the Saharan upwelling region (Hemingson and Bellwood 2018; Spalding et al. 2007). (see **Fig. 5**).

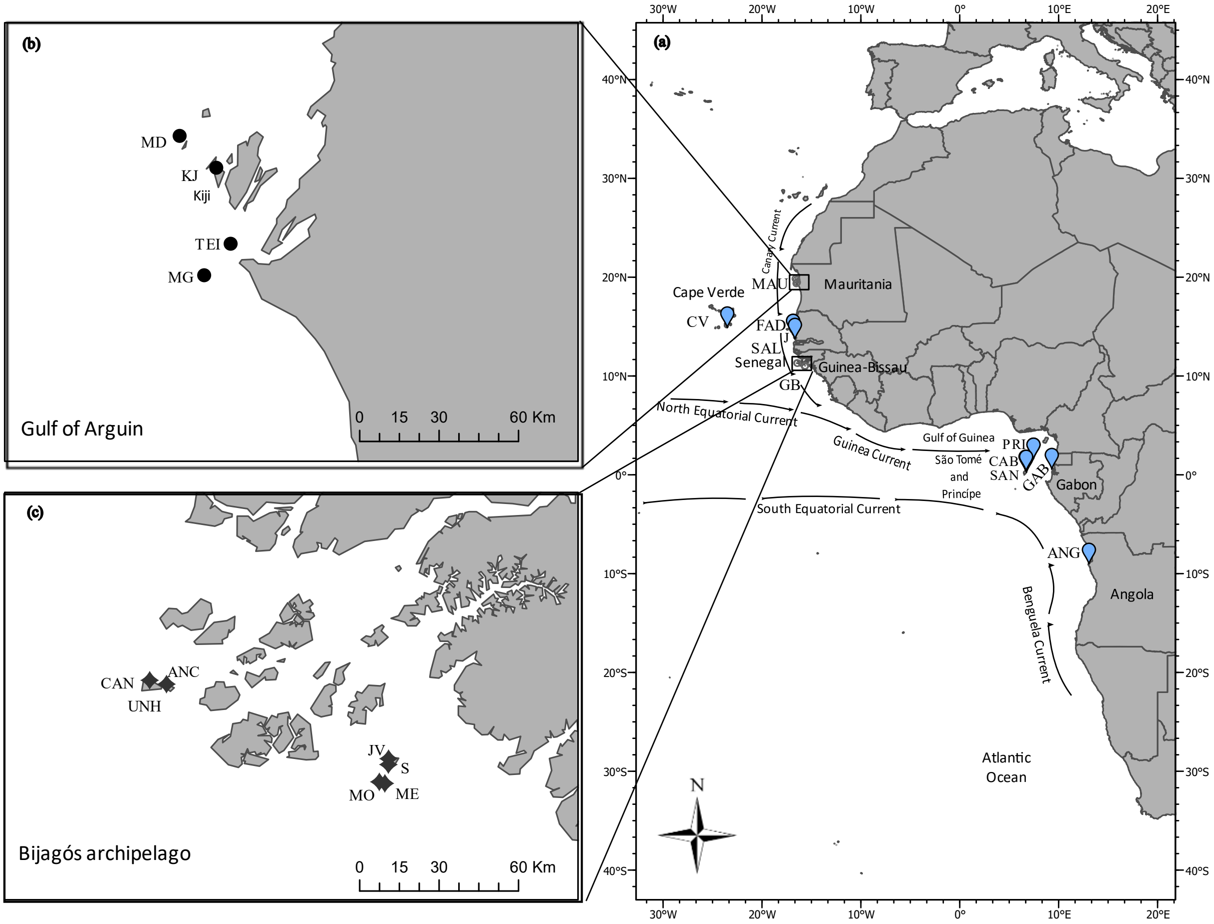

Figure 5. Distribution and sampling of seagrass meadows Halodule wrightii: (a) sample collection throughout the western African coast. (b) location of the four-core population examined on the Banc d'Arguin (Mauritania) and (c) location of the seven-core population examined on the Bijagós archipelago (Guinea-Bissau). Details of geographic location can be found in supplementary table 6.

4.2 Sampling design

A total of 22 populations of *H. wrightii* were collected in seven countries throughout ETA, including Mauritania, Senegal, Guinea Bissau, Cape Verde, São Tomé and Príncipe, Gabon, and Angola (**Fig. 5**). In addition, some sampling sites (e.g., JVPNB samples) were located in conservation hotspot areas. At each site, individual plants (between 3-25 individual shoots) of *H. wrightii* were randomly collected by snorkelling or free diving in subtidal waters (up to 5 m depth) at a distance of 2-5 m apart to minimize sampling of genetically identical clones. Samples collected before 2018 were mainly retrieved from herbarium sheets (**Table 1**). After 2018, targeted sampling of ≥ 15 shoots (conditional on visibility, depth, and cover area) was carried out randomly (**Fig. 6**), maintaining a minimum distance of 1-2 m between each sampling unit. We selected young, fresh leaves cleaned from epiphytes, dried, and stored in silica crystals in a zippered plastic bag at room temperature.

Figure 6. Fieldwork for sampling the species Halodule wrightii on Ilhéu das Cabras, north of São Tomé.

4.3 DNA extraction and Genotyping

DNA extraction was performed using the NucleoSpin Plant II kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's protocol. All populations were genotyped with eight microsatellites. Primer sequences and microsatellite development for the eight markers used were found in Larkin et al. (2012) and Larkin et al. (2017) for loci Hw180, Hw188, Hw190, Hw196, Hw200, Hw212, Hw190b and Hw228 (**Supplementary Table 7**). The samples were PCR-amplified into two duplexes (HW190; HW190b) and (HW180; HW188) and four simplexes (HW196; HW200; HW212; HW228) in a GeneAmp ABI2720 Thermal Cycler (Applied Biosystems, Waltham, MA, USA). The volume of final amplification was 15 µL, consisting of 1x Colorless GoTaq Flexi Buffer (Promega, Madison, WI, USA), 10 mM forward and reverse primers, 2 mM $MgCl_2$ (2.5 mM), 1U GoTaq" G2 Flexi DNA Polymerase (Promega, USA), 0.2 mM dNTPs and 5 µL of template DNA (different dilutions for several populations). PCR conditions consisted of an initial denaturation step at 95°C for 5 min, combined with 30 cycles at 95°C for 30 s, *T*a for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 10 min (**Supplementary Table 9**). 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) in 9.75 µL of Hi-Di formamide, after denaturation at 95°C for 5 min.

Table 1. Locations, genotypic and genetic diversity parameters were calculated for sampled populations of H. wrightii in Western Africa.

Code	Location	Country	N	N 5:8	G	R	A_6	A_3	P $\hat{A}_3$	H_e	H_o	F_{is}
MAU-MG	Mamghar	Mauritania	11	10	7	0,667	1,849±0,051	1,692±0,103	0	0,2852±0,2528	0,4286±0,5043	-0,57895
MAU-KJ	Kiji	Mauritania	10	10	7	0,667	1,8±0,092	1,526±0,139	0	0,149±0,2063	0,204±0,3567	-0,41209
MAU-MD	Mojd Dakhna	Mauritania	12	12	6	0,455	2±0	1,68±0,151	0	0,2576±0,2834	0,4±0,5215	-0,65517
MAU-TEI	Teichott, Banc d'Arguin	Mauritania	24	24	9	0,348	1,84±0,089	1,679±0,107	2	0,2958±0,2094	0,4306±0,3682	-0,49849
SEN-J	Joal	Senegal	22	22	6	0,238	1,75±0	1,505±0,099	0	0,133±0,2462	0,1042±0,2946	0,28571 *
SEN-SAL	Saloum	Senegal	16	12	11	0,909	2,136±0,228	1,834±0,293	2	0,16±0,2255	0,2272±0,3404	-0,4586
SEN-FAD	Fadioth	Senegal	30	30	8	0,241	1,591±0,056	1,482±0,11	1	0,2104±0,2109	0,2813±0,3187	-0,36957
CV	Gamboa, Praia (Santiago)	Cape Verde	21	21	9	0,400	1,601±0,049	1,508±0,083	1	0,2239±0,2162	0,3056±0,3293	-0,39683
GB-ME	Meio – Este Poilão	Guinea-Bissau	14	11	9	0,800	1,891±0,128	1,649±0,156	0	0,1035±0,1964	0,131±0,263	-0,29412
GB-MO	Meio - Oeste Poilão	Guinea-Bissau	23	16	6	0,333	1,75±0	1,521±0,149	0	0,1083±0,2076	0,1417±0,2905	-0,36
GB-S	João Vieira - Sede	Guinea-Bissau	15	14	7	0,462	1,978±0,048	1,762±0,144	0	0,1273±0,236	0,1833±0,3413	-0,51724
GB-ANC	Ancante	Guinea-Bissau	29	29	10	0,321	1,969±0,135	1,686±0,171	0	0,1253±0,1949	0,1747±0,3073	-0,43305
GB-CAN	Ilha do Unhocomo, Canneronho	Guinea-Bissau	30	30	10	0,310	1,9±0,119	1,599±0,148	0	0,1291±0,2122	0,1906±0,3556	-0,5641
GB-JV	Ilha João Vieira	Guinea-Bissau	29	29	11	0,357	1,95±0,169	1,674±0,19	1	0,1781±0,1835	0,2363±0,3015	-0,35348
GB-UNH	Acampamento, Unhocomo	Guinea-Bissau	26	26	15	0,560	2,018±0,203	1,695±0,187	2	0,2555±0,2203	0,325±0,3642	-0,28471
STP-ABA-PRI	Abade beach, Pagué, Principe	STP	5	4	4	1,000	NA	1,712±0,058	1	0,3438±0,2926	0,4688±0,4105	-0,45161
STP-CAB	Cabras Islet	STP	33	31	27	0,867	2,192±0,239	1,764±0,241	2	0,2205±0,1878	0,2232±0,1629	-0,01217
STP-SAN	Santana	STP	6	6	5	0,800	NA	1,644±0,096	0	0,2778±0,2648	0,4±0,4276	-0,52381
GAB	Corisco Bay	Gabon	3	3	3	1,000	NA	1,625±0	1	0,2667±0,2417	0,3333±0,3563	-0,33333
ANG	Mussulo Bay	Angola	39	39	4	0,079	NA	1,651±0,106	0	0,3036±0,2361	0,4375±0,4173	-0,55556

Number of ramets sampled (N); number of ramets used with 5 to 8 loci each (N 5-8); number of genets found (G); genotypic richness (R) R = (G-1)/(N-1); allelic richness (±SE) estimated after standardising G to 6 (G = 6) and G to 3 (G = 3) (except where G < 3) ($\hat{A}_3$ & $\hat{A}_6$); standardised number of private alleles (P$\hat{A}$); gene diversity or expected heterozygosity (He); observed heterozygosity (Ho); and multi-locus inbreeding coefficient (F_{is}).

Alleles sizes were scored manually in STRAND analysis software (Veterinary Genetics Laboratory, University of California, Davis; http://www.vgl.ucdavis.edu/STRand) and binned using the R package 'MsatAllele' (Alberto, 2009). Samples with missing amplifications or ambiguous peaks for all microsatellite loci were removed.

A comprehensive and complete manual revision of microsatellite amplification and scoring consisted of positive and negative controls and controls testing allele amplification in various African samples. The final genotyping scores were taken from each sample after a double-check reading process to reduce scoring errors and guarantee that the peaks were accurately interpreted.

4.4 Statistical analyses

When analyzing partially clonal organisms, it is important to discriminate ramets from genets. Ramets are the modular units of the same genetic individual (N), while genets are distinct genetic individuals originating from different sexual recombination events (G), which can be composed of several ramets. The analysis is done by identifying genets based on Multi-Locus Genotypes (MLGs) (Arriesgado et al. 2015) using the software GenClone v 2.0 (Arnaud-Haond et al. 2007). In addition to the number of distinct genotypes (genets), clonal diversity was calculated as $R = (G-1)/(N-1)$, ranging from one single clone (R = 0) to a different genet (R = 1) (Dorken and Eckert 2001).

Prior to comparing the mean number, the amount of microsatellite genetic diversity within each group was measured to standardise sample size with a small genet number of alleles (A) per locus and estimation among populations with the package StandArich2 (http://alberto-lab.blogspot. nl/p/code.html#!/p/code.html) in Rstudio 4.1.3 (Alberto et al. 2006). The adjustment for the sample size was standardized for private alleles (PÂ) A_3 and A_6 as the minimum values of each sampled population. To calculate observed heterozygosity (H_o) and expected heterozygosity (H_e), the mean number of alleles per locus (A), the number of private alleles (PÂ) and The Fis estimator of departure from Hardy-Weinberg expectation, we used GENETIX 4.052 software (Belkhir et al., 1996).

4.5 Population differentiation

The relationship of genetic divergence between sites over geographic distance is known as isolation by distance (IBD). We regressed genetic differentiation (Fst and Jost'D) against geographical distance to investigate a putative IBD relationship. To estimate how genetic variance was portioned between countries, populations within countries, and within populations, we ran an AMOVA implemented in Arlequin 3.5.2.2 (Excoffier et al., 2010).

The measurement of the geographic distance of the sampling site was performed with Google Earth Pro 7.3.4.8642 software through the geographic coordinates of sampled sites without crossing the land (**Table 4**). To determine IBD, we performed it for core populations, where we compared groups (countries) and among and within populations on the northwest African coast (Mauritania, Senegal, and Guinea-Bissau). Subsequently, we conducted a Mantel test using the Rstudio package *ncf* (Bjornstad 2009), and the matrices were resampled 10,000 times with a significance level of 0,05. Finally, estimates of Pairwise differentiation (F_{st} and Jost'D) using Keenan et al. (2013) methodology were calculated with the R package *diveRsity* 1.9.

The genetic structure across the entire range of H. wrightii was investigated using a Factorial Component Analyses (FCA) as implemented in the software GENETIX. This analysis spreads individual genotypes in a bi-dimensional plane according to their similarity/differences, allowing the detection of genotypic clusters.

Genetic structure was also investigated through Bayesian analysis using STRUCTURE 2.3.2 (Pritchard et al., 2000; http://pritchardlab.stanford.edu/structure.html). This software aims to find the most probable number of genetic clusters K that minimize Hardy-Weinberg and linkage disequilibria. Finally, STRUCTURE HARVESTER web v0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester/) was used to summarize STRUCTURE results and infer, based on values of ΔK, the most likely number of genetic clusters (Earl et al., 2012).

5. Results

A total of 398 sampling units (seagrass shoots) were collected for 20 populations from 7 countries along the Western African coasts and genotyped for eight microsatellite loci.

Unfortunately, the loci Hw196 and Hw190b could not be obtained for the populations of Mamghar, Kiji and Modj Dakhna in Mauritania. Therefore, individuals with missing data in at least four loci were removed from the dataset, with the trimmed dataset consisting of 380 individuals. The final dataset consisted of 174 individual genets or MLGs (G), many of which were shared between populations. Allelic diversity was overall low, the number of alleles per locus ranging from 2 (in locus Hw190) to 7 (in locus Hw212), the mean number of alleles per locus is 4.25 and the allelic richness (A), 1.779. Genclone analyses showed that the probability that individuals with the same MLG occurred by chance was low ($P < 0.01$). Therefore, the criteria to consider or not the same genet was assumed with the Psex value.

5.1 Genetic diversity

Genclone revealed variable levels of genotypic diversity ($0.2 < R < 1$) within populations throughout the range of *H. wrightii*, but no obvious *G* pattern (**Table 3**). Notably, clonal richness (R) was very low in Angola (R= 0.079), as this population revealed only 4 MLGs in 39 samples.

All microsatellite loci revealed some insight into the genetic variability of this Halodule taxon. Although several genotypes were detected, these contained only a few mixtures of alleles typical of the western Atlantic (Tavares et al., 2022). On the other hand, several unique alleles were present for the region (**Fig. 7**). Besides, all loci (except for loci Hw196 in Senegal) were present in every population, often with high frequency. However, not all microsatellite markers displayed two loci (e.g., Hw180, Hw196, Hw200), or when they did, the numbers were deficient (e.g., Hw188, Hw212), demonstrating the possibility of low diversity in those regions (**Fig. 7**).

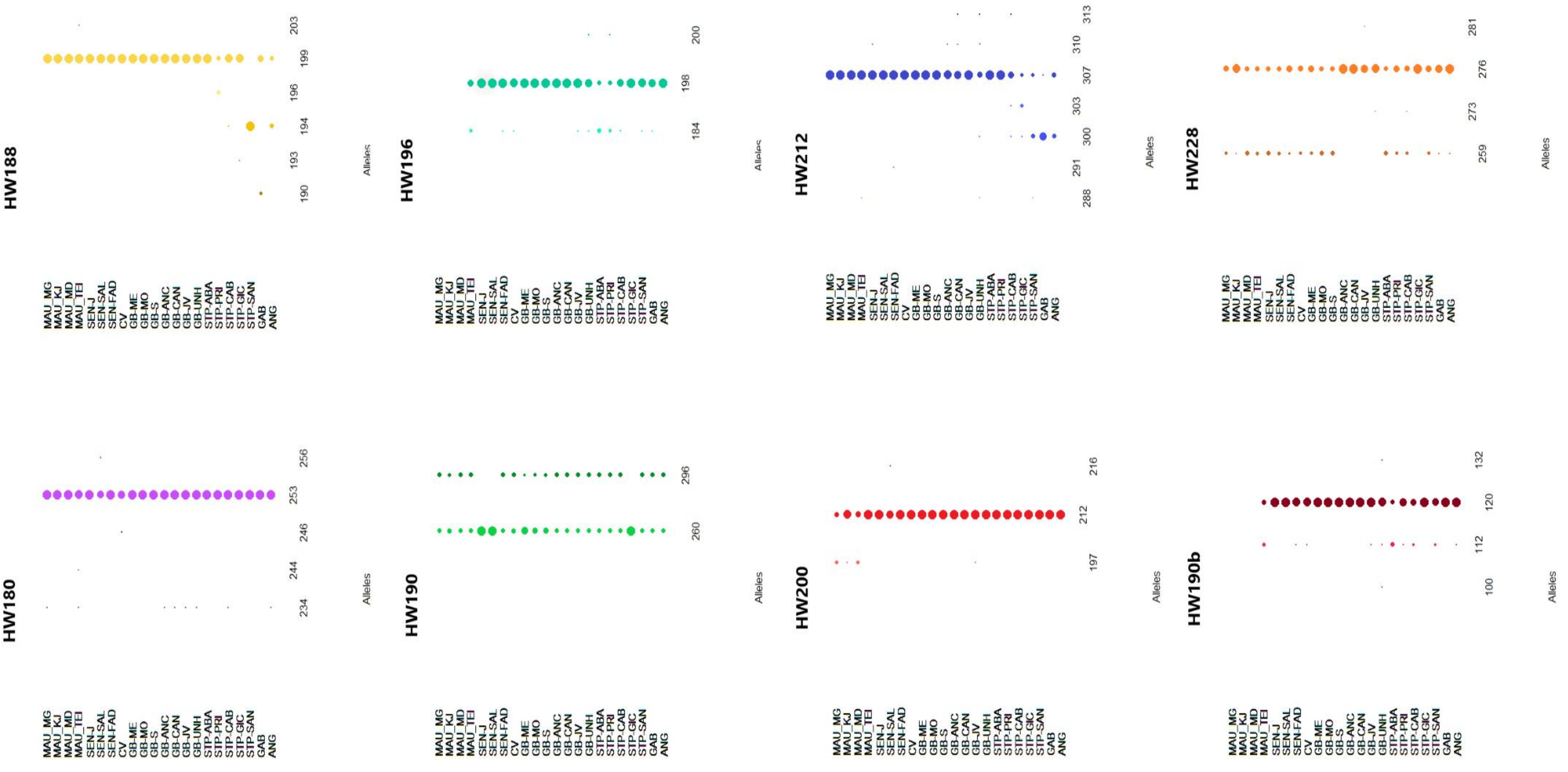

Figure 7. Allele frequency plots per population for all loci. Sample size was standardized, and samples are sorted from north to south of western Africa starting with the samples from the Mauritania followed by the southern west Africa. The number of samples for each analysed population is indicaded on table 1.

Within populations, the standardized allelic richness (based on the smallest common sample sizes *N* = 3 and *N* = 6) varied significantly but was always very low ($A_{3/6} < 3$; mean $\hat{A}_3$ = 1,6625 and mean $\hat{A}_6$ = 1,8955) (**Fig. 8; Table 3**).

Private alleles (*PÂ*) were found in nine populations, with the highest values (*PÂ* = 2) on the northern samples in Mauritania (Teichott), Senegal (Saloum) and Guinea Bissau (Unhocomo), again without a clear geographical pattern. Genetic diversity within populations was overall low (He < 4), with the highest values in Princípe island - São Tomé and Princípe (*He* = 0,3438) and Mussulo bay – Angola (*He* = 0,3036).

