UNIVERSIDADE TÉCNICA DO ATLÂNTICO
INSTITUTO DE ENGENHARIA E CIÊNCIAS DO MAR

**WEST AFRICAN SCIENCE SERVICE CENTRE ON CLIMATE CHANGE
AND ADAPTED LAND USE**

Master Thesis

COMPARATIVE STUDY OF SEAGRASS MEADOWS BETWEEN CAPE VERDE AND SENEGAL: HIGHLIGHT TO SEAGRASS STRUCTURE, EPIFAUNA, AND ECOSYSTEM SERVICES

Gnilane Diogoye DIOUF

Master Research Program on Climate Change and Marine Sciences

São Vicente
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**Comparative study of seagrass meadows between Cape Verde (Gamboa Bay) and Senegal
(**Bétenty**): highlight to seagrass structure, epifauna, and ecosystem services**

Gnilane Diogoye DIOUF

Master's thesis presented to obtain the master's degree in Climate Change and Marine Sciences by the Institute of Engineering and Marine Sciences, Atlantic Technical University, in the West African Science Service Centre on Climate Change and Adapted Land Use.

Supervisor

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Gnilane Diogoye DIOUF

Panel defense

President

Examiner 1

Examiner 2

São Vicente
2023

Financial support

Dedication

To all my family members.

Acknowledgements

Resumo

Os ervas marinhas encontram-se na maioria dos mares do mundo, excepto na Antárctida, e fornecem importantes serviços ecossistémicos tanto ao sistema marinho como aos utilizadores locais. Apesar de estudos recentes e esporádicos, a distribuição e ecologia dos ecossistemas de ervas marinhas ao longo da costa da África Ocidental são ainda pouco compreendidas. Neste contexto, este estudo comparou duas comunidades de ervas marinhas no continente da África Ocidental (Bétenty, Senegal) e numa ilha (Baía de Gamboa, Cabo Verde). Foram comparados conjuntos de espécies, biomassa e parâmetros físico-químicos. Ainda foram utilizadas estatísticas descritivas para identificar diferenças nos dados biológicos, de acordo com factores abióticos locais. Três espécies foram analisadas: *Cymodocea nodosa*, *Zostera noltei* e *Halodule wrightii* (a única presente na Baía de Gamboa GB). O limite sul de *Z. noltei* foi confirmado como estando em Bétenty e não no PNBA mauritanico. As espécies mostraram distribuídas de acordo com o tipo de sedimento, que varia de arenoso com a maior granulometria em Gamboa [66,54 a 950,3 µm] e arenoso lamacento em Bétenty. A cobertura e o DW foram mais de 4 e 6 vezes mais elevados, respectivamente, em Bétenty do que na Gamboa. Além disso, as temperaturas, pH e salinidade estavam dentro da tolerância da espécie em ambos os locais, mas a Gamboa apresentou pouca claridade da água e as folhas das ervas marinhas eram minúsculas (LL= 4,1 ± 0,03 versus 6,5 ± 2,4 cm), cobertas por epífitas filamentosas. Elevadas concentrações de nitrato foram encontradas em ambos os locais. Estas diferenças abióticas poderão explicar a ausência das espécies temperadas *C. nodosa* e *Z. noltei* na ilha. As práticas insustentáveis de pesca no caso de Bétenty e urbanização em Gamboa foram identificadas como as principais ameaças.

Palavras-chave: Biomassa, *Halodule wrightii,* Ilha-Vs-continente, Parâmetros físicos, Sedimentos,

Abstract

Seagrass beds are found in most of the world's seas except the Antarctic and provide important ecosystem services to both the marine system and local users. Despite recent and sporadic studies, the distribution and ecology of seagrass ecosystems along the West African coast are still poorly understood. In this context, this study compared two seagrass communities on the West African mainland (Bétenty, Senegal) and on an island (Gamboa Bay, Cape Verde). Species assemblages, biomass and physico-chemical parameters were compared. Descriptive statistics were used to identify differences in biological data according to local abiotic factors. Three species: *Cymodocea nodosa*, *Zostera noltei* and *Halodule wrightii* (the only one present in Gamboa Bay (GB). The southern limit of *Z. noltei* was confirmed to be in Bétenty and not in the PNBA of Mauritania. The species were distributed according to the type of sediment, which was sandy with the largest grain size in Gamboa [66.54 to 950.3 µm] and sandy muddy in Bétenty. Cover and DW were more than 4 and 6 times higher, respectively, in Bétenty than in Gamboa. Furthermore, temperatures, pH and salinity were within species tolerance at both sites, but Gamboa had low water clarity and its seagrass leaves were tiny (LL= 4.1 ± 0.03 versus 6.5 ± 2.4 cm) and covered by filamentous epiphytes. High nitrate concentrations were found at both sites. These abiotic differences could explain the absence of the temperate species *C. nodosa* and *Z. noltei* on the island. Unsustainable fishing practices in the case of Bétenty and urbanization in Gamboa were identified as the main threats.

Keywords: Biomass, *Halodule wrightii,* Island-vs-mainland, Physical parameters, Sediment

Abbreviations and acronyms

AGDW	Above-ground dry weight
BGDW	Below-ground dry weight
DNA	Deoxyribonucleic acid
DNA	Direcao Nacional do Ambiente
DPN	National Parks Department
DW	Dry weight
GB	Gamboa Bay, island setting
GEOMAR	Helmholtz Centre for Ocean Research Kiel
ITCZ	Intertropical Convergence Zone
ITS	Internal Transcribed Spacer
IUCN	International Union for Conservation of Nature
LL	Leave-Length
LW	Leave-width
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
PE	Elution Buffer
PL	Lysis Buffer
PW	Wash Buffer
RAMPAO	Réseau Régional d'Aires Marines Protégées en Afrique de l'Ouest
RNA	Ribonucleic acid
RD	Rhizome diameter
TON	Total Oxidized nitrogen
UCAD	Université Cheikh Anta Diop
UNEP	United Nations Environment Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
UV	Ultraviolet ray
WoRMS	World Register of Marines Species

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1 Introduction

1.1 Background and Context

Seagrasses, or marine angiosperms, are a polyphyletic group of terrestrial origin that have adapted to a submerged lifestyle in marine waters (Waycott et al., 2006). They consist of about 72 species divided into four families with only marine species (Cymodoceaceae, Zosteraceae, Ruppiaceae) and one family with both marine and brackish water species (Hydrocharitaceae) (Short et al., 2011). Seagrass meadows extend from the temperate zones to the equator and are divided into six bioregions: 1. Temperate North Atlantic; 2. Tropical Atlantic; 3. Mediterranean; 4. Temperate North Pacific; 5. Tropical Indo-Pacific; 6. Temperate Southern Oceans (Short et al., 2007). They have a predicted global coverage of about 1,646,788 km^2 (Jayathilake & Costello, 2018), excluding Antarctica and large areas that remain unmapped. The temperate regions are more important in terms of species than the northern subtropics (Orth et al., 2006).

The distribution of seagrasses from temperate to tropical zones may be the result of a variable mode of reproduction, both sexual and asexual (clonal, primary method), with most species being perennial (Larkum et al., 2006). Meanwhile, in annual populations, survival is only ensured by seed recruitment (Chefaoui et al., 2021). Most seagrasses grow in the shallow coastal zone to receive sufficient light, which is the main factor regulating depth distribution (10% irradiance requirement). In addition, several other biotic and abiotic environmental parameters influence their growth and distribution (Greve & Binzer, 2004). Among these main factors are temperature, the productivity determinant (*C. nodosa* in cold water 10-30°C), salinity, the osmotic pressure trigger (in concentration < 60 ‰), nutrient supply for a healthy population structure and finally a suitable substrate (Congdon et al., 2003).

Seagrass meadows are among the most productive areas that support marine organisms and provide several goods and services: as a primary food source for fish and grazers (urchins, sea turtles), protection of the seabed and coastline from erosion, carbon sequestration and valuable nutrient cycling (US $19,000/ha/year) (Gullström et al., 2002) (Gullström et al., 2002). Therefore, estimating these services provides an idea of their contribution to human well-being and contributes to seagrass conservation (Nordlund et al., 2016). Due to their proximity to the coast, seagrass meadows are affected by both anthropogenic and natural stressors. The main anthropogenic threats are nutrient enrichment, algal blooms, sediment disturbance and increased turbidity (Buckee et al., 2021). Natural impacts include overgrazing, disease and desiccation due to drought in intertidal seagrass meadows (De Fouw et al., 2016). Seagrass

meadows have declined by 29% since their area was first recorded in 1879, with an accelerated rate of 7% per year since 1990 (Waycott et al., 2006). This decline may be exacerbated by climate change (Björk et al., 2008). The main impacts are reductions in light availability, growth and productivity problems, and the extinction of species at their thermal tolerance limit due to sea level rise and temperature increase respectively (Turner & Schwarz, 2006).

1.2   Problem Statement

The West African region is part of the Tropical-Atlantic bioregion) and remains the least known in terms of seagrass studies worldwide (Short et al., 2007), with rudimentary publications mainly covering Mauritania (El-Hacen et al., 2020) and few in Senegal (Cunha & Araújo, 2009) and recently Cape Verde (Creed et al., 2016; Martínez-Garrido et al., 2017). Apart from *Ruppia maritima* (only in Cape Verde), three species inhabit the West African coast, including *Halodule wrightii*, *Zostera noltei* and *Cymodocea nodosa* (Short et al., 2007). The first is the common species in this region, sometimes mixed with other seagrass species (Araujo & Campredon, 2018) or often alone (Polkinghome, 2022).

Knowing that (i) Cape Verde and Senegal are only 658.96 miles (1,060.50 km) apart and belong to the same climatic zone, and (ii) that their mean water temperature is within the tolerated range of the three thermohaline and euryhaline tropic species (T > 24ºC) (Short, 2003), (iii) there is nevertheless a noticeable difference in the occurrence of seagrass species between the two locations. Senegal has three species, including two species present in both marine and bay areas from the north (Dakar) to the south (Joal-Fadiouth and the Bamboung-Sourou areas) (Amara et al., 2018; Cunha & Araújo, 2009), with a tendency for the genus *Zostera* to occur in the Saloum Delta (Elouard & Rosso, 1977), recently authenticated (Sidi Cheikh et al., 2023). Gamboa Bay, on the other hand, contains only *H. wrightii*, recently discovered in a scattered and patchy distribution (Creed et al., 2016). Therefore, based on these differences, studying the drivers would help to explain this different seagrass distribution between the two environments and improve the knowledge of this unknown ecosystem in West Africa.

1.3   Research Questions

Many historical and current factors have determined the current distribution patterns of seagrasses as reflected in the six biogeographical regions (Short et al., 2003). Therefore, the following questions helped to identify the nature of the local factors and the settlement pattern of seagrasses on the mainland (Bétenty) and the island (Gamboa Bay) within the same tropical Atlantic bioregion:

2

1- What are the local abiotic and biotic factors that determine the difference in seagrass distribution between the two sites?

2- What are the structural and morphometric differences of the species between the two sites?

3- Is there a difference in the genetic code of the species *Halodule wrightii* common to the two sites?

As a hypothesis:

H0= The biotic and abiotic parameters of the seagrass meadows do not differ between Bétenty (mainland) and Gamboa Bay (island).

H1= The biotic and abiotic parameters of the seagrass meadows differ between Bétenty and Gamboa Bay.

1.4   Relevance and Importance of the research

This research is of particular importance as it aligns with the objectives of exploring the seagrass beds of the most studied West African bioregion in the entire world. The seagrass beds of two sites, Cape Verde (Gamboa Bay) and Senegal (Bétenty) are recently reviewed in detail (Cunha & Araujo, 2009; Potouroglou & Vegh, 2018). Thus, it would be necessary to know the factors that govern their distribution in this West African region, where most species are in their southern or northern distribution limit (Short et al., 2007).

Results from this first comparative study on the West African coast will document the main drivers of the stability of seagrass meadow structure and community between mainland and island settings. Furthermore, this study will highlight the seagrass ecosystem services, the threats and the need for seagrass management in both Cape Verde and Senegal meadows of West Africa.

1.5   Objectives of the work

The main objective was to compare seagrass beds in the mainland (Bétenty) and island (Gamboa Bay) settings by analyzing species, structural similarities, and dissimilarities. As pioneering research on the West African coast, specific objectives were drawn up to seek further justification for similarities and dissimilarities, namely:

- To compare species assemblages between the two settings,
- To conduct an extensive study of biological parameters, namely: seagrass percentage cover, shoot density, epiphytes cover, biomass,

- To document physical and chemical parameters: pH, salinity, temperature, nutrients, water clarity,

- To describe and compare epifauna assemblages,

- To list the ecosystem services provided in each location,

- To undertake an initial DNA barcoding comparison of the common species, *Halodule wrightii*.

1.6   Structure of the work

The work followed the structure below:

- The first section reviewed the literature that provided a general overview of seagrass beds, the gaps in existing knowledge, particularly on the West African coast, and the different parameters determining their global distribution and those within a geographical region.

- The second section described the methods used for data collection to achieve the objectives.

- Results were presented and discussed in the third and fifth sections.

- The final section (four) provided the conclusion and future work recommendations based on the results.

2   Literature review

This section gives a general overview of seagrass beds, including description and classification, ecosystem services, threats, distribution, and the main drivers of dispersal.

    ✓ **Description and functions of seagrass beds**

Seagrass or marine phanerogams are the only angiosperms (Travassos, 2012) adapted to the marine environment (de Los Santos et al., 2013; Waycott et al., 2006). these terrestrial-originated plants (Larkum et al., 2006) evolved in the marine ecosystem through 4 central adaptations such as (i) an anchorage system made up of rhizomes and roots, (ii) air lacunae to supply roots with oxygen, (iii) flowers with hydrophilous pollination and., in some species, (iv) vivipary (Bandeira & Björk, 2001; Duarte et al., 2008). They are classified into 12 genera, 72 species (Short et al., 2011; Björk et al., 2008) and five families inventoried on the whole: Cymodoceaceae, Zosteraceae, Hydrocharitaceae, Posidoniaceae and Ruppiaceae (Duarte et al., 2008; Kuo & den Hartog, 2001). Of the five families, three are only composed of marine phanerogams (Zosteraceae, Cymodoceaceae and Posidoniaceae), and the fourth (Hydrocharitaceae) includes both marine and freshwater species (Larkum et al., 2006; F. Short & Coles, 2001).

Although their few numbers, seagrasses fulfil a critical socioeconomic, ecological and cultural role (Santos et al., 2020; UNEP, 2020) by acting as a nursery grounds, limiting erosion, improving water clarity, giving food security, purifying and oxygenating water (McKenzie et al., 2020; Wallner-Hahn et al., 2022). Their biomass represents a habitat refuge and direct food source for many marine species, some of which have an immediate commercial value (Guidetti & Bussotti, 2000; Santos et al., 2020). Emblematic and Endangered species such as green turtles, seahorses, and marine mammals (manatees) are found in their meadows, giving this ecosystem a *Heritage Status* to be preserved (Hays et al., 2018; Tavares et al., 2022). Moreover, seagrasses can capture and store a large amount of atmospheric carbon or through the water column to build their leaves and roots. At the same time, their sediments are never saturated (McLeod et al., 2011): up to 83 million metric tons of carbon are captured each year (Short et al., 2011), including salt marshes and mangroves (Nordlund et al., 2016). Moreover, seagrass beds bury carbon three times faster than tropical forests (McKenzie et al., 2020).

Seagrasses contribute to 23 ecosystem functions, except pollination; however, only some seagrasses provide all services, not in all bioregions, and only for some seagrass species (Nordlund et al., 2016).

✓ **Disturbance sources of seagrass beds**

The IUCN Red List of Threatened Species stressed that 14% of all seagrass species are at high risk of extinction, and 24% are Threatened or Near Threatened (Short et al., 2011). Seagrass beds have been declining globally at 7% per year since 1990 (Waycott et al., 2006). Human activities, such as clam harvesting (the primary source of threat to intertidal seagrass beds at low tide (Nordlund et al., 2016), land reclamation, and coastal aquaculture and pollution (through nutrients run-off) pose the main anthropogenic threats to seagrass beds (Turner & Schwarz, 2006; Xu et al., 2021).

Climate change, through its global effect, exacerbates the loss of seagrass beds due to rising temperatures (Björk et al., 2008). Thus, the impacts are mainly due to the increase in sea level, which leads to a decrease in light availability resulting: firstly, in more extended immersion periods; secondly, changes in the tidal regime; and finally, in shoreline regression and sediment erosion (Ondiviela et al., 2014). A temperature of 40-45°C might damage the photosynthetic mechanism of tropical seagrasses, while an increase of 5°C leads to a significant loss of *Zostera marina's* shoot density (temperate species) (Björk et al., 2008). On the other hand, long-term climate change, including a steady temperature increase, could affect seagrass beds' growth and productivity over time and even drive the decline of some species at their thermal tolerance limit (Turner & Schwarz, 2006). The threats and loss of seagrass ecosystems on a global scale impact natural resources and people's livelihoods who depend directly or indirectly on these systems (Nordlund et al., 2016).

✓ **Seagrass distribution and drivers**

Covering about 0.1 to 0.2% of the ocean floor (Hemminga & Duarte, 2002), seagrasses worldwide grow on temperate and tropical coasts in salty and brackish waters, typically along gently sloping, protected coastlines (Larkum et al., 2006) except for Antarctic Continent (Short, 2003). At the most fundamental level, the response of seagrass beds to their environment depends on their genetic composition (where high genetic diversity within seagrass beds increases their recovery from temperature extremes (Reusch et al., 2005) and the interactions of these genes with the environment (Procaccini et al., 2007). Interactions with organisms can also affect the distribution and growth of seagrass beds (Greve & Binzer, 2004), where an association with grazing animals could be a source of seagrass dispersal over a wide geographical distribution along the sea current (Tavares et al., 2022). Furthermore, the determinism of the global distribution of seagrass beds depends on the environmental conditions necessary for species development and diversity.

Therefore, seagrasses' salinity range variation is between 5 ‰ and 45 ‰; and can withstand sudden and wide variations depending on the species (Koch et al., 2007). However, salinity values above 60 ‰ can severely affect seagrass beds in shallow waters (Greve & Binzer, 2004). Seagrass beds mainly occur in intertidal and subtidal areas above 12 m depth (where 10% of sunlight's irradiance can reach the water column). However, *Halophila decipiens* has been reported in depths up to 50 m (Short et al., 2007). Therefore, light and temperature are leading factors that drive the productivity and distribution of seagrass (Cuvillier, 2016). They define seagrass extension's depth and geographical growth limits (Greve & Binzer, 2004). Although tolerant to temperature changes (with species' specific tolerance range), temperate seagrasses have an optimal growth range of 11.5°C to 26°C (Greve & Binzer, 2004; Short et al., 2007), whereas tropical seagrasses can be affected at above 43°C can affect tropical seagrasses (Congdon et al., 2003). Although seagrasses can live in nutrient-limited areas (Bertelli et al., 2020; Greve & Binzer, 2004), sediment type also plays a crucial role in regulating seagrass propagation, with soft substrates preference so that allowing rhizome elongation and root establishment (Kenworthy et al., 2018).

Therefore, six bioregions have been identified worldwide: two tropical and four temperate (Short et al., 2007). The tropical bioregions include the Atlantic and the Indo-Pacific (with more than tenth species (Gullström et al., 2002). Meanwhile, the temperate bioregions include the temperate North Atlantic, the temperate North Pacific, the temperate Southern Oceans and the Mediterranean (Short et al., 2007). Western Africa remains the least-known and studied region (Tavares et al., 2022) and has just a handful of publications (Cunha & Araújo, 2009; Potouroglou & Vegh, 2018). Apart from *Ruppia maritima* only found in Cape Verde (Martínez-Garrido et al., 2017), at least one or two species among the three (*Zostera noltei*, *Cymodocea nodosa* and *Halodule wrightii)* are identified in the West African coast (Alexandre et al., 2017; El-Hacen et al., 2020). The latter species is generally common in this Tropical Atlantic bioregion occurring in a single or intermixed species (Cunha & Araujo, 2009). In this carbonate-rich bioregion, species can be found up to 20-50 m depth (Short et al., 2007).

3   Materials and Methods

3.1   Study areas description

The study area covers the shallow coastal waters of two countries on the West African coast, Cape Verde and Senegal (Fig. 1). The latter is the westernmost country in Africa, extending between 12°N and 17°N, and lies along the North Atlantic Ocean (Amara et al., 2018). The Republic of Cape Verde is located further in the central Atlantic Ocean between 16.0021° Latitude North and 24.0132° Longitude West at 570 km from Senegal (Duarte & Romeiras, 2006). Cape Verde archipelago counts ten volcanic islands and eight islets classified into two groups: Windward in the North and Leeward in the South (Duarte & Romeiras, 2006) according to the prevailing winds influenced by the North Equatorial Counter-Current (González, 2018). Senegal is, contrarily, mostly flat without any pronounced relief (Dia, 2012).