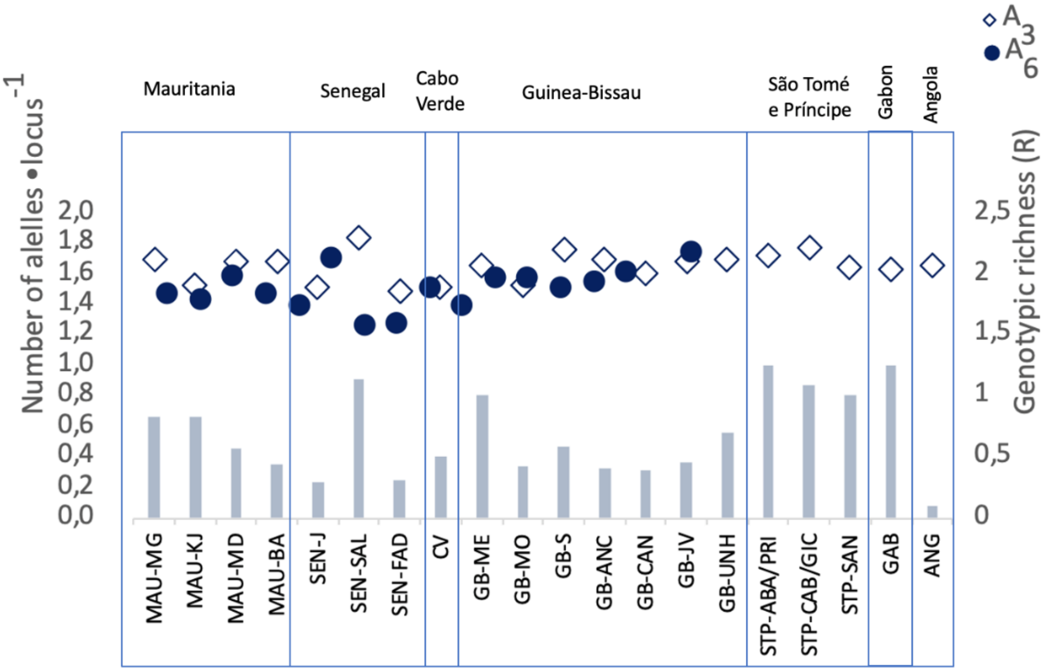

*Figure 8. Allelic richness, shown as points (Â), is the mean number of alleles per locus standardized for the number of genets G = 6 and genotypic richness (R), shown as grey bars, found for all sampled populations of Halodule wrightii. Samples with < 6 genets are shown with open symbols to indicate that $\hat{A}_3$ are the observed values in the sample, as these could not be standardized to G = 6 (see **Table 1**).*

The Mauritania populations contained 6-9 genets out of 10-24 ramets genotyped. The average genotypic richness (*R*) across the area sampled was 0.56 (ranging from 0.3 to 0.67). Mean clonal richness (*R*) was highest in Mamghar and Kiji region in the north, followed by Mojd Dakhna and Teichott in the PNBA in south Mauritania (**Table 2**). On the other hand, the Guinea-Bissau populations revealed 6-15 genets out of 11-30 ramets. Few genets were detected in most Bijagós populations, with *R* ranging from 0.310 to 0.800 (**Table 2)**, indicating a predominance of clonal growth in the Poilão island (JVPNB) and Unhocomo and Canneronho region in the north Bijagós.

Table 2. Standardized allelic richness means Mauritania's core populations (blue) and Guinea-Bissau (green).

Code	Mean A6	Mean A3
MAU-MG	1,8445	1,6795
MAU-KJ		
MAU-MD		
MAU-TEI		
GB-ME	1,95	1,674
GB-MO		
GB-S		
GB-ANC		
GB-CAN		
GB-JV		
GB-UNH		

Allele richness in the nearshore and offshore regions of the Gulf of Arguin showed similarity between their populations, except for the highest value in the Teichott (Banc d'Arguin) population, while genotypic richness was more evident in Mamghar and Kiji populations (**Fig. 9a; Table 3**). A comparison of the Northern limit and Southern limit range edge populations of the Bijagós archipelago showed similar gene diversity values (Acante – north and João Vieira Sede – south) (**Fig. 9b**). However, the allelic richness (A_6) and the number of private alleles ($P\hat{A}_3$) were higher in the northern islands (Unhocomo and Canneronho, $P\hat{A}_3 = 2$) than in the southern island (Sede, Meio-Este and Meio-Oeste, $P\hat{A}_3 = 0$). On the other hand, genotypic richness showed more substantial evidence on Meio-Este island in the southern (**Fig. 9b; Table 3**).

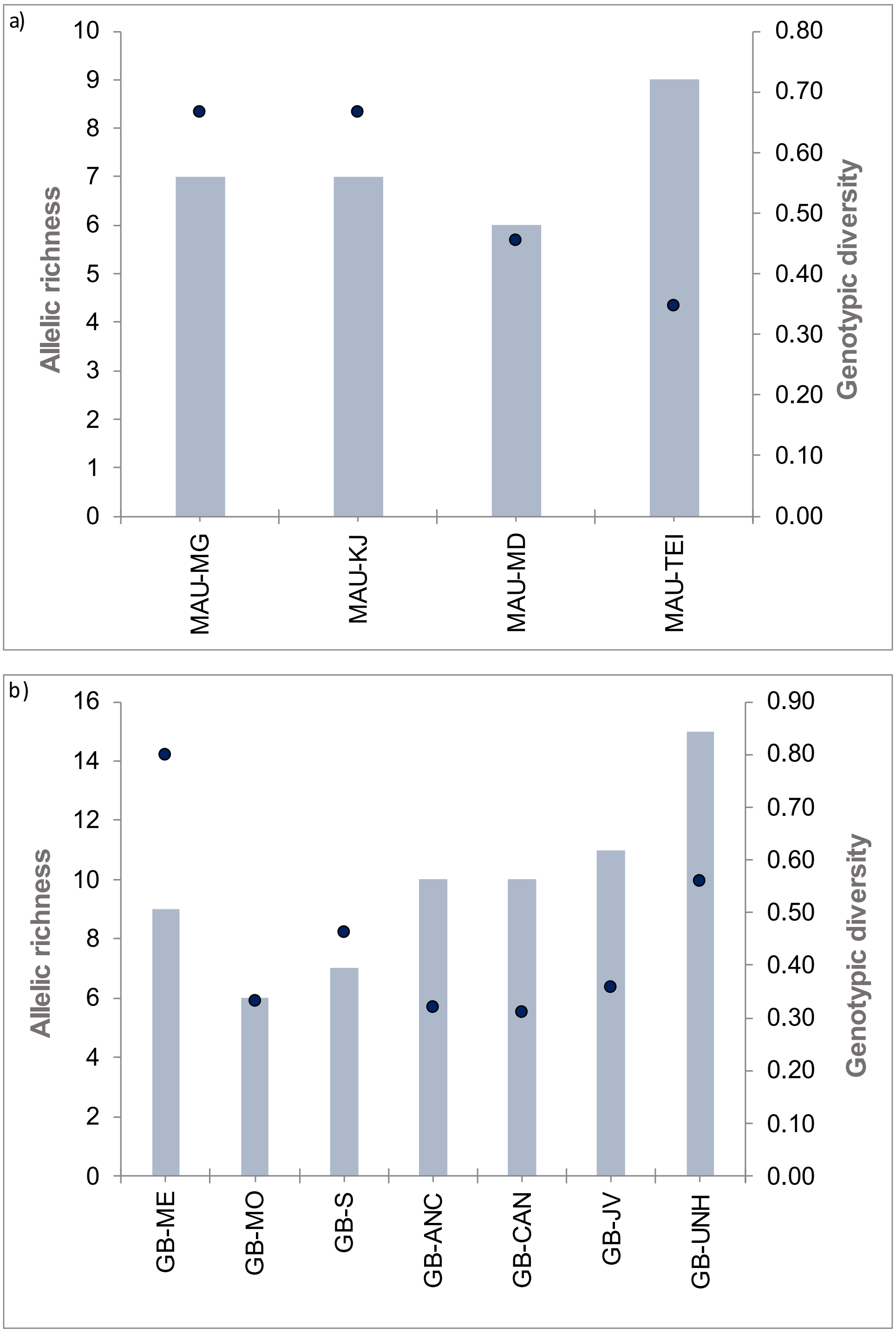

Figure 9. Genetic diversity for the core populations highlighted on the northwest African coast a) Mauritania and b) Guinea-Bissau. Allelic richness (Â) is represented with blue bars; Genotypic diversity (R) is shown with blue circles.

Table 3. Central genetic diversity values for the two study sites in question that compare with each other are the zones of nearshore vs offshore (Mauritania) and Northern vs Southern (Guinea-Bissau) (within populations).

Code	Location	Region	Country	N 05:08	G	R	A_6	A_3	$P\hat{A}_3$	H_e	H_o	F_{is}
MAU-MG	Mamghar	Banc d'Arguin nearshore	Mauritania	10	7	0,667	1,849±0,051	1,692±0,103	0	0,2852±0,2528	0,4286±0,5043	-0,57895
MAU-TEI	Teichott, Banc d'Arguin	Banc d'Arguin nearshore		24	9	0,348	1,84±0,089	1,679±0,107	2	0,2958±0,2094	0,4306±0,3682	-0,49849
MAU-KJ	Kiji	Banc d'Arguin offshore		10	7	0,667	1,8±0,092	1,526±0,139	0	0,149±0,2063	0,204±0,3567	-0,41209
MAU-MD	Mojd Dakhna	Banc d'Arguin offshore		12	6	0,455	2±0	1,68±0,151	0	0,2576±0,2834	0,4±0,5215	-0,65517
GB-UNH	Island Unhocomozinho - Acampamento	Bijagós North	Guinea-Bissau	26	15	0,560	2,018±0,203	1,695±0,187	2	0,2555±0,2203	0,325±0,3642	-0,28471
GB-ANC	Ancante	Bijagós North		29	10	0,321	1,969±0,135	1,686±0,171	0	0,1253±0,1949	0,1747±0,3073	-0,43305
GB-CAN	Ilha do Unhocomo, Canneronho	Bijagós North		30	10	0,310	1,9±0,119	1,599±0,148	0	0,1291±0,2122	0,1906±0,3556	-0,5641
GB-ME	Meio – Este Poilão	Bijagós South		11	9	0,800	1,891±0,128	1,649±0,156	0	0,1035±0,1964	0,131±0,263	-0,29412
GB-MO	Meio - Oeste Poilão	Bijagós South		16	6	0,333	1,75±0	1,521±0,149	0	0,1083±0,2076	0,1417±0,2905	-0,36
GB-S	João Vieira - Sede	Bijagós South		14	7	0,462	1,978±0,048	1,762±0,144	0	0,1273±0,236	0,1833±0,3413	-0,51724
GB-JV	Ilha João Vieira	Bijagós South		29	11	0,357	1,95±0,169	1,674±0,19	1	0,1781±0,1835	0,2363±0,3015	-0,35348

5.2 Genetic differentiation and population structure

The FCA analyses showed two genetic clusters with a clear spatial signal (**Fig. 10**). One comprised the northern populations from Mauritania, Cape Verde, Senegal, Guinea-Bissau, and Principe Island near the equator. In contrast, the other comprised equatorial populations of São Tomé Island and Gabon as Angola further south. The Cabras islet area, just north of São Tomé Island, showed genotypes from the two clusters and intermediate genotypes. (**Fig. 10**). Consequently, the relationship of these populations does not reach a gradual increase in gene enlargement with increasing geographical distance. However, it is mainly motivated by the difference in genetic differentiation estimates at a tiny scale (within Sao Tomean regions) vs a considerable scale (among regions). No such relationship is observed at a small scale (among islands), and there is an apparent lack of relationship when comparing large-scale differentiation, with northern being much less genetically differentiated than Southwestern or Northwestern African populations at similar geographical scales.

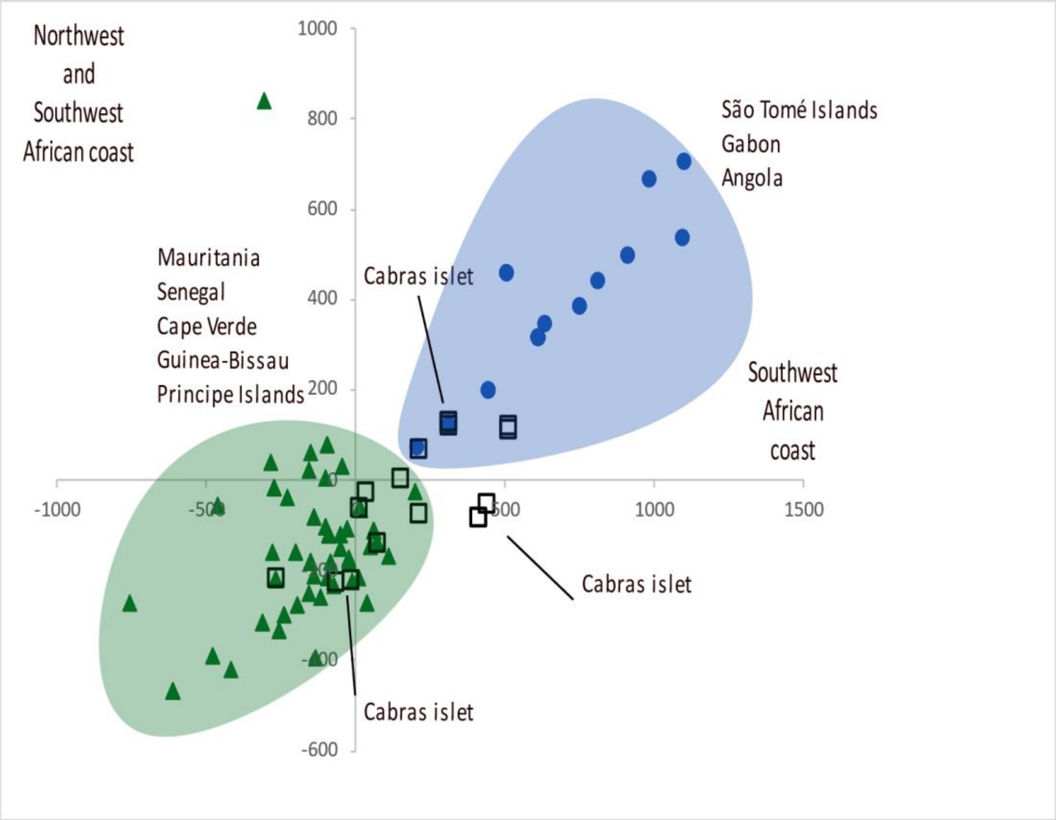

Figure 10. Genetic differentiation of Halodule wrightii is illustrated by factorial correspondence analysis (FCA).

Unlike the FCA, the STRUCTURE analyses did not recover any spatial pattern of genetic structuring (**Fig. 11**). The model evaluation with the ΔK method (Evanno, Regnaut, and Goudet 2005) showed consistently low values of ΔK < 3 for K= 2-5, failing to support any K in particular.

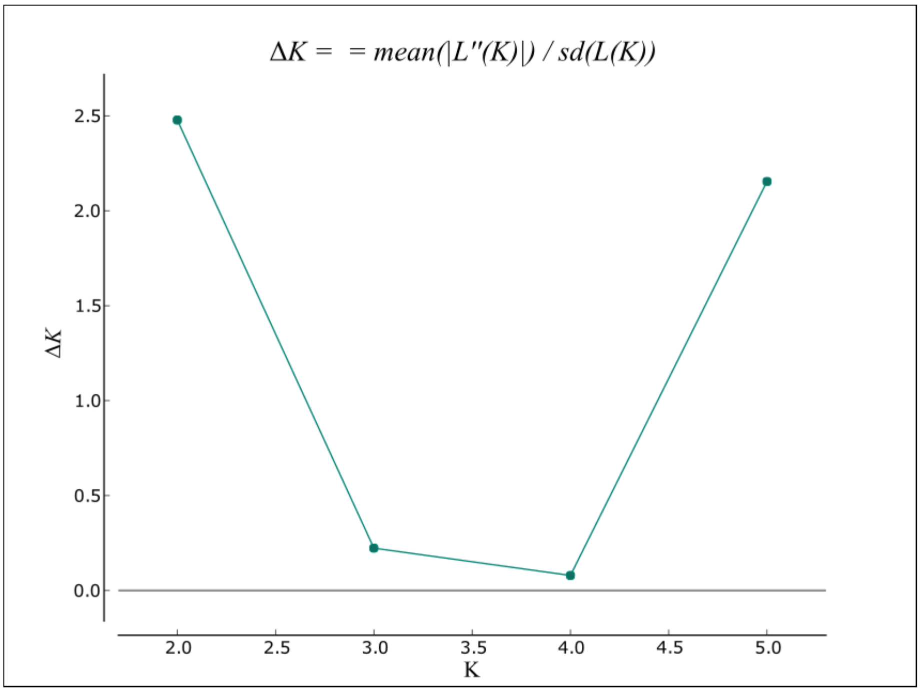

Figure 11. Delta K of cluster number K in genetic clustering analysis using STRUCTURE HARVESTER (linkage disequilibrium (LD)-based pruning was used for K from 1 to 10. The delta K analysis suggests K = 2, K = 3 and K = 4 as the most likely number of distinct genetic ancestries among the 10 Ks (delta K = 2.5).

Highlighting these results, Structure plots for K = 2-5 showed that all individuals of *H. wrightii* possessed even assignments to every genetic cluster considered. In contrast, no individual was recovered as having "pure" reference genotypes (**Fig. 12**). They demonstrated no spatial or incipient population divergence that could result from recent colonization or settlement.

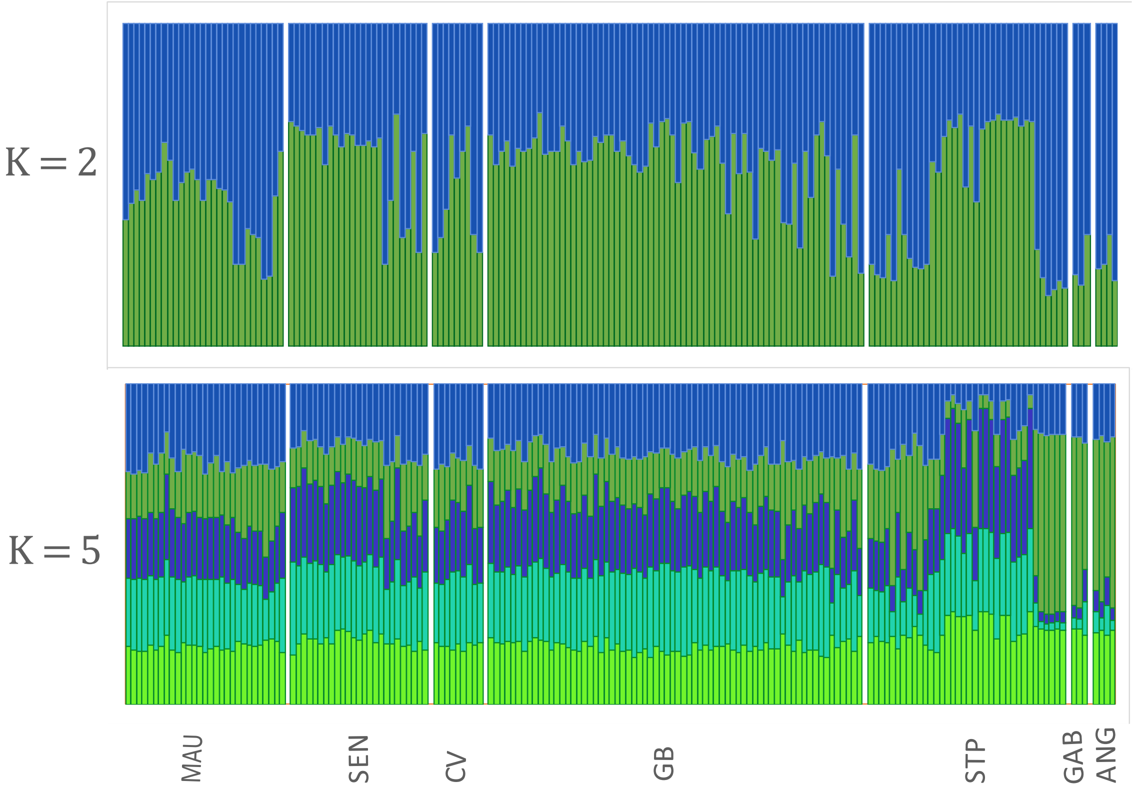

Figure 12. Genome constitution of the 378 genotypes inferred by STRUCTURE to 2 and 5 inferred the best number of clusters based on 8 microsatellite loci of Halodule wrightii individuals (k estimated under STRUCTURE HARVESTER). A column represents each individual; different colours within columns indicate the maximum likelihood probability of belonging to a different cluster. Population codes are in ***Table 1****.*

The results obtained of population differentiation due to genetic structure from fixation index (Fst) and the molecular analysis of variance tested here assessed results between the two core regions of this study: Mauritania and Guinea-Bissau. The results were compared within populations in each country. Pairwise Fst ranged from 0.003 (Modji vs Sede, Mauritania, and Guinea-Bissau, respectively) and 0.3556 (Meio Este and Guinea-Bissau, respectively). As measured by Jost'D (**Supplementary Table 12**), absolute population differentiation ranged substantially, much lower when compared with Fst (**Table 4**). Within regions, pairwise population differentiation was higher in Mauritania than in Guinea-Bissau (**Fig. 13**). The significant signal of differentiation-by-distance in the northwest African coast (P = 0.01).