Both sites are included in the African Sahelian arid and semi-arid climatic regions, primarily (semi-) arid with two distinct seasons: dry season and wet season (Descroix et al., 2020; Duarte & Romeiras, 2006). Harmattan and trade winds influence the dry season and are (sometimes) accompanied by dust- or sand-laden, hot, dry wind blowing from the southern Sahara Desert (between November and May in Cape Verde). Meanwhile, the wet season depends on the northward movements of the Inter-Tropical Convergence Zone (monsoon) (Descroix et al., 2020; Duarte & Romeiras, 2006). It lasts from June to October in the South of Senegal and one month later (July to September) in the North (Amara et al., 2018). In Cape Verde, on the other hand, it is from July to October in the southwest islands. Annual precipitations are unevenly distributed within the regions of both countries. Thus, rainfalls are from 80–300 mm in the arid coastal zones of Cape Verde to 1200–1600 mm (seldom) in the mountain islands (Duarte & Romeiras, 2006); in Senegal, rainfall increases southward, with 263 mm in the North and more than 1200 mm in the South of the country (Amara et al., 2018). However, in some years, Cape-Verde experiences dry summers resulting from the high- and low-pressure zone oscillations (driven by Azores High) (González, 2018). Therefore, annual and monthly precipitations are usually low with an evaporation excess (Duarte & Romeiras, 2006).

Mean annual temperatures range from 23–27 °C at sea level to 18–20 °C at high altitudes, where the maxima (high as 35–40 °C) occur in inner regions of the arid Eastern Islands (Duarte & Romeiras, 2006). Senegal's temperatures vary from 16°C to 25°C along the coast during the cold season and 20°C to 32°C in the countryside. The rainy season records the highest temperatures from 25°C to 40°C (Amara et al., 2018).

✓ **Particularities of each sampling site**

In each country (described above), a specific seagrass sampling site is chosen, including Gamboa Bay for Cape Verde (island) and Bétenty for Senegal (mainland). A description of some of the distinct features is given below.

The island setting: Gamboa Bay

Gamboa Bay is located in Praia city- Santiago (15°03'N15°03'N 23°39'W23°39'W), the capital of Cabo Verde, the most populated island of the Cabo Verde archipelago (Fig. 1a). The subtidal seagrass bed is near seaside tourism complexes, fishing structures and the production farmer (DNA, 2020). Santiago is part of the Leeward Islands (Duarte & Romeiras, 2006). A *Halodule wrightii* seagrass bed was recently described by Creed et al. (2016) over an area of 4000 m^2, according to DNA (National Environment Directorate) monitoring report in February 2020.

The mainland setting: Bétenty

Bétenty, on the other hand, is a village (within part of a small island) located in the rural zone of the Saloum Delta World Heritage Site (13°35" and 14°10'N14°10'N, 16°50" and 17°00'W17°00'W) (RAMPAO, 2015; UNESCO, 1992) due to its impressive mangrove forests (Ndour et al., 2012) (Fig. 1b). Unlike Gamboa, Bétenty is located approximately 100 km south of the capital Dakar (Dia, 2012) and the presence of two seagrass species was confirmed by Cunha & Araújo in 2009, including Halodule wrightii and Cymodocea nodosa (Cunha & Araújo, 2009). A recent regional project has identified a third species in this area (Delta Saloum National Park (PNDS), although its full extent is still unknown (Cheikh et al., 2012).

At low tide, the area presents a different landscape: an exposed meadow towards the high bank (intertidal) and a submerged meadow (subtidal) on the canal side. In addition, the area is crossed by the Diombos (30 km long), one of the saline tributaries (reverse estuaries with high salinity), including the Saloum (110 km long), the Bandiala (18 km long) (Dia, 2012) as well. The mangrove-seagrass complex is rich in biodiversity and provides an economically attractive environment for local people. The latter depend on this natural resource, with fishing and its by-products being their main activities (DPN, 2021).
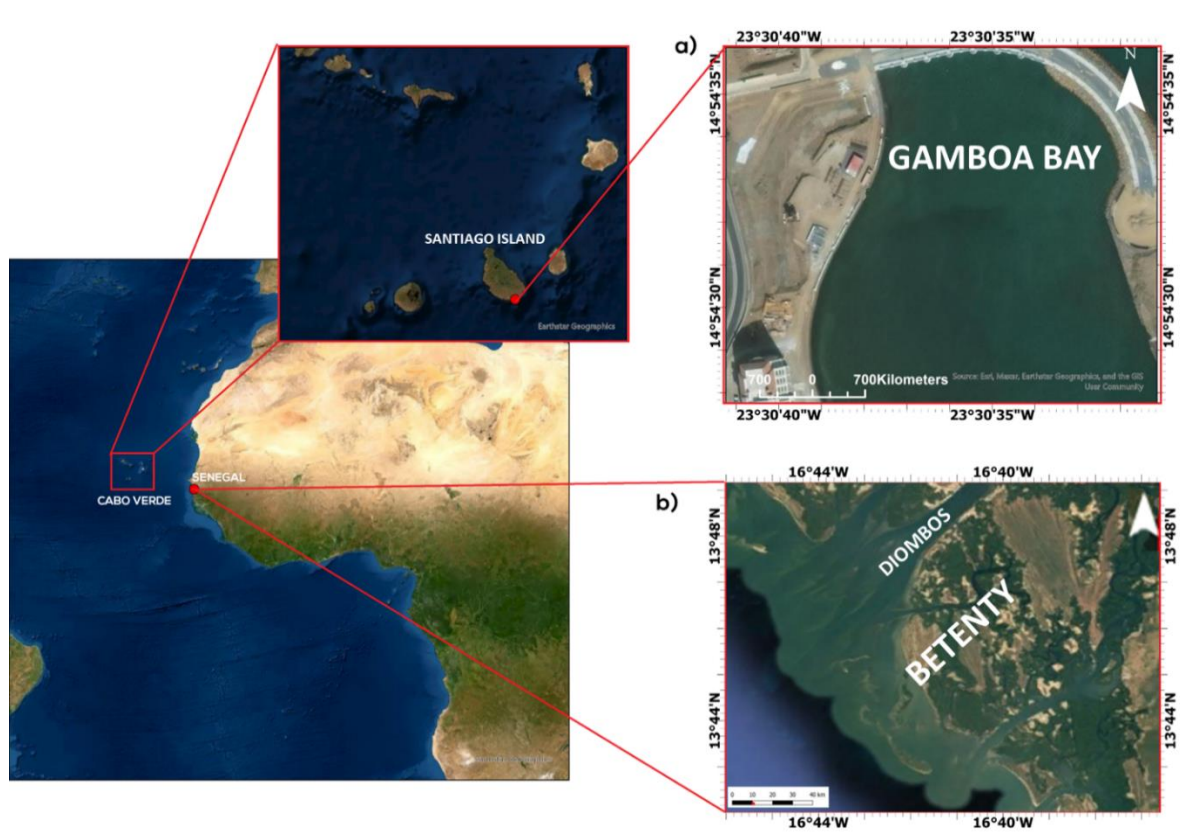
Figure 1: Study area. Map showing the geographical location of (a) Gamboa bay in Santiago Island and (b) Bétenty in the Saloum Delta (with Diombos as one of the three river mouths).

3.2   Sampling design

A field inspection during the post-sampling phase at low tide revealed that the Bétenty area had two parts: a submerged and an emerged part, just as in Gamboa, where the seabed had different levels. Thus, based on this seabed elevation gradient, each meadow was divided into three parts: lower, middle, and upper (correspondences in Table 1 and Fig. 2).

For sampling, 25x25 cm and 20x20 cm quadrats were used (depending on the wanted measurements), and, in each section defined according to the elevation gradient (lower, middle, and lower), six quadrats were randomly launched. Thus, 18 samples were taken in each setting (mainland and island).

Table 1: Corresponding sample points in each meadow, n=6 random thrown quadrats.

Settings	Correspondence of the meadow elevation gradient at low tide.		
	Lower	**Middle**	**Upper**
Bétenty: mainland setting	1.Channel edges	2.Transition zone	3. on the elevated bank
Gamboa Bay: island setting	1. Towards the bridge	2.Center of the meadow	3. Near the rocky shore
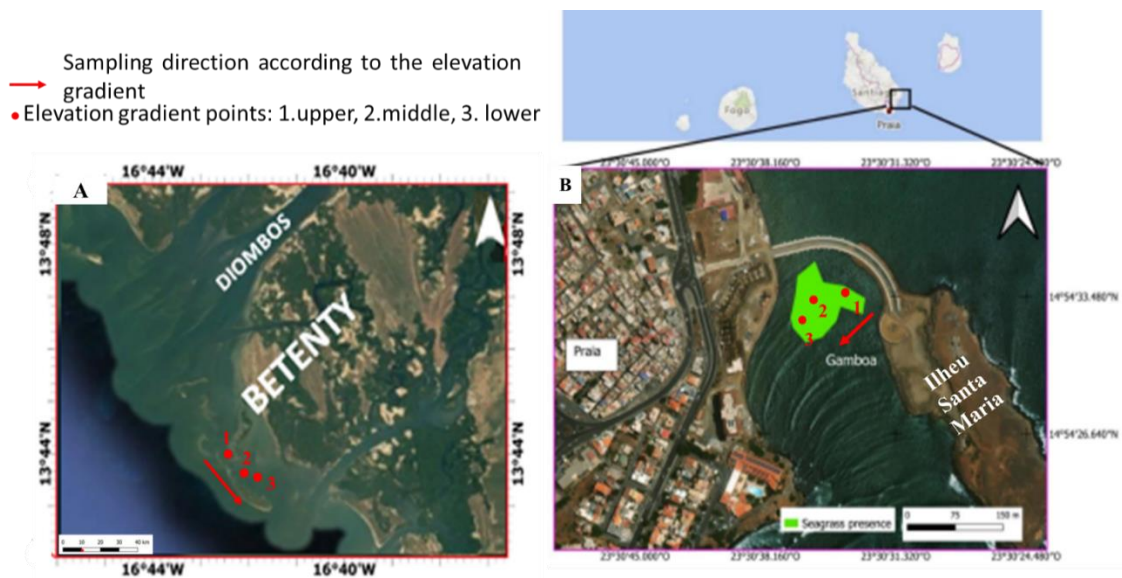
Figure 2: Sampling design according to the elevation gradient (red arrow) at low tide: (A) in Bétenty, mainland setting ; (B) in Gamboa bay, island setting (modified map B, (source: Cheikh et al., 2023).

3.3 Seagrass structural measurements

The study carried out the following structural measurements: percentage seagrass cover, shoot density, biomass, percentage epiphyte cover, morphometry measurement, quantification of associated epifauna and sediment characterization following the adopted seagrass monitoring protocol of McKenzie (2003) (McKenzie et al., 2003). All sampling was conducted during the dry season at low spring tide (end of January, during three days in each site).