Table 4. *Pairwise genetic differentiation among Halodule wrighti sampling sites (Mauritania and Guinea-Bissau). Values below the diagonal represent FST values, estimated with diveRsity. Values above the diagonal represent the distance (km)* measured with Google earth. *See Table 1 for site abbreviations.*

	mau_mg	mau_kj	mau_md	mau_tei	gb-me	gb-mo	gb-s	gb-anc	gb-can	gb-jv	gb_unh
mau_mg	-	57.48	56.28	17.65	1263.04	1289.48	2410.63	1152.21	1229.47	1353.54	1465.31
mau_kj	0,072	-	17.75	43.93	1465.31	1558.19	1774.04	1399.24	1252.2	1470.84	1218.5
mau_md	-0,0156	0,0787	-	44.34	1446.43	1502.3	1508,09	1113.52	1244.38	1331.13	1125.04
mau_tei	0,0827	0,0581	0,0403	-	1284.92	1357.49	1346.46	1143.39	1165.55	1363.47	1194.59
gb-me	0,1704	0,1095	0,1242	0,1204	-	8,3	9.89	113.1	115.4	6.98	114.55
gb-mo	**0,1684**	0,1335	0,1211	0,1224	-0,0975	-	9.63	104.7	120.72	8.94	101.8
gb-s	0,1134	0,0789	0,056	0,0812	-0,0286	-0,0313	-	111.67	117.23	2.50	104.2
gb-anc	0,1413	-0,0093	0,1658	0,1455	0,11	0,174	0,1268	-	6.3	110.6	5.98
gb-can	0,1594	**-0,003**	0,1815	0,163	0,1403	0,2193	0,1481	-0,0215	-	127.5	8.9
gb-jv	0,1044	0,0153	0,1119	0,0958	0,1117	0,1193	0,076	0,0244	0,047	-	103.14
gb_unh	0,1546	0,0307	0,1604	0,0889	0,1027	0,1499	0,106	0,0235	0,0235	0,0614	-

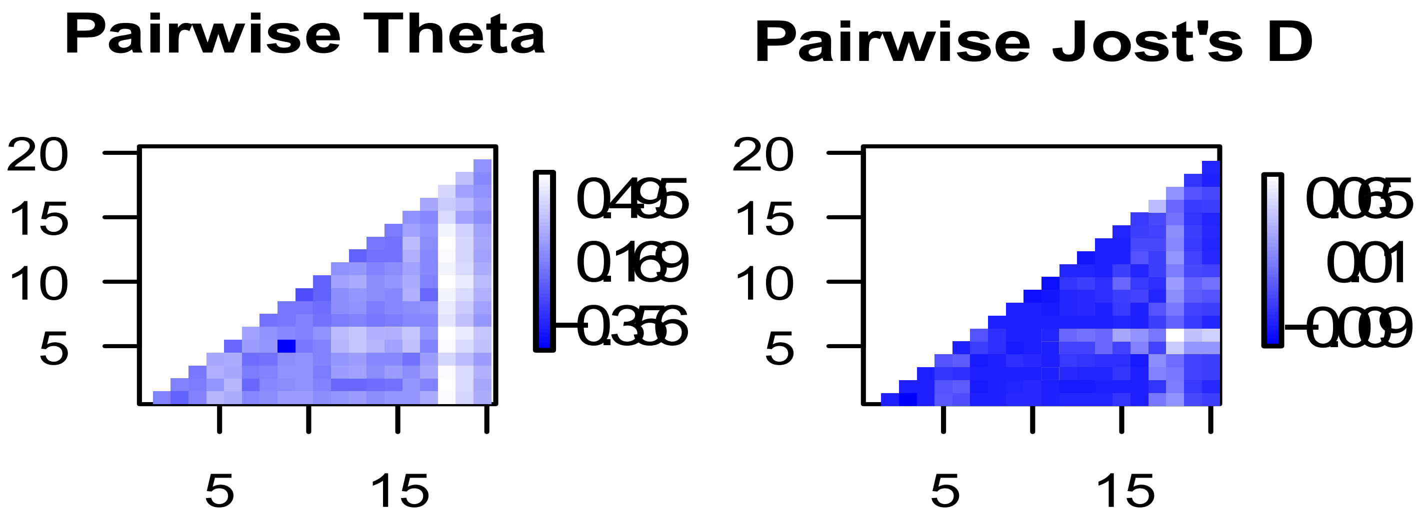

Figure 13. Heatmaps of pairwise differentiation between Halodule wrightii populations. Lighter tones indicate more robust genetic differentiation. Left: Fst; Right: Jost's D data.

Analysis of Isolation by Distance (IBD) correlated the "marine geographic distances" between populations and their degree of genetic differentiation. Distances between populations ranged from ~6.3 (GB_ANC to GB_CAN) to ~1,508 km (MAU_MD to GB_S). Furthermore, The variation in pairwise Fst was conducted by a lack of IBD among the Mauritania sites ($R^2 = 0.0052$). Population assignment results supported this finding (**Fig. 14**).

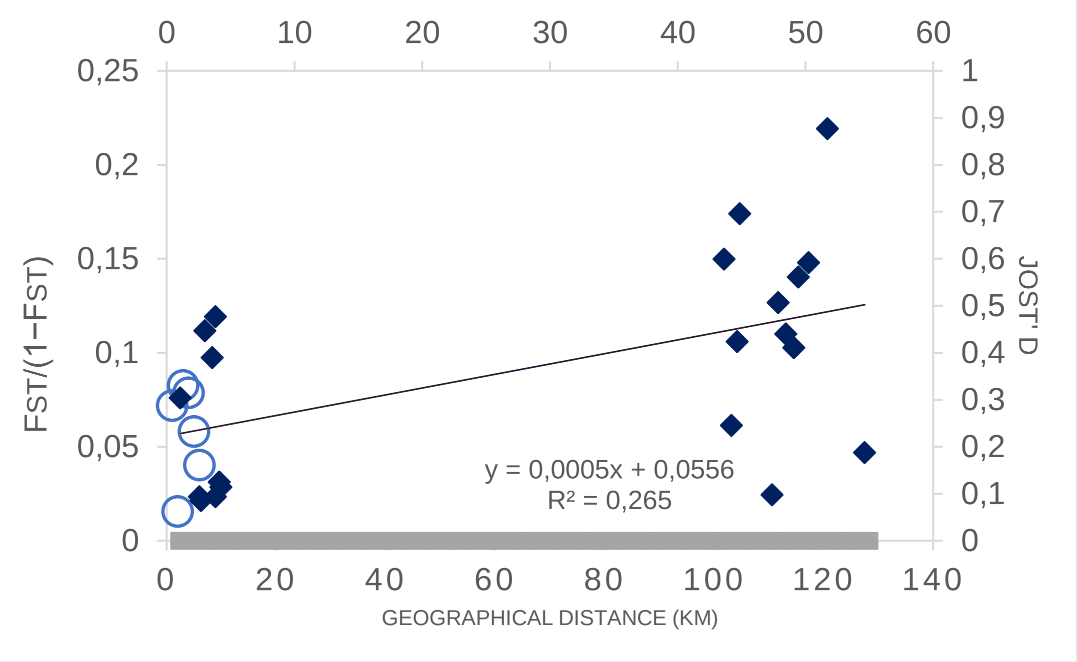

Figure 14. Isolation by distance (IBD) between sites within selected countries (Mauritania and Guinea-Bissau). Linearized F_{ST} *(i.e., Fst/(1-Fst)) values were regressed against geographic distance (in kilometers). The linear relationship and R2 are shown. In addition to populations within clusters (blue circle= Mauritania, blue rhombus = Guinea-Bissau), populations belonging to different clusters were regressed separately and are depicted in three different shades. Jost's D is on the secondary axis and demonstrated no significant values.*

Concerning the hierarchical AMOVA analyses, when populations were grouped according to country (Mauritania and Guinea-Bissau), approximately 89% of genetic variation was contained within populations (as inter-individual differences), whereas only a trivial amount of genetic variation was found between groups (5%): Fst = 0.11 (**Table 4**) among populations within groups (5.47%; Fst = 0.08) (**Table 5**).

Table 5. Hierarchical analysis of molecular variance (AMOVA) of spatial genetic structure for the 14 populations of H. wrightii based on six microsatellite loci. 95% confidence interval of F-statistics were obtained through bootstrapping over loci with 10,000 iterations.

Source of Variation	Sum of squares	Variance Components	Percentage Variation
Among groups	6.844	0.03570	5.14095
Among Populations Within groups	12.815	0.03798	5.46953
Within populations	117.266	0.62077	89.38953
Total	136.924	0.69445	

6. Discussion

Our results showed little genetic connectivity and structure between all populations, so we can assume that hypothesis (i) of influences on the oceanic currents between the divergent zones is not significant to demonstrate genetic connectivity in the species *H. wrightii*. Nevertheless, the genetic differentiation presented diversity among the northern (Mauritania to Principe Islands) and southern groups (São Tomé islands to Angola), with the most significantly differentiated group sharing both genetic diversity located at the range limit of central western Africa (Cabras islet – São Tomé and Princípe). Thus, we can assume the hypothesis of rafting drift among short distances and independent colonization with predominance coming from islands.

We investigated hypothesis (ii) of the influence of ocean currents (CC and GC) on genetic variation in two hotspot zones: in the Gulf of Arguin along nearshore and offshore zones; and in the northern and southern Bijagós Archipelago. Our results showed that the populations from these regions form distinct groups (topologically separated). However, the comparison within populations demonstrated similar levels of genetic variation, with few differences, so we cannot assume the hypothesis of ocean currents as the primary mechanism of seed dispersal.

We found somewhat divergent results between clonal richness across all populations, ranging from minimal values to high ones. Thus, hypothesis (iii) of clonal richness having similar levels was not supported. However, the IBD within populations on the core zones highlighted in this study (iv) expecting seed dispersal across regional scales supports the hypothesis of seed dispersal through ocean currents.

6.1 Dispersal and recruitment dynamics

The results obtained in this study show that populations of *H. wrightii* near the Northern limit of distribution are characterized by low genetic diversity at microsatellite loci, high levels of inbreeding and high genetic divergence. In addition, the number and frequency diversity distribution of alleles revealed low levels of heterozygosity and allelic richness for both distributional limits, lower in both Northern (Northwest Africa coast) and Southern (Southwest Africa coast) limits compared with core populations from all locations.

In general, a non-significant allelic diversity towards the edge of the regional distribution margin was detected among populations (**Fig. 10**). These patterns support the formerly reported general tendency for lower allelic diversity in populations at distributional limits, mainly observed with the spatial modelling distributional of propagules by ocean currents (Tavares et al. 2022) in contrast to the highly structured intraspecific biodiversity by habitat connectivity driven by ocean currents and barriers along the coastlines of southwestern Africa. (Assis et al. 2021; Ovenden et al. 2011).

Differences between the sampling schemes of populations could have impacted the results. Random samples in Senegal, São Tomé and Príncipe and Gabon, with small numbers of shots collected, may show divergent values compared to those populations with a more significant sampling effort, both in area and quantity. Samples with small numbers sampled (Gabon) showed higher diversity than those populations with larger numbers (Angola), but this may have been due to erroneous numbering probabilities (e.g., clonal richness).

The result indicated that dispersal does not affect genetic drift and gene flow in *H. wrightii* populations. There is likely strong limation to dispersal without an actual pattern of IBD, as shown by the lack of significance of spatial patterns of genetic structure found for the seagrass in the ETA range (~6250 km). Consequently, we did not support our central hypothesis (i) of ocean currents as the main dispersal vectors facilitating MPC between sites on the PNBA in Mauritania and the Bijagós archipelagos in Guinea-Bissau. The genetic results revealed no significant population differences, with a few exceptions for nearby sites and a general genetic decoupling between the equatorial line (north and south). Furthermore, the results showed that the equator line (São Tomé and Princípe) populations were genetically distinct although close, referring to origination from independent colonisation events through seeds from origins genetically differentiated. This dysfunction among the predicted connectivity patterns expected and the observed genetic structure, if dispersal was based on ocean currents, infers that biotic transportation (i.e., seed dispersal via marine species) may perform an essential function in the dispersal and establishment of the species rather than ocean currents. However, more research is necessary to study the hypothesis mentioned.

6.2 Genetic diversity based on allelic richness and heterozygosity

Populations of *H. wrightii* had low genetic diversity on the Western African coast (mean A_6 = 1.89 and A_3 = 1.66). The results from this project were similar to other seagrass studies with microsatellite markers (Tavares et al., 2022). However, compared to studies previously made with the same species in the western Atlantic, we had different values (3.26 in Reynolds et al., 2019 and 4.08 in Larkin et al., 2017), presenting a variation in the species-specific traits. With that, hypothesis (i) of the connectivity between the northwest regions (Mauritania and Guinea-Bissau) being genetically divergent due to the oceanic currents passing through the regions does not support our results. Indeed, these patterns may be supported by the hypotheses of low genetic variation lost over time, where uncontrolled use of coastal areas could directly impact these alterations.

The potential to adapt to an environment is directly related to genetic diversity; thus, a decrease in genetic diversity could lead to an accelerated chance of biodiversity loss. For example, the genetic diversity among seagrass populations in São Tomé and Principe, and Senegal was significantly higher than in the other groups (comparison includes A_6 and A_3 due to the sample size). This significant genetic variation could indicate that Principe and Senegal's populations demonstrate more influence on gene flow and resilience to anthropogenic impacts on coastal disturbances. Consequently, the richer diversity in those places increases the genetic diversity of those populations, becoming more diversified compared to other regional locations. However, the high value seen needs to be considered due to the low sample size sampled and tested with standardized alleles.

As clonal plants, seagrass meadows in decline form clonal aggregations, reducing their genetic variability when declining. Moreover, the species *H. wrightii*, by forming seed banks and not being free-floating, could also form isolated aggregations since germination would take longer to start, and there would be no genetic admixture in the long term. Moreover, depending on the distance the pollen disperses, this would result in inbreeding and genetic drift when a microevolutionary mechanism randomly modifies the allele frequencies over time, increasing the Fst values (genetic drift). However, there are not many studies related to *H. wrightii* pollen dispersal yet, needing more accessibility on the subject to be tested.

The low genetic diversity at higher latitudes is also seen in mangroves and coral studies (e.g., Arnaud-Haond et al., 2006; Ridgway et al., 2008). Thus, coastal species can support the low genetic diversity event on seagrass meadows. Generally, the average value of allelic richness among seagrass meadows is low (e.g., Jahnke et al., 2019; Nguyen et al., 2014: Tanaka et al., 2011), although the value found in our project is among the lowest in the world. When genetic variation is very low (e.g., Arriesgado et al. 2015; Becheler et al. 2010; Olsen et al. 2004), the populations have been colonised recently. Consequently, with our low genetic variability, we can say that the populations sampled on the West African coast may have been recently colonised. Furthermore, the seagrass *H. wrightii* is the only taxonomic group suggesting to happen on both sides of the Atlantic (Tavares et al. 2022).

The proportion of variance in the subpopulation in individuals (F_{is}) exhibited a small degree of inbreeding. Thus, it is possible to say that the low inbreeding coefficient means that our populations have higher observed heterozygosity than expected ($H_o > H_e$). This phenomenon could be due to bottleneck effects caused by decreased population sizes and random environmental events based on the observed allele number at the Hardy-Weinberg Equilibrium (Guo et al., 1992). Consequently, we do not assume the Hardy-Weinberg Equilibrium with our negative results. With the results obtained about the coefficient of inbreeding, it is possible to state that there is an unbalanced coming from external factors affecting the West African *H. wrightii* populations, such as environmental disturbances or anthropogenic threats, declining the species, and leading to a loss of biodiversity.

Since tropical areas influence genetic diversity, which has a high biodiversity with different environmental and ecological interactions, we can assume that sites with various organisms interacting with each other tend to have greater diversity. An example of this interplay is the hotspot we are investigating in the Bijagós archipelago. The islands of this archipelago have shown a higher value of genetic diversity than other populations and are a centre of hotspots due to their high migration of marine species. This pattern could hypothesize the interrelationship between different trophies. Animals such as grazers or seabirds migrate every season for breeding and feeding, carrying particles and fragments of organisms. Thus, by interacting with different organisms, they transfer genetic material, tending to increase the genetic richness interaction of this ecosystem. Furthermore, investigating genetic diversity in hotspot areas is crucial in seagrass conservation and fit and acts as an experimental model of biodiversity monitoring for integrating coastal dynamics to mitigate anthropogenic threats and climate change (Olsen et al. 2004).

6.3 Genotypic diversity

Elongation through horizontal rhizomes is essential for population maintenance in seagrasses (van Dijk and van Tussenbroek 2010). In dioecious species such as *H. wrightii*, maintaining a diverse genotypic population is also important for mating chance and breeding performance. Monoclonal populations of dioecious herbaria exist and can persist for thousands of years (Alberto et al. 2008), though only by clonal reproduction, as a single male or single female could not form seeds sexually. Therefore, the genotypical richness and genetic diversity may also play a significant role in the resilience of seagrass ecosystems to maintain recruitment functions (Massa et al., 2013). The genotypic richness observed on the western African coast (*R* = 0.45) is quite variable among sites (R ranges from 0.07 to 1) (see Larkin et al., 2017) and presented similar genotypic richness to previous studies of *H. wrightii* in the WAT (R = 0.46 Tavares et al., 2022). As the genetic analysis of our study showed different levels of genotypic richness occurring along the coastline of the WAT, the results do not support our hypotheses (iii) of similar levels of clonal richness. The heterogeneity of clonal richness values for locations in WAT indicates a notable variation in the investment pattern in sexual vs clonal reproduction at the regional scale. Clonal richness was less relevant in some groups, such as Angola (*R* = 0.07; Fis = -0.55) and Senegal (*R* = 0.2; Fis = 0.28). Furthermore, clonal diversity was higher in the northwestern populations (Senegal = 0.9, Guinea-Bissau = 0.8) and the central/southwest populations (Gabon = 1, São Tomé and Principe = 1), suggesting that sexual reproduction is enhanced. However, the results must be evaluated carefully with the predictions due to the low availability of samples in Gabon.

Bijagós archipelago revealed low values, similar to the islands' north and south (Acante, Meio Oste, Canneronho). The low clonal richness can also indicate low genetic diversity, which does not support our hypothesis (ii) of genetic diversity diverging among the limits between the ranges of those places. However, the patterns still need more evaluation and investigation to understand better clonal diversity patterns and their fitness to predict where greater sexual reproduction occurs in different regional populations than outlying populations. While the core populations of Banc d’Arguin demonstrate lower clonal richness in the nearshore zone (Teichott population). Nevertheless, the four core populations shared similar clonal richness values ranging from 0.3 to 0.66 (Manghar population); Consequently, clonal richness indicated slight divergence among nearshore and offshore zones.

Seagrass beds typically exhibit highly variable levels of clonal richness in separate populations within the same species; thus, it is not a species-specific trait. (Arnaud-Haond et al. 2007; Boyé et al. 2022). Concerning levels of genotypic diversity and the number of adverse events within a population, seagrass recovery can occur through sexual or asexual reproduction when a disturbance of their habitats occurs (Boyé et al., 2022; Jarvis, Moore, and Kenworthy, 2012).

Clonal populations commonly comprise genotype variations (Ellstrand and Roose 1987), where most (terrestrial) populations present intermediate levels of genotypic diversity. In a recent study of *H. wrightii* in Florida Bay, Larkin et al. (2020) conducted a research where clonal diversity was analysed, taking into account the richness, evenness and architecture based on the plant’s clonal growth. The study has found that there is variation in clonal diversity between areas, i.e., clone richness varying between small and large scales. In this context, the study revealed that asexual reproduction plants broadly vary in clonal size and growth pattern (Eckert, Dorken, and Barrett 2016). Furthermore, Ruggiero et al. (2005) found high clonal richness and biased distribution of clones’ length among *Cymodocea nodosa* populations in the Mediterranean Sea. It hypothesized that these clonal processes could be associated with a balance among genets and sexual recruitment, low seed dispersal, and guerrilla enlargement strategy. With these two species sharing similarities, particularly the low seed dispersal (Darnell et al. 2015), the pattern seen with *Cymodoceae nodosa* could be applied to *H. wrightii* too. Thus, it is possible to say with our results that clonal diversity can vary over space and that we must consider the whole context of clonal diversity and not only its richness, which increases the need for greater overall insight into the relationship between area and species diversity (Rosenzweig, 1995).

Another hypothesis regarding genotypic diversity is the traits of the biological specie. *H. wrightii* carries seed bank characteristics, and their density differs among sites. The locations examined in nearshore and offshore zones (Banc d’Arguin) demonstrated a low level of variety, although it is predictable to consider the spatial scale of those regions and the relationship of seed bank density they present. Consequently, it could determine where recruitment could increase under the pressure of seagrasses, even more in areas of decline due to climate change (Chefaoui et al., 2021). Another hypothesis regarding genotypic diversity is the traits of the biological specie. *H. wrightii* carries seed bank characteristics, and their density differs among sites. The locations examined in nearshore and offshore zones (Banc d'Arguin) demonstrated a low level of variety, although it is predictable to consider the spatial scale of those regions and the relationship of seed bank density they present. Consequently, it could determine where recruitment could increase under the pressure of seagrasses, even more in areas of decline due to climate change (Chefaoui et al., 2021).

6.4 Genetic Structure and Differentiation

Despite the substantial link between populations demonstrated by low population genetic divergence, *H. wrightii* is believed to have poor dispersal ability due to its non-floating basal seeds. In this study, we show non-significant genetic structure among most of the sampled *H. wrightii* meadows and find a relation between genetic differentiation (Fst and Jost'D) and increasing geographic distance.

The initial expectation was that the linear distribution, physical separation, and distances among sites would result in significant genetic structure and IBD along the western African coast; however, this was not expected, thus not supporting the hypothesis of oceanographic influences by divergent ocean currents. Large-scale currents in the area play a minor role in the observed genetic differentiation and the three different clusters suggested by analysis. Therefore, we hypothesized that the limited range of strong currents, CC, GC, and BC, should influence the divergence of genetic structure from Mauritania to Angola (20 populations), leading to genetic admixture.

In fact, the sites from the northwest coast of Mauritania and to the central west coast of Angola are significantly differentiated from each other and show non-admixture levels, indicating that they do not receive propagules from their sites. São Tomé and Principe, on the other hand, revealed an admixture in the genetic differentiation, where Cabras Islet shared genetic richness among the two sites (**Fig. 10**).

Another factor suggesting the non-significance of our hypothesis is that the microsatellite markers used in the region do not have high variability, demonstrating difficulty in predicting seed dispersal via abiotic mechanisms since they can detect only genetic differentiation of high magnitude (Tavares et al., 2022). Furthermore, it is imperative to consider the unknown seagrass meadows that could exist along the coastline of evaluated sites, predicting high stepping-stone connectivity.

Our results are in line with the recent study of Tavares et al. (2022), which suggested that populations of São Tomé and Principe play a role in rare stochastic colonization of seagrass meadows since this genetic differentiation is located nearby more assigned to distinct northern and southern groups demonstrating little correlation with either oceanographic distance or geographic distance. Furthermore, different reports revealed independent colonisation of islands, although different traits and life stages must be considered (de Souza et al., 2017; Teschima et al., 2021). With this, we suggest that new colonisations of marine plants could be formed independently of their geographical location, although it is necessary to know the mechanisms that lead a species to insert itself in a new habitat and its attributes to this. Our results are in line with the recent study of Tavares et al. (2022), which suggested that populations of São Tomé and Principe play a role in rare stochastic colonization of seagrass meadows since this genetic differentiation is located nearby more assigned to distinct northern and southern groups demonstrating little correlation with either oceanographic distance or geographic distance. Furthermore, different reports revealed independent colonisation of islands, although different traits and life stages must be considered (de Souza et al. 2017; Teschima, Zilberberg, and Nunes 2021). With this, we suggest that new colonisations of marine plants could be formed independently of their geographical location, although it is necessary to know the mechanisms that lead a species to insert itself in a new habitat and its attributes to this. Our results are in line with the recent study of Tavares et al. (2022), which suggested that populations of São Tomé and Principe play a role in rare stochastic colonization of seagrass meadows since this genetic differentiation is located nearby more assigned to distinct northern and southern groups demonstrating little correlation with either oceanographic distance or geographic distance.

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By structure analysis of *Halodule wrightii* in the Gulf of Mexico (Texas, USA), Larkin (2017) conducted that significant genetic structure separates two different geographic spaces, suggesting distinctions between these locations. However, in the same study, greater genetic strength was found in tidal range sites than in sites with geographical barriers or more distant ones. *Halodule wrightii* could then reproduce and migrate via drafting vegetative fragments since some models seen cannot be related between distance and expansion via clonal growth. Furthermore, the mechanisms for genetic structure exhibit barriers, such as water temperature and reduced habitat area, which need to be considered (Arriesgado et al., 2015). Therefore, vegetative dispersal could account for the pattern of genetic structure.

We discovered no pattern of genetically similar populations at the ends of the range. Therefore, we could hypothesize a non-significant gene flow between the regions without breaks between populations. To this end, it is essential to highlight the importance of more samples per site, more sampling sites in a larger area and deeper seagrass genetics to forward step in population genetics of the seagrasses from north to south of the western African coast.

6.5 Isolation by distance

The method of IBD revealed that genetic connectivity, based on Wright's fixation index (Fst), tends to differ from demographic connectivity as it correlates with the effects of migration, selection, and genetic drift. Translating genetic connectivity into demographic estimates of dispersal is not straightforward (Becheler et al., 2010). We can argue that the practical estimation of the population with genetic models can be unrealistic in real-world situations (Manel, Loiseau, and Puebla 2019). In addition, gene flow over long geographical distances might result from stepping-stone dispersal over multiple generations without implying direct long-distance dispersal events (Saura, Bodin, and Fortin 2014). Isolated populations may not have the gene flow to create high enough levels of variation to adapt to new environments (Zimmerman et al. 1991).

Our results showed a relatively close geographical distance between some populations of Guinea Bissau and Mauritania (see **Fig.14**), which, apart from geographical similarity, share similar Fst values. Therefore, the relationship between the close genetic diversity between these two groups can be hypothesized as areas which are not isolated from each other and which, therefore, may present similar genetic variation.

IBD previously studies on different seagrasses (*Zostera marina*, *Posidonia oceanica* and *Zostera noltii*) revealed an indication of breakpoints of dispersal at a scale of < 250 km and 50 to 100 km, respectively (Arnaud-Haond et al. 2007; Reusch, and Olsen 2000; Coyer et al. 2004b), inferring that below such a distance, geneflow effectively prevents genetic differentiation among populations (Olsen et al., 2004). Seeds' low distance travel underwater (Orth et al., 1994) makes gene flow by rafting fruiting shoots possible (Harwell and Orth 2002; Harwell & Orth, 2002). That shoot dispersal represents a new genotype location of new populations and has been studied under genetic markers using assignment tests (see Reusch, 2002). One interpretation of such a new genotype is that clonal reproduction is likely greater than sexual reproduction, leading to lower genetic diversity; this gene exchange is seen in South Portugal (Billingham et al. 2003). Another zone is applied for the exact inference to a lesser extent in the Black Sea, where IBD relationships immediately increase with geographic distance, contrasting the north of Europe (Coyer et al. 2004b). As inferred from IBD graphs, population connectivity may differ among regions and is crucial to understanding the study area's location. On the other hand, the seagrass studied, *H. wrightii,* is a subtropical and tropical marine plant that needs to be interpreted differently.

This study showed that *H. wrightii* from the Western African coast are not as closely related. IBD estimates for *H. wrightii* are influenced by barriers to oceanographic currents in the core zones (Arnaud-Haond et al., 2007). Within each zone (Mauritania and Guinea-Bissau), geographic and genetic distance correlations are distinct, suggesting stochasticity in gene flow and dispersal patterns at hundreds of kilometres. McMillan (1987) studied seagrass movement and revealed a latitude gradient when comparing northern populations of *H. wrightii* to southern populations. However, it highlighted their biological traits' influence and persistence to remain in seed banks. Complex circulation patterns may also be inferred in enclosed basins, life history, and potential for seed recruitment. Thus, the biology of *H. wrightii* also impacts their dispersal due to its non-buoyant basal seed. Similarly, there is no correlation between gene flow and geographic distance within the region. With that, it is imperative to increase the model tests in regions less studied, such as the western African coast, to understand these patterns.

7. Conclusions and potential for future work

In this thesis, the study aimed to assess genetic data to interpret population connectivity and structure of relatively isolated and fragmented seagrass populations along tropical and subtropical coastlines. We tested three hypotheses with our data: connectivity along the West African coast, connectivity between tidal zones in the Gulf of Arguin, and connectivity between the north vs south islands of the Bijagós Archipelago. Thus, we explored the genetic structure and differentiation through different habitats between the sampled locations. It is expected that *H. wrightii* populations share genetic similarities that may be triggered by the transport and dispersal of fragments via abiotic mechanisms, such as the oceanographic currents of the region. We also expected that the populations of seagrass meadows in migratory regions of megaherbivores might also impact them to transport seeds or fragments. Thus, we estimated a range of genetic and genotypic diversity and isolation by distance approaches amongst *H. wrightii* populations exhibiting possibly seagrass population routes

Our results discovered that the genetic diversity is very low between those regions and within populations. We found low allelic diversity and integration of this data origin distinguished that genotypic rather than genetic diversity may be the appropriate level of genetic organisation for clonal organisms. Furthermore, the results exhibited genetic divergence among populations in the Northwest and Southwest Equatorial Atlantic (NEC and SEC). A particularly interesting result discovered in this study is the genetic differentiation in the islands of São Tomé and Príncipe, each displaying either genotypes of NEC or SEC. However, comparisons are necessary since central edge populations could encounter unique biotic and abiotic pressures and have a varied set of reproductive responses. Genetic mosaicism was present in *H. wrightii* clones, and while the processes underlying transport via oceanographic currents and IBD have been investigated, the fitness consequences remain unclear.

Our results do not emphasise the impact of oceanographic currents passing through and diverging in the West African coastal transition zone as an effective dispersal mechanism. Additionally, the poor genetic data do not reinforce the mechanism of biotic transport via megaherbivores in the migratory zones of green sea turtles. On the other hand, we support the hypothesis of connectivity within the population of the Gulf d'Arguin (Mauritania nearshore and offshore areas).

The relationship between dispersal versus abiotic and biotic mechanisms has been predicted by theory (Boulangeat et al., 2015) and supported empirically by various species and ecosystems (Spielman et al., 2004). Nevertheless, the exact resolution of individual case studies has led to scale-dependent findings. Furthermore, we undertook this at a location under short-term monitoring known to suffer localised declines (Chefaoui et al., 2021; Tavares et al., 2022). Seagrass's natural survival approach is an ideal model for testing ecological and population genetics hypotheses. This model has shown informative in both population genetics and population dynamics studies (Connolly et al. 2018; Lobelle et al. 2013), even if this is rarely related.

The sequencing of *H. wrightii* populations for the next generation is valuable for exploring the drivers of mechanistic transport and recruitment, genetic diversity, and gene flow in *H. wrightii*. Dispersal is a particularly important question for Halodule due to discussions over the Atlantic Ocean (Brazil, Florida, Bermudas) (De Oliveira et al., 1983; Murdoch et al., 2007; Phillips, 1967). Genetic comparisons are unreliable for distinguishing Halodule connectivity. Therefore, sequencing of species lineages may be integrated for more accurate population distribution.

By presenting these findings, we hoped to shape source data on genetic diversity and motivate future research on causal relationships between population genetics and abundance routes. We performed our research in one of the largest and pristine seagrass meadows in western Africa, around the Banc d'Arguin. Our results demonstrated that an extensive and sustained seagrass population could persist with low overall genetic diversity. Thus, the study inserts into the growing body of evidence that identifying ecological and genetic indicators based on evolutionary mechanisms is vital for the management and conservation genetic test model. Our research also aids in addressing the urgent need to create integrated ecological frameworks to discuss the evidence based on the planning of this significantly large but threatened group linked to coastal management to lessen core endangered areas.

8. References

8.1 Chapter one – General Introduction

1. Abe, J., & Brown, B. E. (2020). Towards a Guinea Current Large Marine Ecosystem Commission. In Environmental Development (Vol. 36). Elsevier B.V. https://doi.org/10.1016/j.envdev.2020.100590.
2. Alexandre, A., Silva, J., Ferreira, R., Paulo, D., Serrão, E. A., and Santos, R. (2017). First Description of Seagrass Distribution and Abundance in São Tomé and Prıncipe.´ Aquat. Bot. 142, 48–52. doi: 10.1016/j.aquabot.2017.06.008.
3. Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. In Nature Reviews Genetics (Vol. 11, Issue 10, pp. 697–709). https://doi.org/10.1038/nrg2844.
4. Amano, T., & Sutherland, W. J. (2013). Four barriers to the global understanding of biodiversity conservation: Wealth, language, geographical location and security. Proceedings of the Royal Society B: Biological Sciences, 280(1756). https://doi.org/10.1098/rspb.2012.2649.
5. Andersen, T., Carstensen, J., Hernández-García, E., & Duarte, C. M. (2009). Ecological thresholds and regime shifts: approaches to identification. Trends in ecology & evolution, 24(1), 49–57. doi: 10.1016/j.tree.2008.07.014.
6. Araujo, A., & Campredon, P. (2016). Banc d'Arguin (Mauritania). In The Wetland Book (pp. 1–14). Springer Netherlands. https://doi.org/10.1007/978-94-007-6173-5_154-1.
7. Arber, A. (1920). Water Plants: A Study of Aquatic Angiosperms (Cambridge Library Collection - Botany and Horticulture). Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511700675.
8. Arnaud-Haond, S., Duarte, C. M., Diaz-Almela, E., Marbà, N., Sintes, T., & Serrão, E. A. (2012). Implications of extreme life span in clonal organisms: Millenary clones in meadows of the threatened seagrass posidonia oceanica. In PLoS ONE (Vol. 7, Issue 2). https://doi.org/10.1371/journal.pone.0030454
9. Arriesgado, D. M., Kurokochi, H., Nakajima, Y., Matsuki, Y., Uy, W. H., Fortes, M. D., Campos, W. L., Nadaoka, K., & Lian, C. (2015). Genetic diversity and structure of the tropical seagrass Cymodocea serrulata spanning its central diversity hotspot and range edge. Aquatic Ecology, 49(3), 357–372. https://doi.org/10.1007/s10452-015-9529-0
10. Assis, J., Neiva, J., Bolton, J. J., Rothman, M. D., Gouveia, L., Paulino, C., Mohdnasir, H., Anderson, R. J., Reddy, M. M., Kandjengo, L., Kreiner, A., Pearson, G. A., & Serrão, E. A.

(2022). Ocean currents shape the genetic structure of a kelp in southwestern Africa. Journal of Biogeography, 49(5), 822–835. https://doi.org/10.1111/jbi.14338

11. Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C. and Silliman, B.R. (2011), The value of estuarine and coastal ecosystem services. Ecological Monographs, 81: 169-193. https://doi.org/10.1890/10-1510.1.
12. Becheler, R., Benkara, E., Moalic, Y., Hily, C., & Arnaud-Haond, S. (2014). Scaling of processes shaping the clonal dynamics and genetic mosaic of seagrasses through temporal genetic monitoring. Heredity, 112(2), 114–121. https://doi.org/10.1038/hdy.2013.82
13. Beck, M.W., et al., (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience 51: 633–641.
14. Beckman, N. G., Aslan, C. E., Rogers, H. S., Kogan, O., Bronstein, J. L., Bullock, J. M., Hartig, F., HilleRisLambers, J., Zhou, Y., Zurell, D., Brodie, J. F., Bruna, E. M., Cantrell, R. S., Decker, R. R., Efiom, E., Fricke, E. C., Gurski, K., Hastings, A., Johnson, J. S., … Zambrano, J. (2020). Advancing an interdisciplinary framework to study seed dispersal ecology. In AoB PLANTS (Vol. 12, Issue 2). Oxford University Press. https://doi.org/10.1093/AOBPLA/PLZ048
15. Belhabib, D., Doumbouya, A., Diallo, I., Traore, S., Camara, Y., Copeland, D., Gorez, B., Harper, S., Zeller, D., & Pauly, D. (2012). Guinean fisheries, past, present and... future? In D. Belhabib, D. Zeller, S. Harper, & D. Pauly (Eds.), Marine fisheries catches in West Africa, 1950-2010, Part I (pp. 91-104). (Fisheries Centre Research Reports; Vol. 20, No. 3). University of British Columbia.
16. Berković, B., Cabaço, S., Barrio, J. M., Santos, R., Serrão, E. A., & Alberto, F. (2014). Extending the life history of a clonal aquatic plant: Dispersal potential of sexual and asexual propagules of Zostera noltii. Aquatic Botany, 113, 123–129. https://doi.org/10.1016/j.aquabot.2013.10.007
17. Berkström, C., Gullström, M., Lindborg, R., Mwandya, A. W., Yahya, S. A. S., Kautsky, N., & Nyström, M. (2012). Exploring “knowns” and “unknowns” in tropical seascape connectivity with insights from East African coral reefs. Estuarine, Coastal and Shelf Science, 107, 1–21. https://doi.org/10.1016/j.ecss.2012.03.020
18. Berkström, C., Jörgensen, T. L., & Hellström, M. (2013). Ecological connectivity and niche differentiation between two closely related fish species in the mangrove-seagrass-coral reef continuum. Marine Ecology Progress Series, 477, 201–215. https://doi.org/10.3354/meps10171
19. Binet, D., & Marchai, E. (1993). The Large Marine Ecosystem of Shelf Areas in the Gulf of Guinea: Long-Term Variability Induced by Climatic Changes.
20. Birch, W. R., & Birch, M. (1984). Succession and pattern of tropical intertidal seagrasses in cockle bay, queensland, australia: a decade of observations. In Aquatic Botany (Vol. 19).
21. Bjorndal, K.A., Bolten, A.B., (2003). From ghosts to key species: restoring sea turtle populations to fulfill their ecological roles. Mar. Turt. Newsl. 100, 16e21.
22. Bjorndal, K.A., Bolten, A.B. & Chaloupka, M.Y. (2003a). Survival probability estimates for immature green turtles Chelonia mydas in the Bahamas. Mar. Ecol. Prog. Ser. **252**, 273.

23. Blumenthal, J. M., Abreu-Grobois, F. A., Austin, T. J., Broderick, A. C., Bruford, M. W., Coyne, M. S., Ebanks-Petrie, G., Formia, A., Meylan, P. A., Meylan, A. B., & Godley, B. J. (2009). Turtle groups or turtle soup: Dispersal patterns of hawksbill turtles in the Caribbean. Molecular Ecology, 18(23), 4841–4853. https://doi.org/10.1111/j.1365-294X.2009.04403.x
24. Burkholder DA, Heithaus MR, Thomson JA, Fourqurean JW (2011) Diversity in trophic interactions of green sea turtles Chelonia mydas on a relatively pristine coastal foraging ground. Mar Ecol Prog Ser 439:277-293. https://doi.org/10.3354/meps09313.
25. Carruthers, T. J. B., Dennison, W. C., Longstaff, B. J., Waycott, M., Abal, E. G., Mckenzie, L. J., & Long, W. J. L. (2002). Seagrass habitats of northeast Australia: models of key processes and controls. in bulletin of marine science (Vol. 71, Issue 3).
26. Carton, J., and B. Huang, Warm events in the tropical Atlantic., J. Phys. Oceanogr., 24, 888-903, 1994
27. Catry, P., Barbosa, C., Indjai, B., Almeida, A., Godley, B. J., and Vie, J.-C. (2002). First Census of the Green Turtle at Poilão, Bijagós Archipelago, Guinea-Bissau: The Most Important Nesting Colony on the Atlantic Coast of Africa. Oryx 36, 400–403. doi: 10.1017/S0030605302000765.
28. Catry, P., Barbosa, C., Paris, B., Indjai, B., Almeida, A., Limoges, B., Silva, C., Hono´, H., & Pereira, H. (2009). Status, Ecology, and Conservation of Sea Turtles in Guinea-Bissau. http://dx.doi.org/10.2744/CCB-0772.1
29. Chambers, J. C., & MacMahon, J. A. (1994). A Day in the Life of a Seed: Movements and Fates of Seeds and Their Implications for Natural and Managed Systems. Annual Review of Ecology and Systematics, 25, 263–292. http://www.jstor.org/stable/2097313.
30. Chiswell, S. M., & Rickard, G. J. (2011). Larval connectivity of harbours via ocean currents: A New Zealand study. Continental Shelf Research, 31(10), 1057–1074. https://doi.org/10.1016/j.csr.2011.03.012
31. Coles, R., McKenzie, L., De'ath, G., Roelofs, A., & Long, W. L. (2009). Spatial distribution of deepwater seagrass in the inter-reef lagoon of the Great Barrier Reef World Heritage Area. Marine Ecology Progress Series, 392, 57–68. https://doi.org/10.3354/meps08197
32. Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., (1997). The value of the world's ecosystem services and natural capital. Nature 387, 253–260.
33. Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. In Annual Review of Marine Science (Vol. 1, pp. 443–466). https://doi.org/10.1146/annurev.marine.010908.163757
34. Cowen, R. K., Paris, C. B., & Srinivasan, A. (2006). Scaling of connectivity in marine populations. Science, 311(5760), 522–527. https://doi.org/10.1126/science.1122039
35. CUQ, François et al. (2011). Un système d'information géographique pour l'aide à la gestion intégrée de l'archipel des Bijagos (Guinée-Bissau): notice de la carte, constitution et exploitation du SIG. CNRS, Géosystèmes/INEP, IUCN.
36. Dahlgren, R.M.T., Clifford, H.T. and Yeo P.F. (1985). The Families of the Monocotyledons: Structure, Evolution and Taxonomy. Springer Verlag, Berlin.