✓ **Seagrass species, epiphytes percent cover estimation**

The (global) seagrass percentage cover (covered ground) was estimated by taking the average of the three given values (me and my two companions) in each 25x25 cm quadrat. In the mixed stands of Bétenty, the coverage of each species was deduced from the global seagrass percentage. The percentage cover of epiphytes was measured by estimating the "percentage of total leaf area covered by algal growth" according to the following table proposed by McKenzie (2003) in Figure 3. Therefore, a range [0-10%] was set as the maximum value for a convenient work plan. This assessment did not cover epiphytes species identification. Only identification was made without estimating the percentage cover of drifting (macro) algae associated with seagrasses.
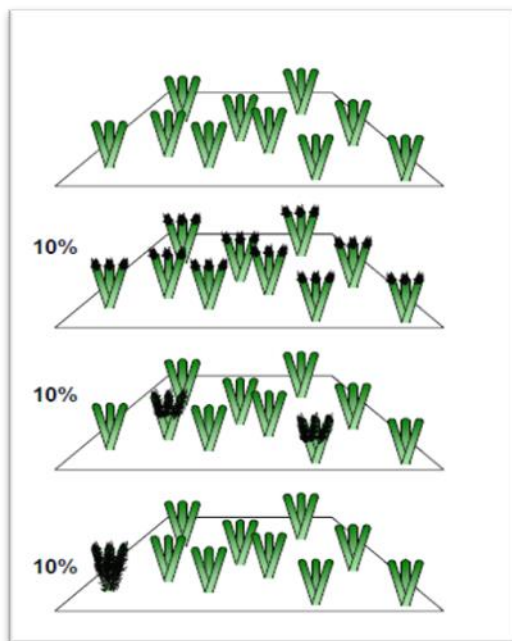
The top quadrat has no epiphytes present.

The second quadrat =10%, of all the leaves in the quadrat, are covered by epiphytes

In the third quadrat =10%, some leaves may be covered by epiphytes, and

In the bottom quadrat =10%, only 1 shoot is totally covered by epiphytes.

Figure 3: Adopted epiphytes' percent cover estimation where 10% set up as maximum. (Source: McKenzie et al., 2003).

✓ **Biomasses quantification**

Shoot density: during this study, shoot density was measured using 20x20 cm quadrats following Hena et al. (2004) methodology (Hena et al., 2004). Five quadrats were used to collect samples. Seagrasses were sampled in each gradient elevation. The shoot density was counted for each quadrat, and the shoot numbers recorded were expressed as density (shoot/m^2). The shoot density was estimated in the mixed stands without separating the different species.

Seagrass biomass: Seagrasses collected by quadrats were rinsed with fresh water to remove epiphytes. Leaves and stems for above-ground biomass were separated from roots and rhizomes for below-ground biomass. There were neither sheaths nor flowers on the seagrass leaves. These samples were then dried in the oven at 65°C for 48 hours to obtain dry weight (gDW). The data were expressed as g/m^2.

✓ **Sediment characterization**

The sediment nature and grain size characterization were according to elevation gradient. Thus, sediment samples (n=18) were collected at about 20 cm depth, labelled (N^o quadrat/site/sampling point/date) and dried in an oven at 60°C for three days (Fig. 4 B).

A standard weight of 100 g of dry soil was placed (on top) in a set of 5 sieves of different diameters stacked in descending order (from top to bottom): 2 mm, 0.5 mm, 0.25 mm, 0.125 mm, 0.063 mm, <0.063 mm (pan) (Fig. 4 A). The set was placed in a mechanical shaker and

stirred for 10 minutes at an amplitude of 1.3 mm (Fig. 4 C). The guide for grain size analysis[1] helped determine the soil weight retained in each sieve. Thus, each sieve's average weight (g) was loaded into the GRADISTAT computer program (Blott & Pye, 2001). GRADISTAT program allowed classification of the nature of the sediment in gravel-sand-mud-silt-clay according to the dominant percentage (%) category. In addition, the diameter size of the soil was also classified by the same program (Appendix A). The cumulative percentile values (the grain size at which a specified percentage of the grains are coarser). However, only D_{10} (grain size at which 10% of the grains are coarser), D_{50} (median), D_{90} (grain size at which 90% of the grains are coarser) and the mean were considered in this study (for simplification).

Figure 4: Sediment size post-processing determination: A: drying labelled sediment samples in the oven, (B) a set of sieves on ascending diameter size; C: disposal for sediment sieving (accurate balance, shaker, amperemeter) (credit photo: G. D. Diouf, 2022).

3.4 Physical and chemical parameters documentation

✓ **Physical parameters: pH, temperature, salinity, Secchi depth**

pH, temperature, and salinity parameters: For this study, salinity, temperature and pH were recorded (3 times) at tide times (low, high) to see their variations during the day. Then, a multi-parameter probe was immersed to a depth of 0.25 m below the water surface.

Secchi depth: For water clarity measurement, a Secchi disk was immersed in the lower, middle, and upper elevation sections during the high tide (3 times) (Fig. 5). This procedure was repeated three times at high tide to have fewer waves influence. Thus, the following formula (Bruckner, 2022) helped estimate the Secchi depth (the depth at which the light rays reach the water column):

$$SD = \frac{M1+M2}{2} \quad (1)$$

Where:

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mac[1] https://www.geoengineer.org/education/laboratory-testing/step-by-step-guide-for-grain-size-analysis.

- SD= Secchi Depth;
- M1= depth at which the Secchi disk is no longer visible when lowered into the water from the shaded side of the boat;
- M2= the depth at which it reappears after being raised.

Figure 5: Lowering of Secchi for Secchi depth measurement in the Bétenty elevated bank during high tide (credit photo: G. D. Diouf, 2022).

✓ **Chemical parameters: nutrients analyses**

Water samples were taken at low and high tides (50 cm depth at high tide). Nutrient analyses were conducted in two laboratories for each site (depending on their expertise). Thus, the obtained nutrients were: ammonia, phosphorus, and nitrates for Bétenty, while in Gamboa Bay, they were TON, phosphate, nitrite and silicate. Therefore, the nitrate and phosphate concentrations were calculated to compare nutrients that exert an important control on primary production (Greve & Binzer, 2004). Based on this, the NO_3: PO_4 ratio was determined based on the Redfield Ratio 16N:1P corresponding to 10 ppm NO_3: 1 ppm PO_4. Therefore, this study's results were compared to the $10NO_3$: $1PO_4$ ratio (see calculation in Appendix B).

3.5 Inventory of the epifauna associated with seagrass meadow

The epifauna associated with the seagrass beds was counted and collected within the quadrats used to estimate the coverage of the seagrass beds. Species were then checked in the species nomenclature of WoRMS[2] (World Register of Marine Species) (WoRMS, 2000) and the Marine Species Identification Portal[3] websites. A list of the encountered species classified according to frequency, family and faunal group helped calculate the biodiversity of epifauna in each area. The different indices calculated were: species richness, Shannon (or Shannon-

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[2] https://www.marinespeciess.org/index.php

[3] http://speciess-identification.org/search.php

Wiener), and evenness according to the formulae in Table 2 below (Davari et al., 2011; Supriatna, 2018).

Table 2: Biodiversity indexes formula: diversity (D), richness (S), and evenness (E) (adapted of Davari et al., 2011 and Supriatna, 2018)

Formula	Component	Index	Definition
$S = \sum_i^1 n$		Richness	-To quantify the number of different species found in particular area.
$H = -\sum (n_i/N) \times ln(n_i/N)$	Shannon-Wiener		-To measure the number of individuals observed for each species in the sample plot.
$D = \sum_{i=0}^{n} n_i(n_i-1) \Big/ N(N-1)$ **Finally: $1-D$**	Simpson (or Gini-Simpson)	Diversity	-To measure the probability that two randomly selected individuals belong to different species.
$E = H/ln(S)$		Evenness	-To measure the abundances of different species in the community.

Where:

- **S***:* The number of different species in the corresponding species list;
- *n_i:* The number of organisms of a particular species *i*;
- *N:* The total number of organisms of all species;
- *Σ*= "sum.", *log:* logarithm.

3.6 Investigating the local functions of seagrass beds

A simplified standard questionnaire was developed for local stakeholders whose primary activities were directly associated with seagrass beds and with expertise in the marine field (Nordlund et al., 2017). This part aimed to inventory the functions played by seagrass beds in each setting and to compare them qualitatively. Thus, face-to-face helped interview fishermen, tourist guides and women fish processors (in Bétenty only). It was supported by a photo of a seagrass meadow and collected species (for quick recognition). Questions included the services perceived by stakeholders and threats (Appendix E).

Thus, starting from the number of fishermen in Gamboa, estimated at 1729 fishermen in Santiago Island (submitted thesis, (Soumah, 2021), the Yamane[4] method was applied (equations 2-3) for the sample size calculation. Thus, 30 fishermen were interviewed in each area. However, since both men and women exploited fisheries resources in Bétenty, the sample size included 20 fishermen and ten women seashells harvesters.

$$n = \frac{N}{1} + N \times e^2 \qquad (2)$$

$$s = \frac{n}{3} \qquad (3)$$

Where:

- **n**=the sample size;
- **N**= the general population;
- **e**= the margin error (0.1,0.05 or 0.01). With a margin of error of 10% and a confidence level of 90%;
- *s*=one-third of the sample size **n**.

3.7 DNA barcoding procedure

This section focused on the seagrass *H. wrightii*, the common species in both sampling areas (Gamboa and Bétenty). These seagrass species were sampled and preserved in silica gel until laboratory analysis at GEOMAR Helmholtz Centre for Ocean Research, Department of Marine Ecology/Marine Evolutionary Ecology. Genetic studies involved understanding the barcoding procedure to highlight similarities and dissimilarities (nucleotide-nucleotide alignments) between *H. wrightii* DNA sequences from the areas. The barcoding procedure was divided into two main phases: DNA extraction and PCR sequencing.

✓ **First step: DNA extraction with the new NucleoSpin protocol**

The plant samples were homogenized by mechanical treatment and then the DNA was extracted with Lysis Buffers based on Macherey-Nagel protocol using NucleoSpin Plant kits (Macherey-Nagel, 2014) following different steps:

- Mechanical sample homogenization (up to 20 mg dry weight leaves),
- DNA extraction with Lysis Buffer PL1, PL2

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[4] HOW TO CALCULATE A RELIABLE SAMPLE SIZE USING TARO YAMANE METHOD | UniProjectMaterials Blog

- Clarification of crude lysates using the NucleoSpin Plant Filters (to remove polysaccharides, contaminations, and residual cellular debris),
- DNA binding to the silica membrane using Buffer PC,
- Silica membrane washing and drying using Buffers PW1 and PW2,
- Genomic DNA elution with Buffer PE.
- The eluted is ready for subsequent reactions like PCR (see the protocol in Appendix C) (Fig.6 A, B).

Figure 6: Material for genomic DNA extraction of seagrass plants: (A) Buffers, (B) Eluded DNA, (C) agarose gel electrophoresis (credit photo: G. D. Diouf, 2022).

✓ **Second step: Control of DNA extraction**

The NanoDrop, a spectral photometric, helped measure the DNA concentration. The quality of the DNA was checked via agarose gel electrophoresis (Fig. 6 C). Therefore, the DNA was stained with GelRed, a fluorescent dye, to make it visible under UV light.