37. Darnell, K. M., Booth, D. M., Koch, E. W., & Dunton, K. H. (2015). The interactive effects of water flow and reproductive strategies on seed and seedling dispersal along the substrate in two sub-tropical seagrass species. Journal of Experimental Marine Biology and Ecology, 471, 30–40. https://doi.org/10.1016/j.jembe.2015.05.006
38. De Oliveira, F.E.C., Pirani, J.R. and Giuletti, A.M. (1983) The Brazilian seagrasses. Aquat Bot 16: 251–267.
39. Den Hartog, C. 1970. The Seagrasses of the World. Amsterdam: North Holland.
40. Dennison, W. C., Orth, R. J., Moore, K. A., Stevenson, J. C., Carter, V., Kollar, S., Bergstrom, P. W., & Batiuk, R. A. (1993). Assessing Water Quality with Submersed Aquatic Vegetation. BioScience, 43(2), 86–94. doi: 10.2307/1311969.
41. Du Petit Thouars LM Aubert (1806) Genera nova madagascariensia secundum methodum Jussiaeanum disposita. p 29. [Paris].
42. Duarte, C. M., Borum, J., Short, F. T., & Walker, D. I. (2008a). Seagrass ecosystems: Their global status and prospects. In Aquatic Ecosystems: Trends and Global Prospects (pp. 281–294). Cambridge University Press. https://doi.org/10.1017/CBO9780511751790.025
43. Duarte, C. M., Borum, J., Short, F. T., & Walker, D. I. (2008b). Seagrass ecosystems: Their global status and prospects. In Aquatic Ecosystems: Trends and Global Prospects (pp. 281–294). Cambridge University Press. https://doi.org/10.1017/CBO9780511751790.025
44. Duartel, C. M., & Ensen~, K. S. (1990). Seagrass colonization: biomass development and shoot demography in Cymodocea nodosa patches (Vol. 67).
45. Dunic, J. C., Brown, C. J., Connolly, R. M., Turschwell, M. P., & Côté, I. M. (2021). Long-term declines and recovery of meadow area across the world's seagrass bioregions. Global Change Biology, 27(17), 4096–4109. https://doi.org/10.1111/gcb.15684
46. Eklf, J. S., McMahon, K., & Lavery, P. S. (2009). Effects of multiple disturbances in seagrass meadows: Shading decreases resilience to grazing. Marine and Freshwater Research, 60(12), 1317–1327. https://doi.org/10.1071/MF09008
47. Ellstrand, N. C. (2014). Is gene flow the most important evolutionary force in plants? American Journal of Botany, 101(5), 737–753. https://doi.org/10.3732/ajb.1400024
48. Endlicher, S. (1836). Genera plantarum secundum ordines naturales disposita (supplementums) /auctore Stephano Endlicher. doi: 10.5962/bhl.title.442.
49. Erftemeijer, P. L., & Lewis, R. R., 3rd (2006). Environmental impacts of dredging on seagrasses: a review. Marine pollution bulletin, 52 (12), 1553–1572. doi: 10.1016/j.marpolbul.2006.09.006.
50. Escribano, R., Daneri, G., Farías, L., Gallardo, V. A., González, H. E., Gutiérrez, D., Lange, C. B., Morales, C. E., Pizarro, O., Ulloa, O., & Braun, M. (2004). Biological and chemical consequences of the 1997-1998 El Niño in the Chilean coastal upwelling system: A synthesis. Deep-Sea Research Part II: Topical Studies in Oceanography, 51(20–21), 2389–2411. https://doi.org/10.1016/j.dsr2.2004.08.011
51. Fang, H., Li, C., & Jiang, L. (2018). Morphology of the immature stages of Adoretus tenuimaculatus Waterhouse, 1875 (Coleoptera: Scarabaeidae: Rutelinae: Adoretini). Journal of Asia-Pacific Entomology, 21(4), 1159–1164. https://doi.org/10.1016/j.aspen.2018.09.001

52. Godley, B. J., Barbosa, C., Bruford, M., Broderick, A. C., Catry, P., Coyne, M. S., Formia, A., Hays, G. C., & Witt, M. J. (2010a). Unravelling migratory connectivity in marine turtles using multiple methods. Journal of Applied Ecology, 47(4), 769–778. https://doi.org/10.1111/j.1365-2664.2010.01817.x
53. Godley, B. J., Barbosa, C., Bruford, M., Broderick, A. C., Catry, P., Coyne, M. S., Formia, A., Hays, G. C., & Witt, M. J. (2010b). Unravelling migratory connectivity in marine turtles using multiple methods. Journal of Applied Ecology, 47(4), 769–778. https://doi.org/10.1111/j.1365-2664.2010.01817.x
54. Goldberg, E. E., & Lande, R. (2007). Notes and Comments Species' Borders and Dispersal Barriers. In Am. Nat (Vol. 170, Issue 2).
55. Grech, A., & Coles, R. G. (2010). An ecosystem-scale predictive model of coastal seagrass distribution. Aquatic Conservation: Marine and Freshwater Ecosystems, 20(4), 437–444. https://doi.org/10.1002/aqc.1107
56. Greene, D. F., & Johnson, E. A. (1996). Wind Dispersal of Seeds from a Forest Into a Clearing. Ecology, 77(2), 595–609. https://doi.org/10.2307/2265633.
57. Gulick, A. G., Johnson, R. A., Pollock, C. G., Hillis-Starr, Z., Bolten, A. B., & Bjorndal, K. A. (2021). Recovery of a cultivation grazer: A mechanism for compensatory growth of Thalassia testudinum in a Caribbean seagrass meadow grazed by green turtles. Journal of Ecology, 109, 3031– 3045. doi: 10.1111/1365-2745.13718
58. Hancock, J. M., Vieira, S., Taraveira, L., Santos, A., Schmitt, V., Semedo, A., Patrício, A. R., Ferrand, N., Gonçalves, H., & Sequeira, F. (2019). Genetic characterization of green turtles (Chelonia mydas) from São Tomé and Príncipe: Insights on species recruitment and dispersal in the Gulf of Guinea. Journal of Experimental Marine Biology and Ecology, 518. https://doi.org/10.1016/j.jembe.2019.151181
59. Harper, J. & Garbary, D. (1997). Marine Algae of Northern Senegal: The Flora and Its Biogeography., 40(1-6), 129-138. https://doi.org/10.1515/botm.1997.40.1-6.129.
60. Harwell, M. C., and Orth, R. J. (2002). Long-Distance Dispersal Potential in a Marine Macrophyte. Ecology 83, 3319–3330. doi: 10.1890/0012-9658(2002)083 [3319:LDDPIA]2.0.CO;2.
61. Heck, K. L., & Valentine, J. F. (2006). Plant-herbivore interactions in seagrass meadows. Journal of Experimental Marine Biology and Ecology, 330(1), 420–436. https://doi.org/10.1016/j.jembe.2005.12.044
62. Hedgecock, D., Barber, P. H., & Edmands, S. (2007). Genetic Approaches to Measuring Connectivity. In Source: Oceanography (Vol. 20, Issue 3).
63. Heileman, S. (2008). I-2 Guinea Current: LME #28.
64. Heithaus M.R., Alcoverro T., Arthur R., Burkholder D.A., Coates K.A., Christianen M.J.A., Kelkar N., Manuel S.A., Wirsing A.J., Kenworthy W.J. and Fourqurean J.W. (2014) Seagrasses in the age of sea turtle conservation and shark overfishing. Front. Mar. Sci. 1:28. doi: 10.3389/fmars.2014.00028.

65. Heller, N. E., & Zavaleta, E. S. (2009). Biodiversity management in the face of climate change: A review of 22 years of recommendations. In Biological Conservation (Vol. 142, Issue 1, pp. 14–32). https://doi.org/10.1016/j.biocon.2008.10.006
66. Hemminga, M. A., & Nieuwenhuize, J. (1991). Transport, deposition and in situ decay of seagrasses in a tropical mudflat area (banc d'arguin, mauritania). Netherlands Journal of Sea Research (Vol. 27, Issue 2).
67. Hemminga, M.A., Duarte, C.M., (2000). Seagrass Ecology. Cambridge University Press, Cambridge.
68. Henderson, C. J., Gilby, B. L., Lee, S. Y., & Stevens, T. (2017). Contrasting effects of habitat complexity and connectivity on biodiversity in seagrass meadows. Marine Biology, 164(5). https://doi.org/10.1007/s00227-017-3149-2
69. Henriques, R., Potts, W. M., Santos, C. v., Sauer, W. H. H., & Shaw, P. W. (2014). Population connectivity and phylogeography of a coastal fish, Atractoscion aequidens (Sciaenidae), across the Benguela Current region: Evidence of an ancient vicariant event. PLoS ONE, 9(2). https://doi.org/10.1371/journal.pone.0087907
70. Horan, R.D., Fenichel, E.P., Drury, K.L.S., Lodge, D.M., (2011). Managing ecological thresholds in coupled environmental–human systems. Proc. Natl. Acad. Sci. U. S. A. 108, 7333–7338.
71. Jaganathan, G.K. Influence of maternal environment in developing different levels of physical dormancy and its ecological significance. Plant Ecol **217**, 71–79 (2016). https://doi.org/10.1007/s11258-015-0560-y.
72. Jarvis, J. C., Moore, K. A., & Kenworthy, W. J. (2012). Characterization and ecological implication of eelgrass life history strategies near the species' southern limit in the western North Atlantic. Marine Ecology Progress Series, 444, 43–56. https://doi.org/10.3354/meps09428
73. Kendrick, G. A., Orth, R. J., Statton, J., Hovey, R., Montoya, L. R., Lowe, R. J., Krauss, S. L., & Sinclair, E. A. (2017). Demographic and genetic connectivity: The role and consequences of reproduction, dispersal and recruitment in seagrasses. In Biological Reviews (Vol. 92, Issue 2, pp. 921–938). Blackwell Publishing Ltd. https://doi.org/10.1111/brv.12261
74. Kendrick, G. A., Waycott, M., Carruthers, T. J. B., Cambridge, M. L., Hovey, R., Krauss, S. L., Lavery, P. S., Les, D. H., Lowe, R. J., Vidal, O. M. I., Ooi, J. L. S., Orth, R. J., Rivers, D. O., Ruiz-Montoya, L., Sinclair, E. A., Statton, J., van Dijk, J. K., & Verduin, J. J. (2012). The central role of dispersal in the maintenance and persistence of seagrass populations. In BioScience (Vol. 62, Issue 1, pp. 56–65). https://doi.org/10.1525/bio.2012.62.1.10
75. Kinlan, B. P., & Gaines, S. D. (2003). Propagule dispersal in marine and terrestrial environments: a community perspective. In Special Feature Ecology (Vol. 84, Issue 8).
76. Kondolf, G. M., Boulton, A. J., O'daniel, S., Poole, G. C., Rahel, F. J., Stanley, E. H., Wohl, E., Bång, A., Carlstrom, J., Cristoni, C., Huber, H., Koljonen, S., Louhi, P., & Nakamura, K. (2006). Process-Based Ecological River Restoration: Visualizing Three-Dimensional Connectivity and Dynamic Vectors to Recover Lost Linkages (Vol. 11, Issue 2). and Society. http://www.ecologyandsociety.org/vol11/iss2/art5/

77. Kool, J. T., Moilanen, A. & Treml, E. A. (2013). Population connectivity: recent advances and new perspectives. LANDSCAPE ECOLOGY, 28 (2), pp.165-185. https://doi.org/10.1007/s10980-012-9819-z.
78. Kowalski, Joseph L. and DeYoe, Hudson R. "Flowering and seed production in the subtropical seagrass, Halodule wrightii (shoal grass)" Botanica Marina, vol. 59, no. 2-3, 2016, pp. 193-199. https://doi.org/10.1515/bot-2015-0099.
79. Lande, R., & Shannon, S. (1996). The Role of Genetic Variation in Adaptation and Population Persistence in a Changing. In Source: Evolution (Vol. 50, Issue 1).
80. Larkum, A.W.D., Waycott, M., Conran, J.G. (2018). Evolution and Biogeography of Seagrasses. In: Larkum, A., Kendrick, G., Ralph, P. (eds) Seagrasses of Australia. Springer, Cham. https://doi.org/10.1007/978-3-319-71354-0_1.
81. Lavery, P. S., McMahon, K., Mulligan, M., & Tennyson, A. (2009). Interactive effects of timing, intensity and duration of experimental shading on Amphibolis griffithii. Marine Ecology Progress Series, 394, 21–33. https://doi.org/10.3354/meps08242
82. Leeney, R. H., & Poncelet, P. (2015). Using fishers' ecological knowledge to assess the status and cultural importance of sawfish in Guinea-Bissau. Aquatic Conservation: Marine and Freshwater Ecosystems, 25(3), 411–430. https://doi.org/10.1002/aqc.2419
83. Lees, K., Pitois, S., Scott, C., Frid, C., Mackinson, S., (2006). Characterizing regime shifts in the marine environment. Fish Fish. 7, 104–127.
84. Les, D. H., Cleland, M. A., & Waycott, M. (1997). Phylogenetic Studies in Alismatidae, II: Evolution of Marine Angiosperms (Seagrasses) and Hydrophily. Systematic Botany, 22(3), 443–463. https://doi.org/10.2307/2419820
85. Les, D., Cleland, M., & Waycott, M. (1997). Phylogenetic Studies in Alismatidae, II: Evolution of Marine Angiosperms (Seagrasses) and Hydrophily. Systematic Botany, 22(3), 443. doi: 10.2307/2419820.
86. Limoges B, Robillard M (1991) Proposition d'un plan d'amenagement de la reserve de la biosphere de l'archipel des Bijagós. Vol. 2 - Les espèces animales, distributions et recommandations. CECI/IUCN. 139pp.
87. Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? Molecular Ecology, 19(15), 3038–3051. https://doi.org/10.1111/j.1365-294X.2010.04688.x
88. McMahon, K., van Dijk, K. J., Ruiz-Montoya, L., Kendrick, G. A., Krauss, S. L., Waycott, M., Verduin, J., Lowe, R., Statton, J., Brown, E., & Duarte, C. (2014). The movement ecology of seagrasses. In Proceedings of the Royal Society B: Biological Sciences (Vol. 281, Issue 1795). Royal Society of London. https://doi.org/10.1098/rspb.2014.0878
89. McMillan, C. (1981). Seed Reserves and Seed Germination for Two Seagrasses, Halodule Wrightii and Syringodium Filiforme, From the Western Atlantic. Aquat. Bot. 11, 279–296. doi: 10.1016/0304-3770(81)90066-8.
90. Muelbert, J. H., Nidzieko, N. J., Acosta, A. T. R., Beaulieu, S. E., Bernardino, A. F., Boikova, E., Bornman, T. G., Cataletto, B., Deneudt, K., Eliason, E., Kraberg, A., Nakaoka, M., Pugnetti,

A., Ragueneau, O., Scharfe, M., Soltwedel, T., Sosik, H. M., Stanisci, A., Stefanova, K., … Zingone, A. (2019). Ilter – the international long-term ecological research network as a platform for global coastal and ocean observation. Frontiers in Marine Science, 6(AUG). https://doi.org/10.3389/fmars.2019.00527

91. Orth, R. J., Carruthers, T. J.B., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., et al. (2006a). A Global Crisis for Seagrass Ecosystems. Bioscience 56, 987–996. doi: 10.1641/0006-3568(2006)56.
92. Orth, R. J., Harwell, M. C., and Inglis, G. J. (2006b). Ecology of Seagrass Beds Seeds and Dispersal Strategies. Seagrasses Biol. Ecol. Conserv. Eds. B. D. Larkum, R. Orth and C. Duarte (Dordrecht: Springer Press, Netherlands), 111–133. doi: 10.1007/1-4020-2983-7_5.
93. Orth, R. J., Luckenbach, M., and Moore, K. A. (1994). Seed Dispersal in a Marine Macrophyte: Implications for Colonization and Restoration. Ecology 75, 1927– 1939. doi: 10.2307/1941597.
94. Patrício, A. R., Hawkes, L. A., Monsinjon, J. R., Godley, B. J., & Fuentes, M. M. P. B. (2021). Climate change and marine turtles: Recent advances and future directions. In Endangered Species Research (Vol. 44, pp. 363–395). Inter-Research. https://doi.org/10.3354/esr01110
95. Patrício, A. R., Varela, M. R., Barbosa, C., Broderick, A. C., Catry, P., Hawkes, L. A., Regalla, A., & Godley, B. J. (2019). Climate change resilience of a globally important sea turtle nesting population. Global Change Biology, 25(2), 522–535. https://doi.org/10.1111/gcb.14520
96. Pennesi, C., Rindi, F., Totti, C., and Beolchini, F. (2015). “Marine macrophytes: biosorbents,” in Springer Handbook of Marine Biotechnology, ed. S. K. Kim (Berlin: Springer), 597–610. doi: 10.1007/978-3-642-53971-8_24.
97. Philander, S. G. (2001). Atlantic Ocean Equatorial Currents. In Encyclopedia of Ocean Sciences (pp. 188–191). Elsevier. https://doi.org/10.1006/rwos.2001.0361
98. Pineda, j., Hare, j. a., & Sponaugle, s. (2007). Larval Transport and Dispersal in the Coastal Ocean and Consequences for Population Connectivity. Oceanography, 20(3), 22–39. http://www.jstor.org/stable/24860094.
99. Poiner, I. R., Staples, D. J., & Kenyon, R. (1987). Seagrass Communities of the Gulf of Carpentaria, Australia. In Mar. Freshw. Res (Vol. 38).
100. Pringle, C. (2003). What is hydrologic connectivity and why is it ecologically important? Hydrological Processes, 17(13), 2685–2689. https://doi.org/10.1002/hyp.5145
101. Reid, K., Hoareau, T. B., Graves, J. E., Potts, W. M., dos Santos, S. M. R., Klopper, A. W., & Bloomer, P. (2016). Secondary contact and asymmetrical gene flow in a cosmopolitan marine fish across the Benguela upwelling zone. Heredity, 117(5), 307–315. https://doi.org/10.1038/hdy.2016.51
102. Ruiz-Montoya, L., Lowe, R. J., Niel, V., and Kendrick, G. A. (2012). The Role of Hydrodynamics on Seed Dispersal in Seagrasses. Limnol. Oceanogr. 57, 1257– 1265. doi: 10.4319/lo.2012.57.5.1257.
103. Schulze, M. J., von der Heyden, S., Japp, D., Singh, L., Durholtz, D., Kapula, V. K., Ndjaula, H. O. N., & Henriques, R. (2020). Supporting Fisheries Management With Genomic Tools: A Case

Study of Kingklip (Genypterus capensis) Off Southern Africa. Frontiers in Marine Science, 7. https://doi.org/10.3389/fmars.2020.557146