✓ **Fourth step: PCR sequencing**

The PCR allowed the making of copies (amplification) of the DNA. For that, three ITS (Internal transcribed spacer) primers (ITS1, ITS4, and ITS2) composed two Mastermixes production (ITS1+ITS2) and (ITS1+ITS4). The PCR was carried out according to White's methodology (White et al., 1990) following the thermocycling program in Table 3.

Table 3: DNA amplification thermocycling program

cycle:	°C	Min
Initial denaturation	94	3 min
Denaturing	94	0:20
Annealing	55	0:20
Extension	72	0:40
N° cycles	34	

The DNA molecules were separated according to their molecular weight (=length) over 100 base pairs (Fig.7). As the fragments in Fig. 7 A (circled in red) were unreadable, a pure PCR

product was required to obtain specific, substantial and high-quality sequencing data. Thus, the BigDye function (with multiple reagents) helped produce short and readable fragments of the PCR product. Subsequently, the PCR amplification series (34 times) made exponentially more exact copies of the target DNA in the Sanger cycle (for strand replication) (see table 3).
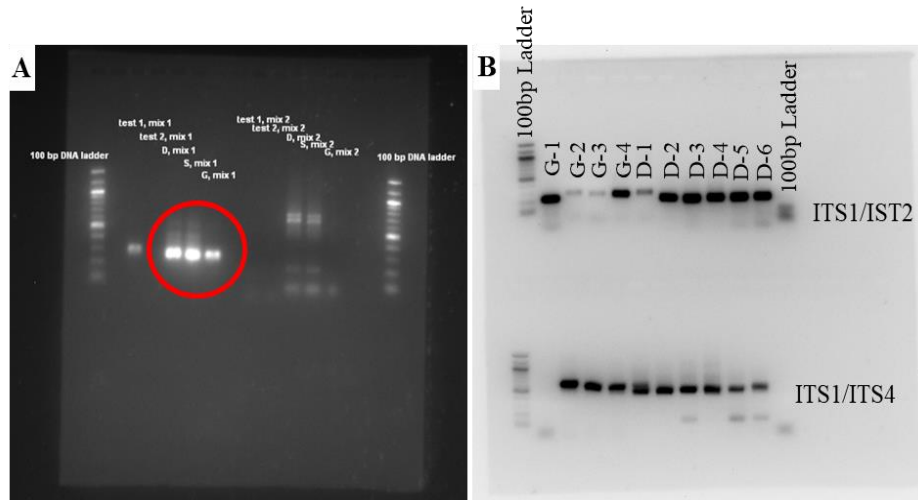
Figure 7: PCR amplification of segments of DNA gene from seagrass: (A) first batch samples with unreadable sequences (in red cercle); (B) second batch samples with good results. Areas of samplings: S: subtidal area of Bétenty; D: intertidal area of Bétenty; G: Gamboa Bay. *Mix:* Mastermix, *ITS 1,2, 4*: primers.

✓ Fifth step: Reading DNA sequences

Of 22 sample tubes that went through the PCR purification and the Sanger cycling phase, only 16 contained pure and good DNA sequences. For the six samples tubes that didn't work, the reasons were: (i) some sequences were too short to be interpreted, (ii) the large fragments with double bands in the PCR amplification screen (Fig.7) were not readable by the *CodonCode Aligner* software. The latter was used to check the alignment of sequences (Fig. 8). The selected lines of sequences were therefore uploaded into the *NCBI* (National Center for Biotechnology

Information) *BLAST* (nucleotide-nucleotide alignments) database for sequences query of high similarities.

Figure 8: Visualization of DNA sequences of seagrass plants through *Codon Code Align* software.

3.8   Data analysis

The Mann-Whitney non-parametric test, also known as the U test, helped to compare the differences in the biological variable between Gamboa bay and Diombos estuary after violating the parametric test assumption, which requires normal distribution (**p >0.05**). The data analysis consisted of descriptive statistics, with emphasis on the determination of the mean and standard deviation of each biological variable. The data analysis was performed using GraphPad Prism 8 software (Mavrevski et al., 2018). On the other hand, the seagrasses' biometrics results and the daily physical and chemical parameters measurements were presented as **mean ± sd** except for the pH, which calculated its **range**. Excel software helped to process data from the questionnaire.

4   Results

This study covered four main sections: (i) seagrass structure highlight to standard seagrass monitoring parameters such as biomass, shoot density, and seagrass percentage cover. On the other hand, (ii) epifauna and elements of ecosystem services, (iii) physical and chemical parameters that might have influenced both structure and fauna assemblages, and (iv) barcoding development of *Halodule wrightii* analyses were also carried out.

4.1   Overview of the seagrass species assemblage between the mainland (Bétenty) and island (GB) settings

Three species of seagrass were identified in this study: Halodule wrightii, Cymodocea nodosa and Zostera noltei. These three-seagrass species formed a mixed meadow at Bétenty, with both subtidal (along the channel edges) and intertidal meadows (Fig. 9 B). The species distribution was therefore different between the two landscapes (see Appendix D).

In GB, *H. wrightii* was the only species found in a subtidal meadow (Fig. 9 A). In both study areas, the seagrass bed was protected on the island side by the islet of "*Ilheu Santa Maria*" to the south, which sheltered it from the sea waves, and by the mangrove plantations surrounding the bed to the north and north-east, and by the islets ("*Ile aux Oiseaux*", "*Ile aux Boeufs*") to the south-east and west of Bétenty (on the mainland setting) (see Fig. 2 in the Methodology section).
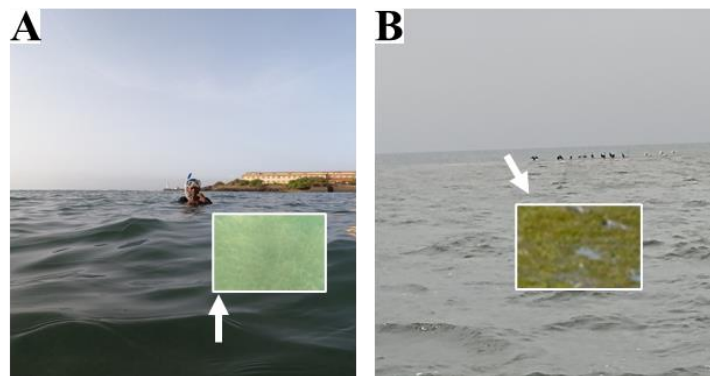
Figure 9: Meadow topography: (A) subtidal meadow at Gamboa Bay, (B) intertidal meadow at Bétenty (on the elevated bank during rising tide) (credit photo: G. D. Diouf, 2022).

4.2   Comparing the DNA barcoding outputs of the common species Halodule wrightii

The barcoding comparison of the sequences of the common species (*H. wrightii*) with the nucleotides (nucleotide-nucleotide alignments) in the NCBI BLAST database using ITS primers showed five samples_ID with high similar sequences query: two from GB and three

from Bétenty (Table 4). The samples were therefore of the same genus, **Halodule**, with the nucleotide *H. wrightii* known from Blast, but the species identification was inconclusive because the sequences with the highest hit probability also selected three species, *Halodule wrightii*, *Halodule pinifolia* and *Halodule uninervis*, with identical scores (Table 4).

Table 4: Sequences producing significant alignments from highly similar sequences query (according to *NCBI Blast* database (selection of megablast). Samples from G= Gamboa Bay; D= Bétenty

16 tubes with pure and good DNA sequences: 9 from Bétenty, 7 from GB								
Area	Samples_ID with high similar sequences query	Max Score	Total Score	Percent Identity	*E-value*	Query-cover	Query length	similar species selected
GB Bay	D01_**G1**_ITS1	117	234	81.10%	1 e $^{-21}$	72%	444	*Halodule wrightii* = *Halodule pinifolia* = *Halodule uninervis*
	C01_**G3**_1(2)	111	111	91.36%	2 e $^{-20}$	40%	202	
	C01_**D1**_ITS1	108	108	90.12%	7 e $^{-19}$	20%	393	
Bétenty	C01_**D1**_ITS1	108	108	20%	7e $^{-19}$	90.12%	310	
	H05_**D2**_4 (2)	418	418	84.86%	4 e $^{-112}$	71%	601	

4.3 Species distribution comparison between the mainland (Bétenty) and island (GB) settings

The 18 sampled quadrats covered a total area of around 3000 m^2. The GB meadow was sparsely covered with seagrass (cover rank = [5-30%]) (Fig. 10 B), with the sole presence of *H. wrightii*. For the species distribution within the meadow, the central part of the GB meadow recorded the highest cover (cover max=30%), while the edges (near the rocky shore and towards the bridge) were quasi-unvegetated (cover max=5%) (Fig. 10 B). On the other hand, Bétenty showed a percentage cover varying from [30 to 85%] (Fig. 10 A) and, according to the gradient of the seabed elevation, *Z. noltei* was mainly present in the intertidal (exposed) bank (mean cover = 30.8 ± 22.8%) (Fig. 10 C, Appendix D), whereas *C. nodosa* was permanently submerged in the channel or some pools of the intertidal zone of Bétenty (mean cover = 8.6 ± 14.6%). *H. wrightii* was mixed with both species on this side. The global mean seagrass cover was more than 4 times higher (*p < 0.0001*) in Bétenty (54.4 ± 17.6%) compared to GB (11.5 ± 10.8%) where the canopy was 3 times lower (8.9 ± 1.5 cm and 3.8 ± 2.1 cm, *p < 0.0001*).

In comparison, the mean cover of the common species *H. wrightii* was not statistically different between the two sites ($p = 0.34$, 15± 13% in Bétenty and 11.1± 10.8% in GB), nor was shoot density ($p = 0.31$, 825± 617 shoots/m^2 in Bétenty and 615.1± 585.6 shoots/m^2 in GB). However, leaf length (LL) was greater in Bétenty than in GB ($p= 3.106^{-7}$, 6.5 ± 2.4 and 4.1 ± 1.9 cm, respectively).
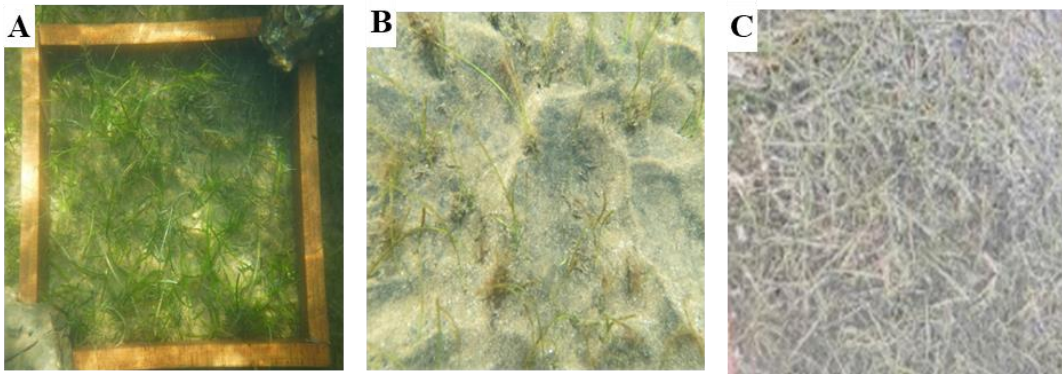

Figure 10: Seagrass meadow appearance at low tide: (A) submerged seagrass beds at the edge of Bétenty channel, (B) submerged seagrass bed in Gamboa Bay (near the constructed bridge), (C) exposed seagrass beds on the intertidal bank of Bétenty (credit photo: G. D. Diouf, 2022).