104. Selkani, I. (2022). Let’s Talk about MPAs: Blue Spaces in Africa—Case of RAMPAO. 32. https://doi.org/10.3390/environsciproc2022015032
105. Selkoe, K. A., Gaggiotti, O. E., Treml, E. A., Wren, J. L. K., Donovan, M. K., & Toonen, R. J. (2016). The DNA of coral reef biodiversity: Predicting and protecting genetic diversity of reef assemblages. Proceedings of the Royal Society B: Biological Sciences, 283(1829). https://doi.org/10.1098/rspb.2016.0354
106. Sheaves, M. (2009). Consequences of ecological connectivity: The coastal ecosystem mosaic. In Marine Ecology Progress Series (Vol. 391, pp. 107–115). https://doi.org/10.3354/meps08121
107. Short, F. T., Polidoro, B., Livingstone, S. R., Carpenter, K. E., Bandeira, S., Sidik Bujang, J., Calumpong, H. P., Carruthers, T. J. B., Coles, R. G., Dennison, W. C., Erftemeijer, P. L. A., Fortes, M. D., Freeman, A. S., Jagtap, T. G., Hena, A., Kamal, M., Kendrick, G. A., Kenworthy, W. J., Nafie, Y. A. la, … Zieman, J. C. (2011). Extinction risk assessment of the world’s seagrass species. In Biol. Conserv (Issue 7).
108. Short, F., Carruthers, T., Dennison, W., & Waycott, M. (2007). Global seagrass distribution and diversity: A bioregional model. Journal of Experimental Marine Biology and Ecology, 350(1–2), 3–20. https://doi.org/10.1016/j.jembe.2007.06.012
109. Sintes, T., Marbà, N. & Duarte, C.M. Modeling nonlinear seagrass clonal growth: Assessing the efficiency of space occupation across the seagrass flora. Estuaries and Coasts: J ERF 29, 72–80 (2006). https://doi.org/10.1007/BF02784700
110. Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, C. A., & Robertson, J. (2007). Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. In BioScience (Vol. 57, Issue 7, pp. 573–583). https://doi.org/10.1641/B570707
111. Steele, M. A., Smallwood, P. D., Albert Spunar, & Elise Nelsen. (2001). The Proximate Basis of the Oak Dispersal Syndrome: Detection of Seed Dormancy by Rodents. American Zoologist, 41(4), 852–864. http://www.jstor.org/stable/3884529.
112. Stokes HJ, Mortimer JA, Hays GC et al (2019) Green turtle diet is dominated by seagrass in the Western Indian Ocean except amongst gravid females. Mar Biol 166:135. https://doi.org/10.1007/s00227-019-3584-3.
113. SWOT Scientific Advisory Board (2011). The State of the World’s Sea Turtles (SWOT) Minimum Data Standards for Nesting Beach Monitoring. Technical Report, 24 pp. (https://static1.squarespace.com/static/5b80290bee1759a50e3a86b3/t/5baba504104c7bbff39ac4ad/1537975557038/SWOT_MinimumDataStandards_TechReport.pdf)
114. Tavares, A. I., Assis, J., Patrício, A. R., Ferreira, R., Cheikh, M. A. S., Bandeira, S., Regalla, A., Santos, I., Potouroglou, M., Nicolau, S., Teodósio, M. A., Almada, C., Santos, R., Pearson, G. A., & Serrao, E. A. (2022). Seagrass Connectivity on the West Coast of Africa Supports the

Hypothesis of Grazer-Mediated Seed Dispersal. Frontiers in Marine Science, 9. https://doi.org/10.3389/fmars.2022.809721

115. Taylor, P. D., Fahrig, L., Henein, K., & Merriam, G. (1993). Nordic Society Oikos Connectivity Is a Vital Element of Landscape Structure (Vol. 68, Issue 3).
116. Terrados, J., Borum, J., Kamp-Nielsen, L., Duarte, C. M., & Fortes, M. D. (1999). Experimental evaluation of the effects 3 of siltation-derived changes in sediment conditions 4 on the Philippine seagrass Cymodocea rotundata 5 6 7 Zayda Halun.
117. Tischendorf, L., Fahrig, L., Tischendorf, L., & Fahrig, L. (2000). MINI-REVIEW On the usage and measurement of landscape connectivity What is landscape connectivity?
118. Tol, S. J., Jarvis, J. C., York, P. H., Congdon, B. C., & Coles, R. G. (2021). Mutualistic relationships in marine angiosperms: Enhanced germination of seeds by mega-herbivores. Biotropica, 53(6), 1535–1545. https://doi.org/10.1111/btp.13001
119. Tol, S. J., Jarvis, J. C., York, P. H., Grech, A., Congdon, B. C., & Coles, R. G. (2017). Long distance biotic dispersal of tropical seagrass seeds by marine mega-herbivores. Scientific Reports, 7(1). https://doi.org/10.1038/s41598-017-04421-1
120. Tomlinson, P. B., (1974). Vegetative morphology and meristem dependence - the foundation of productivity in seagrasses. Aquaculture 4: 107-30.
121. Tomlinson, P.B. (1982). Anatomy of the Monocotyledons, VII Helobiae (Alismatidae), Clarendon Press, Oxford.
122. Trégarot, E., Catry, T., Pottier, A., El-Hacen, E. H. M., Sidi Cheikh, M. A., Cornet, C. C., Maréchal, J. P., & Failler, P. (2021). Coastal protection assessment: a tradeoff between ecological, social, and economic issues. Ecosphere, 12(2). https://doi.org/10.1002/ecs2.3364
123. Trierweiler, C., Klaassen, R. H. G., Drent, R. H., Exo, K. M., Komdeur, J., Bairlein, F., & Koks, B. J. (2014). Migratory connectivity and populationspecific migration routes in a long-distance migratory bird. Proceedings of the Royal Society B: Biological Sciences, 281(1778). https://doi.org/10.1098/rspb.2013.2897
124. Udy, J. W., & Dennison, W. C. (1997). Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. Marine and Freshwater Research, 48(7), 605–614. https://doi.org/10.1071/MF97001
125. Ukwe, C. N., Ibe, C. A., Alo, B. I., & Yumkella, K. K. (2003). Achieving a paradigm shift in environmental and living resources management in the Gulf of Guinea: The large marine ecosystem approach. Marine Pollution Bulletin, 47(1–6), 219–225. https://doi.org/10.1016/S0025-326X(02)00473-3
126. Unsworth, R. K. F., Collier, C. J., Waycott, M., Mckenzie, L. J., & CullenUnsworth, L. C. (2015). A framework for the resilience of seagrass ecosystems. Marine Pollution Bulletin, 100(1), 34–46. https://doi. org/10.1016/j.marpolbul.2015.08.016.
127. Unsworth, R., Collier, C., Waycott, M., Mckenzie, L., & Cullen-Unsworth, L. (2015). A framework for the resilience of seagrass ecosystems. Marine Pollution Bulletin, 100(1), 34-46. doi: 10.1016/j.marpolbul.2015.08.016.

128. van Dijk, K., Bricker, E., van Tussenbroek, B. I., and Waycott, M. (2018). RangeWide Population Genetic Structure of the Caribbean Marine Angiosperm Thalassia Testudinum. Ecol. Evol. 8, 9478–9490. doi: 10.1002/ece3.4443.
129. Walck, J. L., Baskin, J. M., Baskin, C. C., & Hidayati, S. N. (2005). Defining transient and persistent seed banks in species with pronounced seasonal dormancy and germination patterns. Seed Science Research, 15(3), 189–196. https://doi.org/10.1079/ssr2005209
130. Walker, B., Kinzig, A., & Langridge, J. (1999). Plant Attribute Diversity, Resilience, and Ecosystem Function: The Nature and Significance of Dominant and Minor Species. In Ecosystems (Vol. 2). Springer-Verlag.
131. Waples, R. S., & Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. In Molecular Ecology (Vol. 15, Issue 6, pp. 1419–1439). https://doi.org/10.1111/j.1365-294X.2006.02890.x
132. Watkinson, A. R. (1978). The Demography of a Sand Dune Annual: Vulpia Fasciculata: III. The Dispersal of Seeds. Journal of Ecology, 66(2), 483–498. https://doi.org/10.2307/2259147.
133. Waycott, M., Duarte, C. M., Carruthers, T. J. B., Orth, R. J., Dennison, W. C., Olyarnik, S., et al. (2009). Accelerating Loss of Seagrasses Across the Globe Threatens Coastal Ecosystems. Proc. Natl. Acad. Sci. 106, 12377–12381. doi: 10.1073/pnas.0905620106.
134. Waycott, M., Procaccini, G., Les, D. H., and Reusch, T. B. H. (2006). “Seagrass Evolution, Ecology and Conservation: A Genetic Perspective,” in Seagrasses: Biology, Ecologyand Conservation (Dordrecht: Springer), 25–50. doi: 10.1007/ 978-1-4020-2983-7_2.
135. Wieters, E. A., Medrano, A., & Quiroga, G. (2013). Spatial variation in photosynthetic recovery of intertidal turf algae from acute UVB and temperature stress associated with low tides along the central coast of Chile. Journal of Experimental Marine Biology and Ecology, 449, 340-348. https://doi.org/10.1016/j.jembe.2013.10.008.
136. Zarnetske, P.L., Hacker, S.D., Seabloom, E.W., Ruggiero, P., Killian, J.R., Maddux, T.B. and Cox, D. (2012), Biophysical feedback mediates effects of invasive grasses on coastal dune shape. Ecology, 93: 1439-1450. https://doi.org/10.1890/11-1112.1

8.2 Chapter two – Introduction, Material and Methods, Results, Discussion and Conclusions

1. Alberto, Filipe, Sophie Arnaud-haond, Carlos M. Duarte, and Ester A. Serrão. 2006. “Alberto F, Arnaud-Haond S, Duarte CM, Serrao EA (2006) Genetic Diversity of a Clonal Angiosperm near Its Range Limit the Case of Cymodocea Nodosa at the Canary Islands Mar Ecol-P.” 309:117–29.
2. Alberto, Filipe, Sónia Massa, Pablo Manent, Elena Diaz-Almela, Sophie Arnaud-Haond, Carlos M. Duarte, and Ester A. Serrão. 2008. “Genetic Differentiation and Secondary Contact Zone in the Seagrass Cymodocea Nodosa across the Mediterranean-Atlantic Transition Region.” *Journal of Biogeography* 35(7):1279–94.
3. Alexandra H. Cunha and António Araújo. 2009. “New Distribution Limits of Seagrass Beds in West Africa.” *Journal of Biogeography* 36(8):1619–21.

4. Alexandre, Ana, João Silva, Rogério Ferreira, Diogo Paulo, Ester A. Serrão, and Rui Santos. 2017. “First Description of Seagrass Distribution and Abundance in São Tomé and Príncipe.” *Aquatic Botany* 142(March):48–52.
5. Araújo, Miguel B. and Alejandro Rozenfeld. 2014. “The Geographic Scaling of Biotic Interactions.” *Ecography* 37(5):406–15.
6. Arnaud-Haond, Sophie, Marianna Migliaccio, Elena Diaz-Almela, Sara Teixeira, Mirjiam Susanne van de Vliet, Filipe Alberto, Gabriele Procaccini, Carlos M. Duarte, and Ester A. Serrão. 2007. “Vicariance Patterns in the Mediterranean Sea East West Cleavage and Low.Pdf.”
7. Arriesgado, Dan M., Hiroyuki Kurokochi, Yuichi Nakajima, Yu Matsuki, Wilfredo H. Uy, Miguel D. Fortes, Wilfredo L. Campos, Kazuo Nadaoka, and Chunlan Lian. 2015. “Genetic Diversity and Structure of the Tropical Seagrass Cymodocea Serrulata Spanning Its Central Diversity Hotspot and Range Edge.” *Aquatic Ecology* 49(3):357–72.
8. Assis, Jorge, Pierre Failler, Eliza Fragkopoulou, David Abecasis, Gregoire Touron-Gardic, Aissa Regalla, Ebaye Sidina, Herculano Dinis, and Ester A. Serrao. 2021. “Potential Biodiversity Connectivity in the Network of Marine Protected Areas in Western Africa.” *Frontiers in Marine Science* 8(November):1–12.
9. Assis, Jorge, João Neiva, John J. Bolton, Mark D. Rothman, Licínia Gouveia, Cristina Paulino, Hasliza Mohdnasir, Robert J. Anderson, Maggie M. Reddy, Lineekela Kandjengo, Anja Kreiner, Gareth A. Pearson, and Ester A. Serrão. 2022. “Ocean Currents Shape the Genetic Structure of a Kelp in Southwestern Africa.” *Journal of Biogeography* 49(5):822–35.
10. Assis, Jorge, Ester A. Serrão, Nelson Castilho Coelho, Fernando Tempera, Myriam Valero, and Filipe Alberto. 2018. “Past Climate Changes and Strong Oceanographic Barriers Structured Low-latitude.Pdf.”
11. Baskin, Jerry M. and Carol C. Baskin. 2014. “What Kind of Seed Dormancy Might Palms Have?” *Seed Science Research* 24(1):17–22.
12. Becheler, R., O. Diekmann, C. Hily, Y. Moalic, and S. Arnaud-Haond. 2010. “The Concept of Population in Clonal Organisms: Mosaics of Temporally Colonized Patches Are Forming Highly Diverse Meadows of Zostera Marina in Brittany.” *Molecular Ecology* 19(12):2394–2407.
13. Beck, M. W., K. L. Heck, K. W. Able, D. L. Childers, D. B. Eggleston, B. M. Gillanders, B. Halpern, C. G. Hays, K. Hoshino, T. J. Minello, R. J. Orth, P. F. Sheridan, and M. P. Weinstein. 2001. “The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates.” *BioScience* 51(8):633–41.
14. Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. (1996). GENETIX 4.05, Logiciel Sous Windows TM Pour La Génétique Des Populations.Laboratoire Génome, Populations, Interactions, CNRS UMR 5171 (Montpellier (France): Université de Montpellier II).
15. Belkhir, L.C., Raufaste, N. and Bonhomme, F. (1996-2004) GENETIX 4.05 Logiciel Sous Windows TM Pour la Génétique des Populations. In: Laboratoire Génome P, Interactions, CNRS UMR 5171, editor, Université de Montpellier II, Montpellier

16. Billingham, Martin R., Thorsten B. H. Reusch, Filipe Alberto, and Ester A. Serrão. 2003. "Is Asexual Reproduction More Important at Geographical Limits? A Genetic Study of the Seagrass Zostera Marina in the Ria Formosa, Portugal." *Marine Ecology Progress Series* 265:77–83.
17. Boyé, Aurélien, Olivier Gauthier, Ronan Becheler, Vincent Le Garrec, Christian Hily, Marion Maguer, and Jacques Grall. 2022. "Drivers and Limits of Phenotypic Responses in Vulnerable Seagrass Populations: Zostera Marina in the Intertidal." *Journal of Ecology* 110(1):144–61.
18. Buonomo, Roberto, Jorge Assis, Francisco Fernandes, Aschwin H. Engelen, Laura Airoldi, and Ester A. Serrão. 2016. "Habitat Continuity and Stepping-stone Oceanographic Distances Explain Population.Pdf."
19. Van Buskirk, Josh and Larry B. Crowder. 1994. "Life-History Variation in Marine Turtles Author (s): Josh van Buskirk and Larry B . Crowder Life-History Variation in Marine Turtles." *American Society of Ichthyologists and Herpetologists (ASIH)* 1994(1):66–81.
20. Cabaço, Susana, Óscar Ferreira, and Rui Santos. 2010. "Population Dynamics of the Seagrass Cymodocea Nodosa in Ria Formosa Lagoon Following Inlet Artificial Relocation." *Estuarine, Coastal and Shelf Science* 87(4):510–16.
21. Carton, James A. and Bohua Huang. 1994. "Warm Events in the Tropical Atlantic." *American Meteorological Society* https://news.ge/anakliis-porti-aris-qveynis-momava.
22. Chefaoui, Rosa M., Carlos M. Duarte, Ana I. Tavares, Duarte G. Frade, M. A. Sidi Cheikh, M. Abdoull Ba, and Ester A. Serrao. 2021. "Predicted Regime Shift in the Seagrass Ecosystem of the Gulf of Arguin Driven by Climate Change." *Global Ecology and Conservation* 32(May):e01890.
23. Connolly, Rod M., Timothy M. Smith, Paul S. Maxwell, Andrew D. Olds, Peter I. Macreadie, and Craig D. H. Sherman. 2018. "Highly Disturbed Populations of Seagrass Show Increased Resilience but Lower Genotypic Diversity." *Frontiers in Plant Science* 9(June):1–9.
24. Costanza, Robert, Ralph D'Arge, Rudolf de Groot, Stephen Farber, Monica Grasso, Bruce Hannon, Karin Limburg, Shahid Naeem, Robert V. O'Neill, Jose Paruelo, Robert G. Raskin, Paul Sutton, and Marjan van den Belt. 1997. "The Value of the World's Ecosystem Services and Natural Capital. LK - Https://Royalroads.on.Worldcat.Org/Oclc/4592801201." *Nature TA - TT -* 387(6630):253–60.
25. Cowen, Robert K. and Su Sponaugle. 2009. "Larval Dispersal and Marine Population Connectivity." *Annual Review of Marine Science* 1:443–66.
26. Coyer, J. A., G. Hoarau, W. T. Stam, and J. L. Olsen. 2004. "Geographically Specific Heteroplasmy of Mitochondrial DNA in the Seaweed, Fucus Serratus (Heterokontophyta: Phaeophyceae, Fucales)." *Molecular Ecology* 13(5):1323–26.
27. Coyer, J. a, O. E. Diekmann, G. Procaccini, N. Milchakova, G. a Pearson, W. T. Stam, and J. L. Olsen. 2004. "Population Genetics of Dwarf Eelgrass." 281:51–62.
28. Creed, Joel C., Aschwin H. Engelen, Emanuel C. D'Oliveira, Salomão Bandeira, and Ester A. Serrão. 2016. "First Record of Seagrass in Cape Verde, Eastern Atlantic." *Marine Biodiversity Records* 9(1):1–4.

29. Darnell, Kelly M., Dale M. Booth, Evamaria W. Koch, and Kenneth H. Dunton. 2015. "The Interactive Effects of Water Flow and Reproductive Strategies on Seed and Seedling Dispersal along the Substrate in Two Sub-Tropical Seagrass Species." *Journal of Experimental Marine Biology and Ecology* 471:30–40.
30. De Oliveira, F.E.C., Pirani, J.R. and Giuletti, A.M. (1983) The Brazilian seagrasses. Aquat Bot 16: 251–267.
31. Díaz-Abad, Lucía, Natassia Bacco-Mannina, Fernando Miguel Madeira, João Neiva, Tania Aires, Ester A. Serrao, Aissa Regalla, Ana R. Patrício, and Pedro R. Frade. 2022. "EDNA Metabarcoding for Diet Analyses of Green Sea Turtles (Chelonia Mydas)." *Marine Biology* 169(1):1–12.
32. Diekmann, Onno E. and Ester A. Serrão. 2012. "Range-edge Genetic Diversity Locally Poor Extant Southern Patches Maintain a.Pdf."
33. van Dijk, Jent Kornelis and Brigitta I. van Tussenbroek. 2010. "Clonal Diversity and Structure Related to Habitat of the Marine Angiosperm Thalassia Testudinum along the Atlantic Coast of Mexico." *Aquatic Botany* 92(1):63–69.
34. Dorken, Marcel E. and Christoper G. Eckert. 2001. "Severely Reduced Sexual Reproduction in Northern Populations of a Clonal Plant, Decodon Verticillatus (Lythraceae)." *Journal of Ecology* 89(3):339–50.
35. Duarte, Carlos M., Jens Borum, Frederick T. Short, and Diana I. Walker. 2008. "Seagrass Ecosystems: Their Global Status and Prospects." *Aquatic Ecosystems: Trends and Global Prospects* 281–94.
36. Earl, D.A., and Vonholdt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4 (2), 359-361.
37. Eckert, Christopher G. 2001. "The Loss of Sex in Plants." *Evolutionary Ecology* 15(4–6):501–20.
38. Eckert, Christopher G., Marcel E. Dorken, and Spencer C. H. Barrett. 2016. "Ecological and Evolutionary Consequences of Sexual and Clonal Reproduction in Aquatic Plants." *Aquatic Botany* 135:46–61.
39. Ellstrand, Norman C. and Mikeal L. Roose. 1987. "Patterns of Genotypic Diversity in Clonal Plant Species." *American Journal of Botany* 74(1):123–31.
40. Evanno, G., S. Regnaut, and J. Goudet. 2005. "Detecting the Number of Clusters of Individuals Using the Software STRUCTURE: A Simulation Study." *Molecular Ecology* 14(8):2611–20.
41. Evans, R. D., K. M. McMahon, K. J. van Dijk, K. Dawkins, M. Nilsson Jacobi, and A. Vikrant. 2021. "Identification of Dispersal Barriers for a Colonising Seagrass Using Seascape Genetic Analysis." *Science of the Total Environment* 763:143052.
42. EXCOFFIER, L. and LISCHER, H.E.L. (2010), Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10: 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

43. Goldberg, Emma E. and Russell Lande. 2007. “Species’ Borders and Dispersal Barriers.” *American Naturalist* 170(2):297–304.
44. Guo, S. W., & Thompson, E. A. (1992). Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. Biometrics, 48(2), 361–372. https://doi.org/10.2307/2532296
45. Hancock, Joana M., Safiro Furtado, Sonia Merino, Brendan J. Godley, and Ana Nuno. 2017. “Exploring Drivers and Deterrents of the Illegal Consumption and Trade of Marine Turtle Products in Cape Verde, and Implications for Conservation Planning.” *Oryx* 51(3):428–36.
46. Harwell, Matthew C. and Robert J. Orth. 2002. “2002_Harwell_etal_Long Distance Dispersal Potential in a Marine Marcrophyte.PDF.”
47. Heileman, S. 2009. “I-2 Guinea Current : LME # 28.”
48. Hemingson, Christopher R. and David R. Bellwood. 2018. “Biogeographic Patterns in Major Marine Realms: Function Not Taxonomy Unites Fish Assemblages in Reef, Seagrass and Mangrove Systems.” *Ecography* 41(1):174–82.
49. Hemminga, M., Duarte, C.M. 2000. Seagrass Ecology. Cambridge Univ. Press, Cambridge, in press.
50. Hutchings, L., C. D. van der Lingen, L. J. Shannon, R. J. M. Crawford, H. M. S. Verheye, C. H. Bartholomae, A. K. van der Plas, D. Louw, A. Kreiner, M. Ostrowski, Q. Fidel, R. G. Barlow, T. Lamont, J. Coetzee, F. Shillington, J. Veitch, J. C. Currie, and P. M. S. Monteiro. 2009. “The Benguela Current: An Ecosystem of Four Components.” *Progress in Oceanography* 83(1–4):15–32.
51. Jarvis, Jessie C., Kenneth A. Moore, and W. Judson Kenworthy. 2012. “Characterization and Ecological Implication of Eelgrass Life History Strategies near the Species’ Southern Limit in the Western North Atlantic.” *Marine Ecology Progress Series* 444:43–56.
52. Kendrick, Gary A., Robert J. Orth, John Statton, Renae Hovey, Leonardo Ruiz Montoya, Ryan J. Lowe, Siegfried L. Krauss, and Elizabeth A. Sinclair. 2016. “Demographic and Genetic Connectivity: The Role and Consequences of Reproduction, Dispersal and Recruitment in Seagrasses.” *Biological Reviews* 92(2):921–38.
53. Larkin, Patrick D., Ashley M. Hamilton, Anthony I. Lopez, and Sebastian Rubiano-Rincon. 2020. “How Clone Can You Go? Seedbank Density and a Multiscale Assessment of Genotypic Diversity in the Seagrass Halodule Wrightii.” *Aquatic Botany* 163(May 2019):103207.
54. Larkin, Patrick D., Tabitha J. Maloney, Sebastian Rubiano-Rincon, and Michael M. Barrett. 2017. “A Map-Based Approach to Assessing Genetic Diversity, Structure, and Connectivity in the Seagrass Halodule Wrightii.” *Marine Ecology Progress Series* 567:95–107.
55. Larkin, Patrick, Tabitha Schonacher, Michael Barrett, and Mindy Paturzzio. 2012. “Development and Characterization of Microsatellite Markers for the Seagrass Halodule Wrightii.” *Conservation Genetics Resources* 4(2):511–13.
56. Larkum, Anthony W. D., Robert J. Orth, and Carlos M. Duarte. 2006. *Seagrasses: Biology, Ecology and Conservation*.