4.4 Comparing bio-metrics between the mainland (Bétenty) and island settings (GB)

Of the five random quadrats sampled at each site (different from the six in each depth section) to compare the biomass of the two seagrass meadows, DW was 6 times higher in Bétenty than in GB (68.54 ± 18.67 and 11.15 ± 9.30 gDW.m^{-2}, respectively). In both meadows, the BGDW biomass surpassed the AGDW, especially in GB, where was 10 times higher. Concerning the morphometrics comparison, *H. wrightii'* specimen in this bay were tinier (leaf width = <0.1 ± 0.06; rhizome diameter = 0.75 ± 0.25 mm, large internodes (2.83 ± 0.08 cm) (Table 5) and its leaves were covered with a mat of filamentous algae or epibionts (unidentified) at 9.44 ± 0.96% (out of a maximum of 10%) compared to Bétenty (7.2 ± 4.6% (Fig. 11).

Table 5: Biometrics measurements comparison between mainland (Bétenty) and island (GB= Gamboa Bay) settings.

Sites	Bétenty			GB
Mean values	*Cymodocea nodosa*	*Zostera noltei*	*Halodule wrightii*	*Halodule wrightii*
Leaf- length (cm)	12 ± 0.04	8 ± 0.02	6.5 ± 2.4	4,1 ± 0.03
Leaf width (mm)	0,3 ± 0.41	0,1 ± 0.58	Around 0.1 ± 0.15	Les 0,1 ± 0.06
Rhizome diameter (mm)	2.06 ± 0.04	2 ± 0.04	1 ± 0.08	0.75 ± 0.25
Internode (cm)	2.05 ± 0.08	2.21 ± 0.06	2.45 ± 0.12	2.83 ± 0.08
Shoots density (shoots·m^{-2})		1855 ±376.96		310 ±164
AGDW (gDW·m^{-2})		25.55 ±6.11		1.30 ±1.2
BGDW (gDW·m^{-2})		42.15 ±12.98		11.15 ±10.43
DW (gDW·m^{-2})		68.54 ±18.67		11.20 ±9.30

Figure 11: Nature of the epiphytes. (A) epiphytes algae or epibionts in Bétenty (Bétenty mainland setting), and (B) Epiphytic mat dust-like laying on the seagrass blades in GB (Island setting) (credit photo: G. D. Diouf, 2022).

- **Comparing the type of sediment between the mainland (Bétenty) and island (GB) settings**

The sediment was predominantly sandy except for the muddy-sand intertidal bank of Bétenty. There was no gravel or clay found at either site (Fig. 12). The sediment at GB was +50% 'very fine sand' [low diameter size =0.063-0.125 mm] especially in the stable and more covered middle part of the meadow, and less deep with clear water (Secchi depth = 1.55 ± 0.02 m/depth >=5 m at high tide) (Table 6). Conversely, the quasi-unvegetated extremity close to the bridge (the deepest part) possessed the higher sediment diameter sizes: 14% 'medium sand' ([0.25 mm-0.5 mm]) mixed with 9.5% "very coarse sand" ([>1 to 2 mm]) (Fig. 12 B) with less water transparency (Secchi depth=1.25 m/depth > 5m).
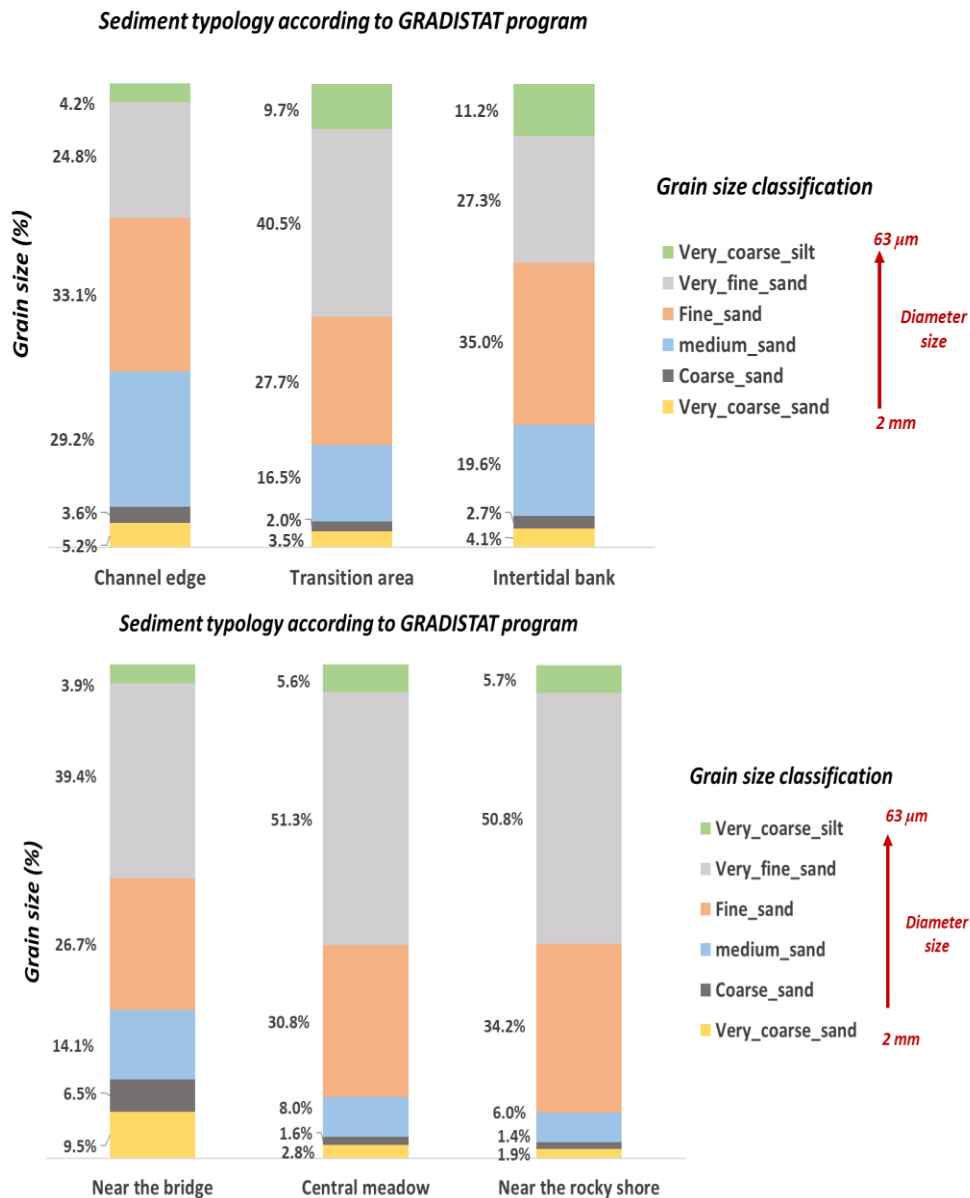

Figure 12: Sediment nature classification based on grain size diameter. (A) Bétenty: sandy-muddy sediment, (B) Gamboa Bay (GB): sandy sediment.

Table 6: Comparative data of the Secchi depth *obtained* during high tide in Bétenty mainland and GB (Gamboa Bay) island settings.

Elevation gradient	Secchi depth (m)		Observations
	Bétenty (mainland)	GB (island)	
Lower	1.85 ± 0.06	1.25 ± 0.02	**Bétenty**= Channel edge: the most profound part and less transparent (>=5 m depth). **GB**= Near the bridge: the deepest and less clear part.
Middle	1.99 ± 0.02	1.55 ± 0.02	**Bétenty**=Transitional area: deepest part before the elevated bank. **GB**=Central meadow, water was more transparent in this part.
Upper	2.1 ± 0.36	1.08 ± 0.12	**Bétenty**=On the top of the elevated bank (3 m depth): Secchi disc touched the bottom while remaining visible contrarily to the channel. **GB**= Near the rocky shoreline and less deep part (~4 m).

Comparing the two settings, the largest sediment size was found at GB, ranging from 66.54 µm (D_{10}) to 950.3 µm (D_{90}), and slightly higher than Bétenty (sediment size range= 61.2- 503.3 µm). In the intertidal zone of Bétenty, 11.2% of "very coarse silt" and 35% of "fine sand" made the sediment sand-muddy, where *Z. noltei* was restricted (Fig. 12 A). In this part, the Secchi depth reached 2.1 ± 0.36 m for a depth of 4 m (±0.5) at high tide (Table 5). The little amount of "very coarse sand" [>1 to 2 mm] was found mainly in the deepest parts of both sites consisting of small rocks at GB (near the bridge) (Fig. 13 A) and empty shells at Bétenty (channel edges) (Fig. 13 B).
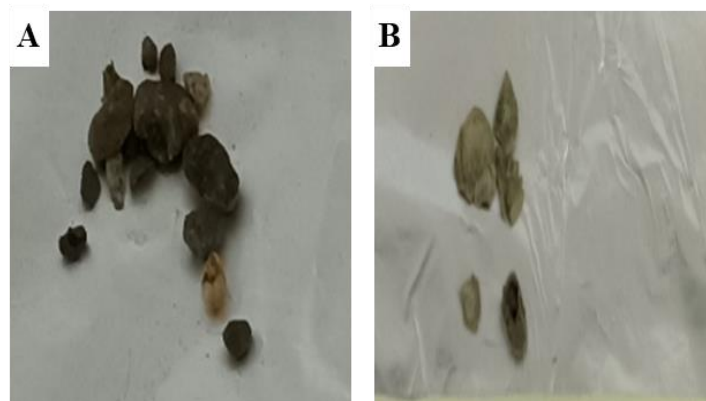

Figure 13: Retained sediment in the largest sieve diameter very coarse sand ([>1 to 2 mm]) composed of (A) small rocks in Gamboa Bay, (B) mollusk shells at Bétenty) (credit photo: G. D. Diouf, 2022).

4.5 Comparing physical and chemical parameters analysis outputs between the mainland (Bétenty) and the island (GB bay)

The high salinity and pH values were characteristic of a bay (GB) and an estuary (Bétenty) while the latter had the highest value (38‰). The water parameters showed the same trend changes during the tidal periods in both sites: high temperatures during high tides (the sunny part of the day) coinciding with low pH values. Salinity remained unchanged. More nutrients were discharged at high tide, especially the Nitrate concentrations 10 times higher at Bétenty than that GB (19.3 ±0.19 and 1.85 ±0.25, respectively). Thus, compared to the calculated standard $10NO_3:1PO_4$ ratio, GB had low ratios (Table 7).

Table 7: Water parameters (physical and chemical) analysis results in Bétenty and Gamboa Bay (GB).

Study areas	Tides	Physical parameters			Chemical parameters		
		pH	T (°C)	Salinity (‰)	Nitrate (mg/L)	Phosphate (mg/L)	$10NO_3/1PO_4$
Bétenty (mainland)	Low tide	(8.3-8.9)	23.13± 0.23	38	10.5 ±0.15	3.28±0.02	11/3
	High tide	(8-8.3)	25.97 ± 0.89	38	19.3 ±0.19	3.03 ±0.07	19/3
GB (island)	Low tide	(8.4-8.8)	22.8 ± 0.12	36	1.2 ±0.26	0.06 ±0.01	1/ 0.06
	High tide	(8.3-8.6)	24.8 ± 0.12	36	1.85 ±0.25	0.05 ±0.04	2/0.05

(Mean value (±sd) except pH (median))

4.6 Biotic parameters analysis outputs a comparison between the mainland (Bétenty) and the island (GB)

In both areas, the seagrass meadows were associated with a common flora and different fauna. Thus, the algal species found in both meadows were those drifting or settling on the seagrass' leaves at low tide (more visible in the intertidal zone of Bétenty), including *Dictyota sp.* and *Caulerpa sp.* (Fig. 15). The identification of associated fauna was limited to species crawling on the ground or buried in the substrate, the epifauna. Thus, the species list included

several groups such as Bivalves, Echinoderms (mainly snails) and Crustaceans. The latter was only observed at Bétenty (Fig. 14).
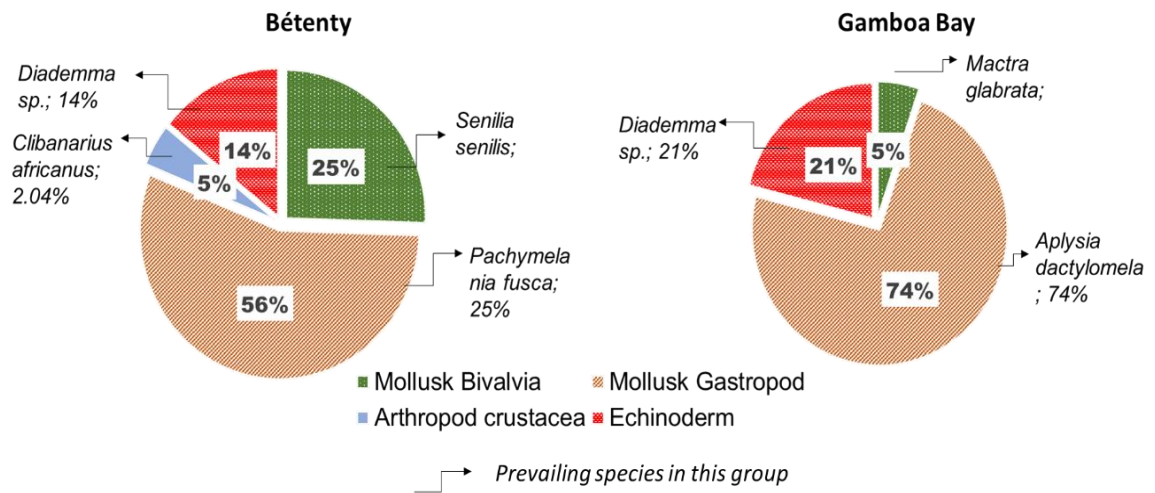

Figure 14: Different epifauna groups present in Bétenty and Gamboa Bay (GB) with the prevailing species in each group.
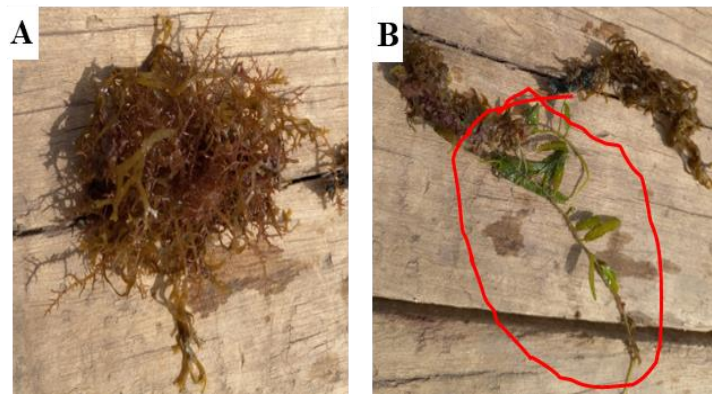

Figure 15:Associated drifting algae found in Bétenty and Gamboa bay (GB) meadows: (A) *Dictyota sp.* (B) *Caulerpa sp.* in red circle) (credit photo: G. D. Diouf, 2022).

The comparison of biodiversity between the two study areas showed a higher diversity (S=22, H=2.54, D=0.89) and a more even species distribution (E=0.82) in Bétenty than GB (S=4, H=1.09, D=0.62, E=0.79) (Table 8). The species distribution within each site showed that many species were mainly submersed and therefore, according to the gradient of the elevation of the seabed, the channel (lower elevation) of the Bétenty had more species (20 out of 22 species and high species richness) than the exposed (intertidal) bank (14 species) (Appendix E).

Table 8: Biodiversity indexes comparison between mainland (Bétenty) and island (GB Bay) settings.

Diversity indexes	BÉTENTY (mainland)	GB (island)
Richness (S)	22	4
Shannon (H)	2.54	1.09
Evenness (E)	0.82	0.79
Simpson (D)	0.89	0.62

The Gastropod family dominated where *Pachymelania fusca* prevailed (Fig. 16 A). In comparison, the central and well-covered part of the meadow in GB was dominated by the grazer *Aplysia dactylomela* (Fig. 16 C), for a total of four species present (S=4).
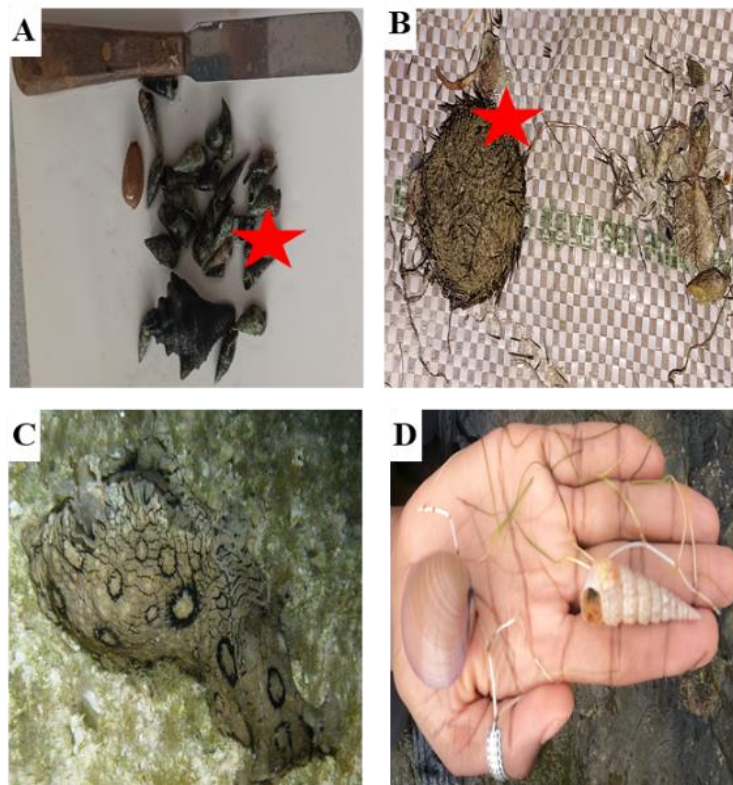

Figure 16: Epifauna associated with the seagrass beds in the two settings comprising mollusk gastropod, Echinoderm (sea-ursin) and Bivalvia including (in red star): (A)*Pachymelania fusca* and (B) *Senilia senilis*, (C) (Diademma sp.) in Bétenty (above), (D) *Aplysia dactylomela*, (E)*Turritella bicingulata* (gastropod) and *Mactra glabrata* (Bivalvia) in GB (below) (credit photo: G. D. Diouf, 2022).

4.7 Findings of the seagrass ecosystem services inventory

The responses of local stakeholders during the interview highlighted the direct link between seagrass beds and the diverse fauna mentioned above. Other services, including coastal protection and water purification (Fig. 17), were explicitly highlighted by fishermen from

Bétenty (22% of the population aged [43-56[years with more than 10 years of experience (Appendix E) while most respondents in GB (86%) knew only about the seagrass's habitat function than the others ecosystems services (Appendix E).
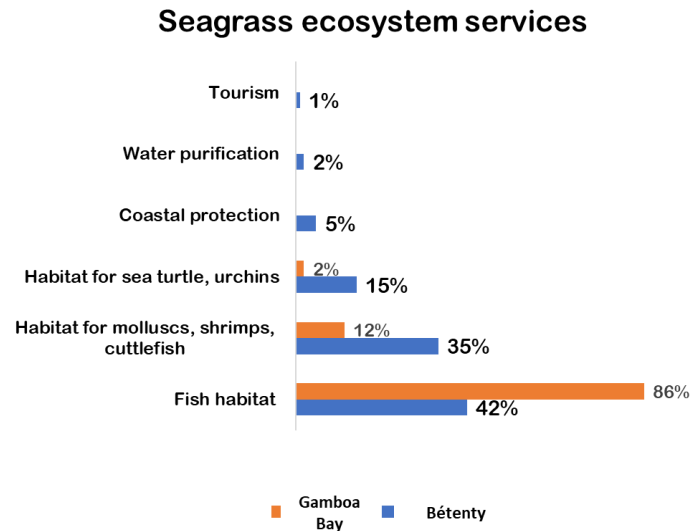
Seagrass ecosystem services

Figure 17: Review of seagrass ecosystem services in the mainland (Bétenty) and island (Gamboa Bay) settings.