57. Les, Donald H., Maryke A. Cleland, and Michelle Waycott. 1997. "Phylogenetic Studies in Alismatidae, II: Evolution of Marine Angiosperms (Seagrasses) and Hydrophily." *Systematic Botany* 22(3):443–63.
58. Lobelle, Delphine, Emma J. Kenyon, Kevan J. Cook, and James C. Bull. 2013. "Local Competition and Metapopulation Processes Drive Long-Term Seagrass-Epiphyte Population Dynamics." *PLoS ONE* 8(2).
59. Lourenço, Carla R., Katy R. Nicastro, Christopher D. McQuaid, Rosa M. Chefaoui, Jorge Assis, Mohammed Z. Taleb, and Gerardo I. Zardi. 2017. "Evidence for Rangewide Panmixia despite Multiple Barriers to Dispersal in a Marine Mussel." *Scientific Reports* 7(1):1–16.
60. Manel, Stéphanie, Nicolas Loiseau, and Oscar Puebla. 2019. "Long-Distance Marine Connectivity: Poorly Understood but Potentially Important." *Trends in Ecology and Evolution* 34(8):688–89.
61. Massa, S.I., Paulino, C.M., Serrão, E.A. et al. Entangled effects of allelic and clonal (genotypic) richness in the resistance and resilience of experimental populations of the seagrass Zostera noltii to diatom invasion. BMC Ecol 13, 39 (2013). https://doi.org/10.1186/1472-6785-13-39
62. McMahon, Kathryn, Kor Jent van Dijk, Leonardo Ruiz-Montoya, Gary A. Kendrick, Siegfried L. Krauss, Michelle Waycott, Jennifer Verduin, Ryan Lowe, John Statton, Eloise Brown, and Carlos Duarte. 2014. "The Movement Ecology of Seagrasses." *Proceedings of the Royal Society B: Biological Sciences* 281(1795).
63. McMillan, Calvin. 1981. "Seed Reserves and Seed Germination for Two Seagrasses, Halodule Wrightii and Syringodium Filiforme, from the Western Atlantic." *Aquatic Botany* 11(C):279–96.
64. McMillan, Calvin. 1987. "Seed Germination and Seedling Morphology of the Seagrass, Halophila Engelmannii (Hydrocharitaceae)." *Aquatic Botany* 28(2):179–88.
65. Murdoch, T.J.T., Glasspool, A.F., Outerbridge, M., Ward, J., Manuel, S., Gray, J., Nash, A., Coates, K.A., Pitt, J., Fourqurean, J.W., others, 2007. Large-scale decline in offshore seagrass meadows in Bermuda. Mar. Ecol. Prog. Ser. 339, 123–130.
66. Olsen, Jeanine L., Wytze T. Stam, James A. Coyer, Thorsten B. H. Reusch, and Martin Billingham. 2004. "Molecular Ecology - 2004 - OLSEN - North Atlantic Phylogeography and Large-scale Population Differentiation of the Seagrass.Pdf."
67. Orth, Robert J., Mark Luckenbach, and Kenneth A. Moore. 1994. "Seed Dispersal in a Marine Macrophyte : Implications for Colonization and Restoration Author (s): Robert J . Orth , Mark Luckenbach , Kenneth A . Moore Reviewed Work (s): Published by : Ecological Society of America Stable URL : Http://Www.Jstor.Org/S." *Ecological Society of America* 75(7):1927–39.
68. Ovenden, Jennifer R., Jess A. T. Morgan, Raewyn Street, Andrew Tobin, Colin Simpfendorfer, William Macbeth, and David Welch. 2011. "Negligible Evidence for Regional Genetic Population Structure for Two Shark Species Rhizoprionodon Acutus (Rüppell, 1837) and Sphyrna Lewini (Griffith & Smith, 1834) with Contrasting Biology." *Marine Biology* 158(7):1497–1509.
69. Palumbi, Stephen R. 2003. "Population Genetics, Demographic Connectivity, and the Design of Marine Reserves." *Ecological Applications* 13(1 SUPPL.):146–58.

70. Petit, Rémy J., Itziar Aguinagalde, Jacques Louis De Beaulieu, Christiane Bittkau, Simon Brewer, Rachid Cheddadi, Richard Ennos, Silvia Fineschi, Delphine Grivet, Martin Lascoux, Aparajita Mohanty, Gerhard Müller-Starck, Brigitte Demesure-Musch, Anna Palmé, Juan Pedro Martín, Sarah Rendell, and Giovanni G. Vendramin. 2003. "Glacial Refugia: Hotspots but Not Melting Pots of Genetic Diversity." *Science* 300(5625):1563–65.
71. Philander, S. G. 2001. "Atlantic Ocean Equatorial Currents." *Encyclopedia of Ocean Sciences* 188–91.
72. Phillips, R.C., 1967. On species of the seagrass, Halodule, in Florida. Bull. Mar. Sci. 17, 672–676.
73. Reusch, T. B. H., W. T. Stam, and J. L. Olsen. 2000. "A Microsatellite-based Estimation of Clonal Diversity and Population Subdivision in.Pdf."
74. Rosenzweig, M.L. (1995) Species Diversity in Space and Time. Cambridge University Press, New York. DOI: 10.1017/CBO9780511623387
75. Ruggiero, M. V., T. B. H. Reusch, and G. Procaccini. 2005. "Local Genetic Structure in a Clonal Dioecious Angiosperm." *Molecular Ecology* 14(4):957–67.
76. Santos, Carmen Ivelize Van-Dúnem do Sacramento Neto dos. 2007. "Comunidades de Macroinvertebrados e Peixes Associadas à Pradaria Marinha de Halodule Wrightii (Ascherson , 1868) Na Laguna Do Mussulo , Angola Carmen Ivelize Van-Dúnem Do Sacramento Neto Dos Santos Dissertação Para Doutoramento (Biologia Marinha e Aqua."
77. Saura, Santiago, Orjan Bodin, and Marie-Josée Fortin. 2014. "EDITOR S CHOICE Stepping Stones Are Crucial for Species Long-distance.Pdf."
78. de Souza, Júlia N., Flávia L. D. Nunes, Carla Zilberberg, Juan A. Sanchez, Alvaro E. Migotto, Bert W. Hoeksema, Xaymara M. Serrano, Andrew C. Baker, and Alberto Lindner. 2017. "Contrasting Patterns of Connectivity among Endemic and Widespread Fire Coral Species (Millepora Spp.) in the Tropical Southwestern Atlantic." *Coral Reefs* 36(3):701–16.
79. Spalding, Mark D., Helen E. Fox, Gerald R. Allen, Nick Davidson, Zach A. Ferdaña, Max Finlayson, Benjamin S. Halpern, Miguel A. Jorge, Al Lombana, Sara A. Lourie, Kirsten D. Martin, Edmund McManus, Jennifer Molnar, Cheri A. Recchia, and James Robertson. 2007. "Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas." *BioScience* 57(7):573–83.
80. Tavares, Ana I., Jorge Assis, A. R. Patrício, Rogério Ferreira, Mohamed Ahmed Sidi Cheikh, Salomão Bandeira, Aissa Regalla, Iderlindo Santos, Maria Potouroglou, Suzana Nicolau, Maria Alexandra Teodósio, Carmen Almada, Rui Santos, Gareth A. Pearson, and Ester A. Serrao. 2022. "Seagrass Connectivity on the West Coast of Africa Supports the Hypothesis of Grazer-Mediated Seed Dispersal." *Frontiers in Marine Science* 9(June):1–13.
81. Teschima, Mariana M., Carla Zilberberg, and Flavia L. D. Nunes. 2021. "Strong Genetic Differentiation Demarks Populations of Favia across Biogeographic Regions of the Atlantic Ocean." *Coral Reefs* 41(3):523–34.

82. Tol, Samantha J., Jessie C. Jarvis, Paul H. York, Bradley C. Congdon, and Robert G. Coles. 2021. “Biotropica - 2021 - Tol - Mutualistic Relationships in Marine Angiosperms Enhanced Germination of Seeds by Mega-herbivores.Pdf.”
83. Ukwe, C. N., C. A. Ibe, B. I. Alo, and K. K. Yumkella. 2003. “Achieving a Paradigm Shift in Environmental and Living Resources Management in the Gulf of Guinea: The Large Marine Ecosystem Approach.” *Marine Pollution Bulletin* 47(1–6):219–25.
84. Vilela, Isabelle, ; Moacyr Araujo, Pedro Tyaquiçã, and Dóris Veleda. 2018. “Empirical Orthogonal Function Analysis of Satellite-Derived Currents in The Tropical Atlantic.” *Tropical Oceanography* 46(2):1–24.
85. Vucetich, John A. and Thomas A. Waite. 2003. “Spatial Patterns of Demography and Genetic Processes across the Species’ Range: Null Hypotheses for Landscape Conservation Genetics.” *Conservation Genetics* 4(5):639–45.
86. Wright, Justin P. and Clive G. Jones. 2006. “The Concept of Organisms as Ecosystem Engineers Ten Years on: Progress, Limitations, and Challenges.” *BioScience* 56(3):203–9.
87. Zimmerman, Richard C., John L. Reguzzoni, Sandy Wyllie-Echeverria, Michael Josselyn, and Randall S. Alberte. 1991. “Assessment of Environmental Suitability for Growth of Zostera Marina L. (Eelgrass) in San Francisco Bay.” *Aquatic Botany* 39(3–4):353–66.

1. Supplementary material

Table 6. Code for each population sampled in this study, integrating geographical locations, location per country, year of collection and geographic coordinates.

Codes	Population	Location	Country	Year of collection	Lat	Long
MAU-MG	MR-MG	Mamghar	Mauritania	-	19,373004	-16,534773
MAU-KJ	MR-KJ	Kiji		-	19,73805	-1649369
MAU-MD	MR-MD	Mojd Dakhna		-	19,84629	-16,61804
MAU-BA	MR-BA	Teichott, Banc d'Arguin		2018	19,48	-16,445
SEN-J	SN	Joal	Senegal	2022	14,159683	-16,860533
SEN-SAL	SN	Saloum		2022	13,758469	-16,667205
SEN-FAD	SN	Fadioth		2017	14,156	-16,841
CV	CV	Gamboa, Praia (Santiago)	Cape Verde	2015	14,912	-23,508
GB-ME	GNB	Meio - Este (Parque Nacional Marinho João Vieira-Poilão)	Guinea-Bissau	2022	10,967711	-15,657463
GB-MO	GNB	Meio - Oeste (Parque Nacional Marinho João Vieira-Poilão)		2022	10,972948	-15,676269
GB-S	GNB	João Vieira - Sede (Parque Nacional Marinho João Vieira-Poilão)		2022	11,051591	-15,645064
GB-ANC	GNB	Ancante		2021	11,3048	-16,398387
GB-CAN	GNB	Ilha do Unhocomo, Canneronho		2021	11,317862	-16,455539
GB-JV	GNB	Ilha João Vieira		2021	11,0317	-15,645437
GB-UNH	GNB	Acampamento, Unhocomo			11,281	-16,382
STP-ABA/PRI	ST-ABA	Abade beach, Pagué, Principe	São Tomé and Principe	2013/2017	1,633	7,455
STP-CAB/GIC	ST-CAB	Cabras Islet		2017, 2021	0,414	6,656
STP-SAN	ST-SAN	Santana		2017	0,255	6,747
GAB	GB	Corisco Bay	Gabon	2015	0,594	9,307
ANG	AO	Mussulo Bay	Angola	2018	-9,006	13,064

Table 7. Primer pairs and characteristics of eight polymorphic microsatellite loci developed from Halodule wrightii.

Locus	**Primer sequence (5' – 3')**	**Size range (bp)**	**Reference**
Hw180	F: GTGGAGGCCGAACTGTATCT R: CGACCTTCATCCTAATCATCG	232-250	Larkin et al., 2012
Hw188	F: ACCTTCATAAATGGCAACTTG R: CAACTTGGTTCTGGTAGTCATC	109-135	Larkin et al., 2012
Hw190	F: ATGACGAATCCCGAGGTAT R: CCAGGTATTGTCGCTTTCA	266-276	Larkin et al., 2012
Hw190b	F: ATGACGAATCCCGAGGTAT R: GTTTCACCCACGTTAAAGCACAAT	120-147	Larkin et al., 2017
Hw196	F: ACAACCTAGATCATCCTCACAC R: AGCAGGAAGTCAAGAGATAGG	178-187	Larkin et al., 2012
Hw200	F: TTATGGGATCTATTTGTGGTCT R: TTTTGCTTTGTAGTCTTGGTG	237-277	Larkin et al., 2012
Hw212	F: ATGGATGTTCATTGAGTTTGAC R: CAAGGCTAAGGTAGTGGACC	280-298	Larkin et al., 2012
Hw228	F: AAGACGGCATTGGAAAATAAG R: GTTTGGTATCATCGGAAGCACTGT	266-290	Larkin et al., 2017

Table 8. Concentrations and volume of PCR reagents for the microsatellite markers. The composition of PCR reagents concentration final concentration Volume (µL) in 15 µL reaction.

Simplex		10ul mix+ 5µl DNA	
	[ ] final	**1x**	**32x**
Buffer (5x)	1x	3	96
MgCl2 (25mM)	2 mM	1,2	38,4
dNTPs (2mM each)	0,07 mM each	1,5	48
Forward (10 uM)	0,05 uM	0,6	19,2
Reverse (10 uM)	0,05 uM	0,6	19,2
Taq (5U/µL)	1 U	0,2	6,4
H2O	-	2,9	92,8
Duplex		10ul mix+ 5ul DNA	
	[ ] final	**1x**	**32x**
Buffer (5x)	1x	3	96
MgCl2 (25mM)	2 mM	1,2	38,4
dNTPs (2mM each)	0,07 mM each	1,5	48
Forward (10 uM)	0,05 uM	0,6	19,2
Reverse (10 uM)	0,05 uM	0,6	19,2
Forward (10 uM)	0,05 uM	0,6	19,2
Reverse (10 uM)	0,05 uM	0,6	19,2
Taq (5U/µL)	1 U	0,4	12,8
H2O	-	1,5	48

Table 9. PCR conditions for the 8 microsatellite markers used during this project performed during laboratory work. The conditions were used for simplex and duplex with previous studies with H. wrightii.

SIMPLEX					
Cycle step	**TºC**	**Time**	**Nº cycles**	**Primer**	**T*a***
Initial desnaturation	95	5 min			
Desnaturation	95	30 sec	35	Hw 212	57
Annealing	Ta	30 sec		Hw 228	55
Extention	72	30 sec			
Final Extention	72	10 min			
Cycle step	**TºC**	**Time**	**Nº cycles**	**Primer**	**T*a***
Initial desnaturation	95	5 min		Hw 196	56
Desnaturation	95	1 min	35	Hw 200	57
Annealing	Ta	1 min			
Extention	72	1 min			
Final Extention	72	10 min			
Note: deixar maq a 10 C					
DUPLEX					
Cycle step	**TºC**	**Time**	**Nº cycles**	**Primer**	**T*a***
Initial desnaturation	95	5 min		Hw 180	53
First desnaturation	95	30 sec	5	Hw 188	
Annealing	Ta	30 sec		Hw 190	
Extention	72	30 sec		Hw 190b	
Second desnaturation	95	30 sec	30		
Annealing	Ta	30 sec			
Extention	72	30 sec			
Final Extention	10	∞			

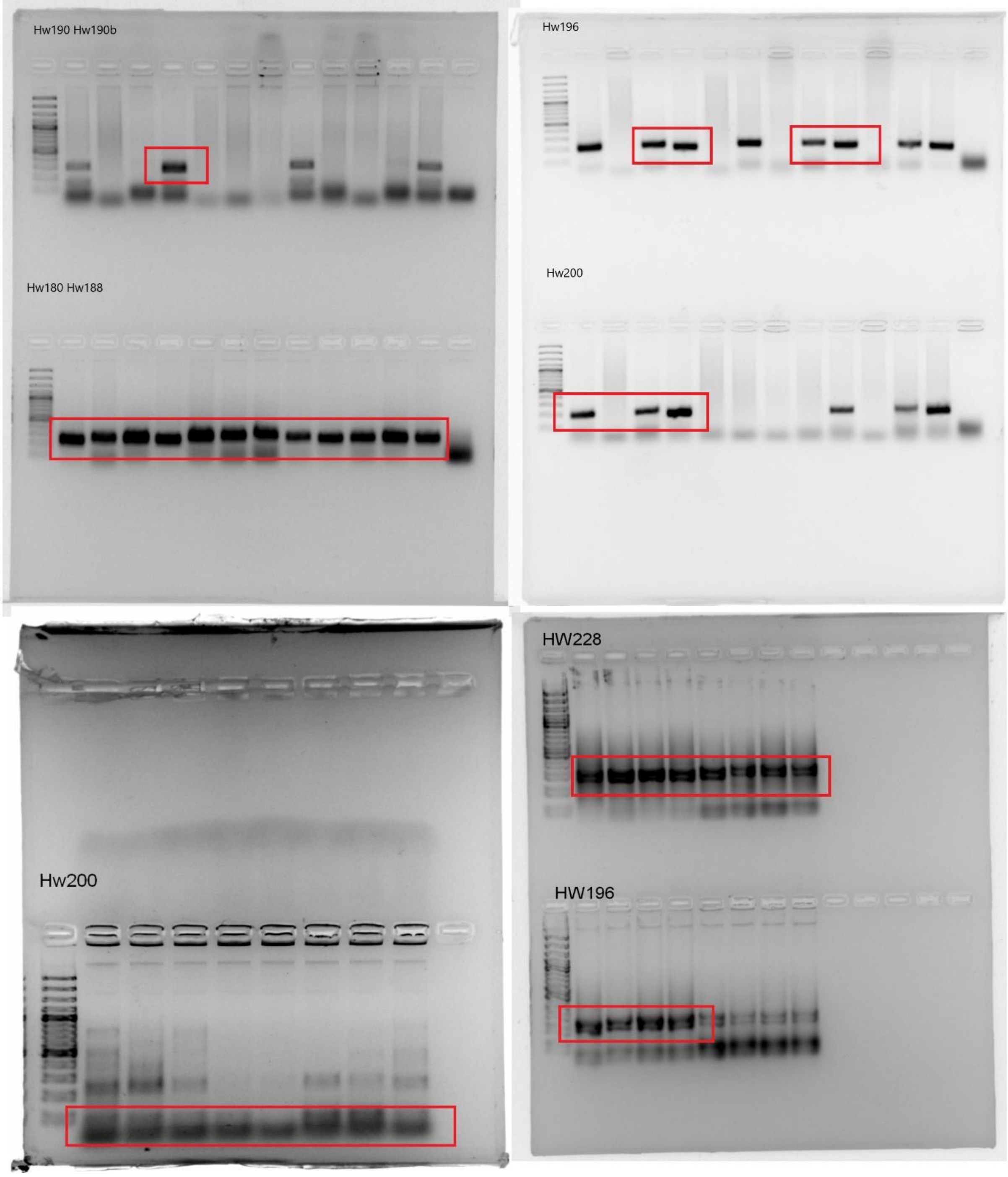

Figure 15. PCR shots of Halodule wrightii sampled in São Tomé and Prícinpe (Cabras islet - STP_GIC) during the laboratory work. The red box highlighted the fragments of subsequent loci, starting above left: HW190 and HW190B; HW 180 and HW188; HW196; HW200; HW228; HW196. Some PCR replications were made to ensure that the markers were highlighted. Note: HW212 missing PCR picture.

Table 10. colour scheme copy for each allele inserted with the specific microsatellite marker with the LIZ 500 on Strand. Note that the numbers in the first column represent where the reads are in the base pairs (bp).

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<tr><th>110</th><th>120</th><th>130</th><th>140</th><th>150</th><th>170</th><th>180</th><th>230</th><th>240</th><th>250</th><th>260</th><th>270</th><th>280</th><th>290</th></tr>
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</table>

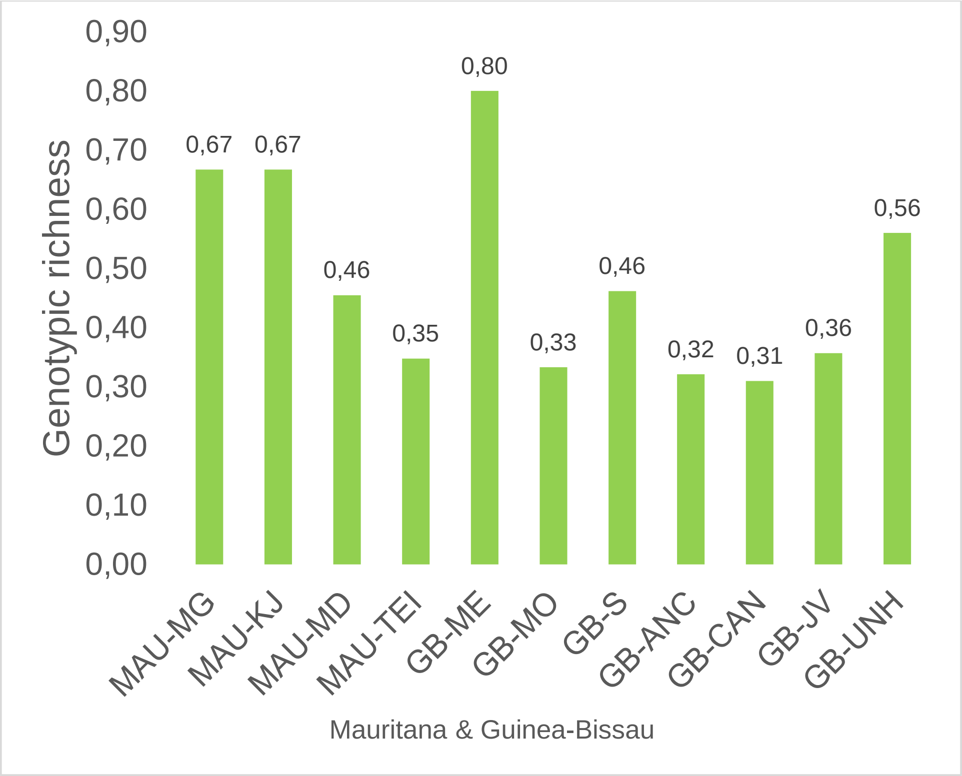

Figure 16. Genotypic richness (R) in bar graphs for the two core populations (Mauritania and Guinea-Bissau). Note that the values did not vary along the divergent places compared (Nearshore vs Offshore; Northern vs Southern). The highest clonal richness is seen in the Southern zone in Meio-Este zone in Guinea-Bissau.

Table 11. quantification of number of Genet (G) to standardize population sample size with a low genet number before comparing the mean number of alleles (A) per locus and estimation among populations with the package StandArich2 in Rstudio 4.1.3.

CODE	G	HW180		HW188		HW190		HW196		HW200		HW212		HW190b		HW228	
MAU_MG	1	234	253	199	199	260	296	999	999	197	212	307	307	999	999	259	276
MAU_MG	2	999	999	199	199	260	296	999	999	197	212	307	307	999	999	259	276
MAU_MG	3	253	253	199	199	260	296	999	999	197	212	307	307	999	999	276	276
MAU_MG	4	253	253	199	199	260	296	999	999	197	212	307	307	999	999	259	276
MAU_MG	5	999	999	199	199	260	296	999	999	212	212	999	999	999	999	276	276
MAU_MG	6	253	253	199	199	260	296	999	999	999	999	307	307	999	999	259	276
MAU_MG	7	253	253	199	199	260	296	999	999	999	999	999	999	999	999	276	276
MAU_KJ	1	253	253	199	199	260	260	999	999	999	999	999	999	999	999	276	276
MAU_KJ	2	253	253	199	199	260	296	999	999	212	212	307	307	999	999	276	276
MAU_KJ	3	253	253	199	199	260	296	999	999	197	212	307	307	999	999	259	276
MAU_KJ	4	253	253	999	999	260	296	999	999	212	212	999	999	999	999	276	276
MAU_KJ	5	253	253	199	199	260	296	999	999	999	999	999	999	999	999	276	276
MAU_KJ	6	253	253	199	199	260	296	999	999	212	212	999	999	999	999	276	276
MAU_KJ	7	253	253	199	199	260	296	999	999	212	212	999	999	999	999	999	999

MAU_MD	1	253	253	199	199	260	296	999	999	197	212	307	307	999	999	259	276
MAU_MD	2	253	253	199	199	260	296	999	999	212	212	307	307	999	999	259	276
MAU_MD	3	253	253	199	199	260	296	999	999	212	212	999	999	999	999	999	999
MAU_MD	4	253	253	199	199	260	296	999	999	197	212	307	307	999	999	276	276
MAU_MD	5	253	253	999	999	260	296	999	999	999	999	307	307	999	999	259	276
MAU_MD	6	999	999	199	199	260	296	999	999	197	212	307	307	999	999	259	276
MAU	1	234	253	199	199	260	296	184	198	212	212	307	307	112	120	276	276
MAU	2	253	253	199	199	260	296	184	198	212	212	307	307	112	120	259	276
MAU	3	253	253	199	199	260	296	198	198	212	212	307	307	112	120	276	276
MAU	4	253	253	199	199	260	296	184	198	212	212	307	307	120	120	259	276
MAU	5	253	253	199	199	260	296	198	198	212	212	307	307	112	120	259	276
MAU	6	253	253	199	199	260	296	184	198	212	212	288	307	112	120	259	276
MAU	7	253	253	199	203	260	296	184	198	212	212	307	307	112	120	259	276
MAU	8	244	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
MAU	9	253	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
SEN-J	1	253	253	199	199	260	260	198	198	212	212	310	310	120	120	259	276
SEN-J	2	253	253	199	199	260	260	198	198	212	212	307	307	120	120	259	276
SEN-J	3	253	253	199	199	260	260	999	999	999	999	999	999	120	120	276	276
SEN-J	4	253	253	199	199	260	260	198	198	999	999	999	999	120	120	259	276
SEN-J	5	253	253	199	199	260	260	198	198	212	212	999	999	120	120	259	276
SEN-J	6	999	999	199	199	260	260	198	198	212	212	307	307	120	120	259	276
SEN-SAL	1	999	999	999	999	999	999	198	198	212	216	307	307	999	999	259	276
SEN-SAL	2	253	253	199	199	260	260	198	198	212	212	307	307	120	120	259	276
SEN-SAL	3	253	256	199	199	260	260	999	999	999	999	999	999	120	120	999	999
SEN-SAL	4	253	256	199	199	260	260	198	198	212	216	999	999	120	120	259	276
SEN-SAL	5	253	256	199	199	260	260	198	198	212	212	999	999	120	120	259	276
SEN-SAL	6	253	253	199	199	260	260	198	198	212	212	999	999	120	120	259	276
SEN-SAL	7	253	253	199	199	260	260	999	999	212	212	999	999	120	120	259	276
SEN-SAL	8	253	253	199	199	260	260	198	198	212	216	999	999	120	120	259	276
SEN-SAL	9	253	256	199	199	260	260	999	999	999	999	999	999	120	120	259	276
SEN-SAL	10	253	253	199	199	999	999	999	999	999	999	999	999	120	120	276	276
SEN-SAL	11	253	256	199	199	999	999	198	198	999	999	999	999	120	120	999	999
SEN-FAD	1	253	253	199	199	260	296	184	198	212	212	307	307	112	120	259	276
SEN-FAD	2	253	253	199	199	260	296	198	198	212	212	291	307	120	120	276	276
SEN-FAD	3	253	253	199	199	260	260	198	198	212	212	307	307	120	120	276	276
SEN-FAD	4	253	253	199	199	260	296	198	198	212	212	307	307	112	120	259	276
SEN-FAD	5	253	253	199	199	260	296	198	198	212	212	307	307	112	120	276	276
SEN-FAD	6	253	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
SEN-FAD	7	253	253	199	199	260	296	198	198	212	212	291	307	112	120	259	276
SEN-FAD	8	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
CV	1	246	253	199	199	260	296	198	198	212	212	307	307	112	120	259	276
CV	2	253	253	199	199	260	296	198	198	212	212	307	307	112	120	259	276
CV	3	246	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
CV	4	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
CV	5	246	253	199	199	260	260	198	198	212	212	307	307	120	120	276	276

CV	6	253	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
CV	7	253	253	199	199	260	260	198	198	212	212	307	307	120	120	259	276
CV	8	253	253	199	199	260	296	184	198	212	212	307	307	120	120	259	276
CV	9	246	253	199	199	260	296	184	198	212	212	307	307	120	120	259	276
GB-ME	1	253	253	199	199	260	260	198	198	212	212	999	999	120	120	259	276
GB-ME	2	253	253	199	199	999	999	999	999	212	212	999	999	120	120	259	276
GB-ME	3	253	253	199	199	999	999	198	198	212	212	999	999	120	120	259	276
GB-ME	4	253	253	199	199	999	999	198	198	999	999	999	999	120	120	999	999
GB-ME	5	253	253	199	199	999	999	198	198	212	212	999	999	999	999	259	276
GB-ME	6	253	253	199	199	999	999	198	198	212	212	999	999	999	999	276	276
GB-ME	7	253	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
GB-ME	8	253	253	199	199	999	999	198	198	999	999	999	999	999	999	276	276
GB-ME	9	253	253	199	199	260	260	999	999	999	999	999	999	120	120	999	999
GB-MO	1	253	253	199	199	260	260	198	198	212	212	307	307	120	120	276	276
GB-MO	2	253	253	199	199	999	999	999	999	212	212	999	999	120	120	999	999
GB-MO	3	253	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
GB-MO	4	253	253	199	199	999	999	198	198	212	212	999	999	120	120	259	276
GB-MO	5	253	253	199	199	260	260	198	198	212	212	307	307	120	120	259	276
GB-MO	6	253	253	199	199	999	999	198	198	212	212	307	307	120	120	259	276
GB-S	1	253	253	199	199	260	296	198	198	212	212	307	307	999	999	259	276
GB-S	2	253	253	199	199	260	296	198	198	212	212	307	307	120	120	259	276
GB-S	3	253	253	199	199	999	999	999	999	212	212	999	999	999	999	276	276
GB-S	4	253	253	199	199	260	296	198	198	212	212	999	999	120	120	259	276
GB-S	5	253	253	199	199	260	260	999	999	999	999	999	999	120	120	999	999
GB-S	6	253	253	199	199	260	296	198	198	999	999	307	307	120	120	999	999
GB-S	7	253	253	199	199	260	260	198	198	212	212	999	999	120	120	259	276
GB-ANC	1	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-ANC	2	999	999	999	999	999	999	198	198	212	212	307	307	999	999	276	276
GB-ANC	3	253	253	199	199	260	296	198	198	212	212	307	307	120	120	999	999
GB-ANC	4	253	253	199	199	260	296	198	198	212	212	307	307	999	999	999	999
GB-ANC	5	234	253	199	199	260	296	198	198	212	212	307	310	999	999	276	276
GB-ANC	6	234	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-ANC	7	253	253	999	999	260	296	198	198	212	212	307	307	999	999	999	999
GB-ANC	8	253	253	199	199	260	296	198	198	212	212	307	310	120	120	276	276
GB-ANC	9	253	253	199	199	999	999	198	198	212	212	307	307	999	999	999	999
GB-ANC	10	253	253	199	199	260	260	198	198	999	999	307	310	999	999	999	999
GB-CAN	1	253	253	199	199	999	999	198	198	212	212	307	310	120	120	276	276
GB-CAN	2	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-CAN	3	234	253	199	199	260	296	198	198	212	212	307	307	999	999	276	276
GB-CAN	4	253	253	199	199	260	296	198	198	212	212	307	310	120	120	276	276
GB-CAN	5	253	253	199	199	999	999	198	198	212	212	307	310	120	120	999	999
GB-CAN	6	999	999	999	999	999	999	198	198	212	212	307	307	999	999	276	276
GB-CAN	7	253	253	199	199	260	296	198	198	212	212	307	313	120	120	276	276
GB-CAN	8	253	253	199	199	999	999	198	198	212	212	307	307	999	999	276	276

GB-CAN	9	999	999	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-CAN	10	253	253	199	199	999	999	198	198	212	212	307	307	120	120	276	276
GB-JV	1	999	999	999	999	999	999	198	198	212	212	307	307	999	999	276	281
GB-JV	2	253	253	199	199	260	296	184	198	212	212	307	307	120	120	276	281
GB-JV	3	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-JV	4	234	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-JV	5	253	253	199	199	260	260	198	198	212	212	307	307	120	120	276	281
GB-JV	6	253	253	199	199	260	296	198	198	197	212	307	307	120	120	276	276
GB-JV	7	234	253	199	199	260	296	198	198	212	212	307	307	112	120	276	276
GB-JV	8	253	253	199	199	260	296	198	198	212	212	307	307	999	999	276	276
GB-JV	9	999	999	999	999	999	999	198	198	212	212	307	307	999	999	276	276
GB-JV	10	253	253	199	199	260	296	198	198	212	212	307	307	999	999	276	281
GB-JV	11	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	281
GB-ACP	1	253	253	199	199	260	296	198	198	212	212	300	307	120	120	276	276
GB-ACP	2	253	253	199	199	260	296	184	198	212	212	307	307	120	120	276	276
GB-ACP	3	253	253	199	199	260	296	198	198	212	212	307	307	120	120	273	276
GB-ACP	4	234	253	199	199	260	296	184	198	212	212	307	310	100	100	276	276
GB-ACP	5	253	253	199	199	260	296	198	198	212	212	307	310	120	120	273	276
GB-ACP	6	253	253	199	199	260	296	184	198	212	212	307	310	120	120	276	276
GB-ACP	7	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-ACP	8	253	253	199	199	260	296	198	198	212	212	307	310	120	120	276	276
GB-ACP	9	234	253	199	199	260	296	198	198	212	212	307	310	120	120	276	276
GB-ACP	10	253	253	199	199	260	296	184	200	212	212	300	307	120	120	276	276
GB-ACP	11	253	253	199	199	260	296	198	198	212	212	307	313	120	120	276	276
GB-ACP	12	253	253	199	199	260	296	198	198	212	212	288	307	120	120	276	276
GB-ACP	13	253	253	199	199	260	296	184	198	212	212	307	313	112	120	273	276
GB-ACP	14	253	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
GB-ACP	15	253	253	199	199	260	296	184	198	212	212	288	307	120	132	276	276
STP-PRI	1	253	253	199	199	260	296	184	198	212	212	307	307	112	120	259	276
STP-PRI	2	253	253	196	199	260	296	184	200	212	212	307	307	120	120	276	276
STP-PRI	3	253	253	196	199	260	296	184	198	212	212	307	307	112	120	259	276
STP-PRI	4	253	253	196	199	260	296	198	198	212	212	307	307	120	120	259	276
STP-CAB	1	253	253	199	199	260	296	184	198	212	212	300	307	112	120	259	276
STP-CAB	2	253	253	199	199	260	296	198	198	212	212	307	313	120	120	276	276
STP-CAB	3	253	253	199	199	260	296	198	198	212	212	300	307	120	120	259	276
STP-CAB	4	253	253	199	199	260	296	184	198	212	212	303	307	112	120	259	276
STP-CAB	5	253	253	194	199	260	296	184	198	212	212	303	303	112	120	259	276
STP-CAB	6	253	253	194	199	260	296	198	198	212	212	307	307	112	120	259	276
STP-CAB	7	253	253	199	199	260	296	184	198	212	212	307	307	112	120	259	276
STP-CAB	8	253	253	199	199	260	296	198	198	212	212	307	307	120	120	273	276
STP-CAB	9	234	253	199	199	260	296	198	198	212	212	307	307	120	120	276	276
STP-CAB	10	253	253	199	199	260	260	999	999	999	999	999	999	120	120	999	999
STP-CAB	11	253	253	199	199	260	260	198	198	999	999	303	307	120	120	276	276
STP-CAB	12	253	253	199	199	260	260	198	198	212	212	303	303	120	120	276	276
STP-CAB	13	253	253	193	199	260	260	198	198	212	212	307	307	120	120	276	276

STP-CAB	14	253	253	199	199	260	260	198	198	212	212	300	303	120	120	276	276
STP-CAB	15	999	999	999	999	260	260	198	198	212	212	303	307	120	120	276	276
STP-CAB	16	253	253	199	199	260	260	198	198	212	212	300	307	120	120	276	276
STP-CAB	17	999	999	199	199	260	260	198	198	212	212	303	303	120	120	276	276
STP-CAB	18	253	253	193	199	260	260	198	198	999	999	303	307	120	120	276	276
STP-CAB	19	253	253	193	199	260	260	198	198	212	212	999	999	120	120	276	276
STP-CAB	20	253	253	199	199	260	260	198	198	212	212	307	307	120	120	276	276
STP-CAB	21	253	253	193	199	260	260	198	198	212	212	999	999	120	120	276	276
STP-CAB	22	253	253	199	199	260	260	198	198	212	212	303	307	120	120	276	276
STP-CAB	23	253	253	199	199	260	260	198	198	212	212	307	307	120	120	999	999
STP-CAB	24	253	253	199	199	260	260	198	198	212	212	999	999	120	120	999	999
STP-CAB	25	253	253	199	199	260	260	198	198	212	212	999	999	120	120	276	276
STP-CAB	26	253	253	193	199	260	260	198	198	212	212	303	307	120	120	276	276
STP-CAB	27	999	999	999	999	260	260	198	198	212	212	300	300	120	120	276	276
STP-SAN	1	253	253	194	194	260	296	198	198	212	212	300	307	120	120	276	276
STP-SAN	2	253	253	194	194	260	296	184	198	212	212	300	307	112	120	259	276
STP-SAN	3	253	253	194	194	260	296	198	198	212	212	300	307	112	120	259	276
STP-SAN	4	253	253	194	194	260	296	198	198	212	212	300	307	120	120	259	276
STP-SAN	5	253	253	194	194	260	296	198	198	212	212	288	300	120	120	276	276
GAB	1	253	253	190	199	260	296	198	198	212	212	300	300	120	120	276	276
GAB	2	253	253	190	199	260	296	184	198	212	212	300	300	120	120	276	276
GAB	3	253	253	199	199	260	296	198	198	212	212	300	307	120	120	259	276
ANG	1	234	253	194	199	260	296	198	198	212	212	300	307	120	120	276	276
ANG	2	253	253	194	199	260	296	198	198	212	212	300	307	120	120	276	276
ANG	3	253	253	194	199	260	296	198	198	212	212	307	307	120	120	276	276
ANG	4	253	253	194	199	260	296	198	198	212	212	300	307	112	120	259	276

Table 12. Pairwise Jost'D differentiation index values. The abbreviations are the same as in the Table 6.

Jost'D	mau_mg	mau_kj	mau_md	mau_tei	gb-me	gb-mo	gb-s	gb-anc	gb-can	gb-jv	gb_unh
mau_mg											
mau_kj	1e-04										
mau_md	-0.0094	0									
mau_tei	1e-04	1e-04	-2e-04								
gb-me	0.004	0.0025	6e-04	0.0051							
gb-mo	0.0037	0.0029	2e-04	0.0041	-0.0048						
gb-s	4e-04	1e-04	-1e-04	5e-04	-0.0032	-0.0035					
gb-anc	0.0022	-7e-04	0.003	0.0082	0.0025	0.0056	0.0011				
gb-can	0.0015	-0.001	0.0017	0.0063	0.0035	0.0086	0.0016	-0.0027			
gb-jv	0.0011	-2e-04	0.0014	0.0041	0.0046	0.0049	8e-04	0	0		
gb_unh	0.0061	0	0.007	0.0049	0.0092	0.0215	0.0099	1e-04	0	0.0015	