Despite these services, seagrass meadows were reported to be under various threats. Natural threats such as siltation and seasonal swell were only reported in Bétenty (Fig. 18). Anthropogenic threats were also mentioned such as: fishing activities (anchoring, dredging) (Fig. 18) in addition to the continuous seagrass shoots uprooting due to women's shellfish harvesting method in Bétenty (Fig. 19A). Indirectly threats pollution and coastal infrastructure development (construction) mainly impacted the urban seagrass meadow of GB (Fig. 19B).
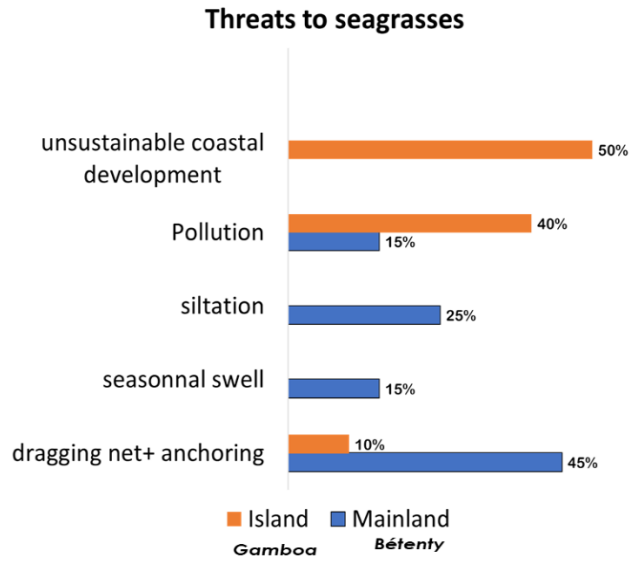
Figure 18: Review of seagrass threats in the mainland (Bétenty) and island (Gamboa Bay) settings
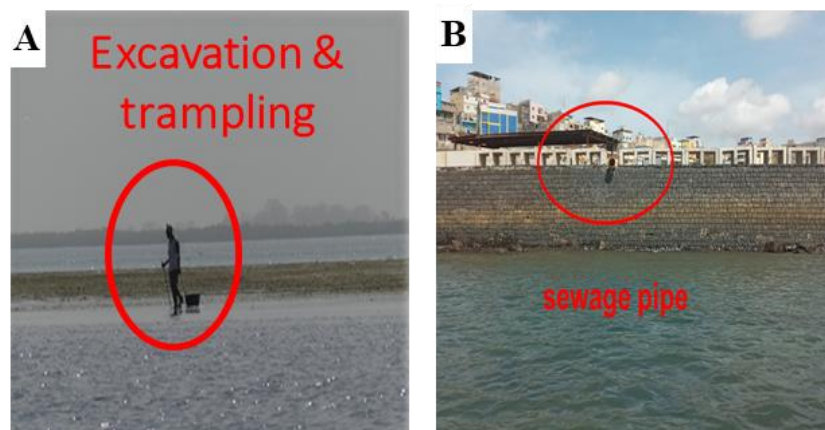

Figure 19: Anthropogenic threats to the seagrass meadows. (A) seasonal seashells harvesting in the seagrass meadow of Bétenty; (B) Permanent installation of a sewage pipe towards the seagrass meadow of GB (credit photo: G. D. Diouf, 2022).

5   Discussion

5.1   Species assemblages' comparison between mainland and island settings

Three seagrass species were documented in this study: *Halodule wrightii*, *Cymodocea nodosa* and *Zostera noltei* which represent the full seagrass diversity of the entire West African coast (Cunha & Araújo, 2009; Palazot S., 2020), except for *Ruppia maritima* found in Cape Verde yet (Martínez-Garrido et al., 2017). The southernmost West African distribution of the dwarf seagrass *Zostera noltei* is now in the Saloum delta instead of its previously established limit in PNBA of Mauritania (Chefaoui et al., 2021; Cheikh et al., 2023) where *H. wrightii* has its northern limit in (PNBA) in Mauritania (Chefaoui et al., 2021). These results thus confirm the work carried out by Elouard & Rosso indicating the presence of *Zostera nana* (former name of *Z. noltei*) in the Saloum Delta since 1977 (Elouard & Rosso, 1977). . This species together with *C. nodosa*, two temperate species (Short et al., 2007), occur close to their southern limits in Senegal. *H. wrightii* whereas, is a pioneer and common species in the West African coast (Bertelli et al., 2020; Short et al., 2007).

5.2   Highlights of the Halodule wrightii DNA barcode findings

The DNA barcode analysis of *H. wrightii* revealed a common genus, **Halodule,** between the two *H. wrightii* populations from Bétenty (mainland) and Gamboa Bay (island). However, the species identification needed to be more precise because a significant resemblance was also found for two other species *Halodule pinifolia* and *Halodule uninervis*. This ambiguity could be due to:

- (i) the different (but synonymous) names given to the same species by the collectors that deposited the sequences as the input is non-curated in GenBank genetic sequence database (Federhen, 2012; Keele et al., 2014),

-(ii) GenBank may also provide the only incomplete match to any database with ITS sequence compared to the random lines of this study,

- (iii) finally, due to the real differences among the populations of the target species (*H. wrightii*) that's currently not resolved because those data have not yet been obtained and deposited in GenBank. Seagrass genetic studies on the West African coast remain rudimentary (Tavares et al., 2022) compared to a handful of ecological studies in this region (Creed et al., 2016; El-Hacen et al., 2020). Therefore, to fully resolve the species status of the samples from this study, a comprehensive inventory of all possible barcoding sequences of West African

Halodule species would be required, along with the proposed taxonomic determination of the voucher material.

5.3 Highlights of seagrass species distribution and drivers between mainland and island

Seagrasses are distributed globally, except in Antarctica, according to their adaptation to local environmental parameters (Orth et al., 2006). This need for adaptation explains the species number and distribution differences observed within and between the two study areas.

Indeed, on the mainland side (Bétenty), the species were distributed according to their ability to withstand physical exposure (sun) during low water levels in combination with the sediment type affinity (Greve & Binzer, 2004). In this response, the species *Z. noltei* and *H. wrightii* dominated the muddy-sand intertidal bank thanks to the morpho-physiological adaptation of both genera consisting in forming large, homogeneous meadows with tightly packed and elongated leaves on the substrate to avoid desiccation in a sun-exposed meadow during water levels (Job et al., 2015). Comparatively, *C. nodosa* known to adapt to high hydrodynamic conditions through hard and extensible leaves (de los Santos et al., 2013) was therefore permanently submersed either in the subtidal and sandy channel edges as observed in marine waters of Joal-Fadiouth MPA (Cunha & Araújo, 2009; DJACBOU, 2013). On the other hand, the species was confined in small water pools of the exposed intertidal bank similar to the neighboring estuarine meadow of PNBA in Mauritania (Pottier et al., 2021)

In Gamboa Bay, on the island side, the unique *H. wrightii* species inhabited the sandy meadow in a patchy distribution mode which might be due to water movement and sediment instability. The sheltered center of the meadow was more well-covered (cover max=30%) than the edges (cover max=5%) exposed either to wave action in the rocky shore side or to a microcurrent towards the constructed bridge, especially during rising tides. These continuous movements, in the long term, could cause sediment instability (evidenced by sand waves) including easy sediment resuspension, therefore leading to water transparency reduction. Furthermore, the continuous transport of sediments has resulted in the leaching of fine sediments, causing a less consolidated substrate, coarse sediment. This aspect could enable the attachment of *H. wrightii* shoots namely in the deeper part of the meadow (near the bridge) (De Boer, 2007; Greve & Binzer, 2004).

The disturbance of the basic requirements for seagrass growth (suitable substrate and less exposure to wave action) as noted in Gamboa Bay could explain the absence of the two temperate species *C. nodosa* and *Z. noltei* which became more vulnerable at their southern

distribution limit on the West African coast (Greve & Binzer, 2004; Seddon & Murray-Jones, 2001). These species thrive in an environment with a stable substrate and clear water (Yates et al., 2011), like in the Mediterranean (Mutlu et al., 2022) and European waters (Short et al., 2007). Seen on a sub-regional scale, the strong Northeasterly trade winds and swells of Cape Verde might also prevent the establishment of *C. nodosa*, as noted in the northern part of the Canary Islands (together belonging to the Macaronesia region) (Duarte & Romeiras, 2006; Rosell-Fieschi & Polifrone, 2014), and thus, explaining the absence of *C. nodosa* in Gamboa. Meanwhile, the absence of *Z. noltei* in this bay may be related to the that the species often colonizes the intertidal zone or shallow waters in dominance over other species in intermixed meadows (Greve & Binzer, 2004).

5.4   Highlights of physicochemical parameters analysis findings

Temperature, pH, and salinity were within the tolerance range of the three-seagrass species (Greve & Binzer, 2004) although high temperatures were recorded during high tides (on a sunny day). On a long time scale, climate change-induced increasing temperatures (Turner & Schwarz, 2006) more effective on the island side which records less rainfall (Duarte & Romeiras, 2006) in addition to high sea surface water evaporation could lead to intense thermo-hydric stress and species mortality of the temperate *C. nodosa* and *Z. nodosa* (Lee et al., 2007; Turner & Schwarz, 2006) and instead favor the growth of the warm-water, opportunistic *H. wrightii* species (Biber et al., 2009; Tuya et al., 2017) in Gamboa Bay through seed germination (Short & Neckles, 1999).

Concerning the chemical parameters, high observed nutrient concentrations during high tide, especially nitrate (NO_3) could come from fertilized lawns and cropland runoff, rudimentary septic systems of dwellings in the Saloum delta on the mainland side and sediment-eroded runoff, pollution (surrounding hotels) for the urbanized zone of Gamboa Bay (Burkholder et al., 2007; Duarte et al., 2008; Gallup et al., 2020). As a result, epiphytic algal overgrowth (Pérez-Ruzafa et al., 2012) mainly on the *H. wrightii* leaves of the Bay, therefore providing evidence of nutrient-enriched conditions (Cabaço et al., 2013). Subsequently, nutrient decrease during ebb suggested either nitrate uptake via roots and leaves or an N-limitation (taken from obtained $10NO_3:1PO_4$ ratios (a proxy for Redfield N/P ratio) for seagrass growth (Kowalski et al., 2009). The N limiting factor is mainly found in the carbonate sediments of the Atlantic Bioregion (Larkum et al., 2006) to which these two study areas belong. Significant loss and fragmentation of seagrass habitat, as observed in this site (Schepanski et al., 2009), could also be a consequence of these high nutrient concentrations.

High belowground biomass at the expense of aboveground, elongated horizontal rhizomes (large internode distance), and tiny rhizome diameter (Short & Neckles, 1999), all these adaptations allowed the fast-growing *H. wrightii* species to increase its nutrient acquisition capacity and colonize Gamboa meadow (Kenworthy et al., 2018).

5.5   Highlights of Biomasses and morphometrics findings

Comparison of some parameters during this study to those of Creed done in 2016 (Creed et al., 2016) in Gamboa Bay showed that the SD and DW biomass decreased 19 and 20 times, respectively, both AGDW and BGDW became smaller, and LW 6 times tinier. The patchy distribution of the seagrass distribution still observed though there was an increase (from 10 patches of 20 m^2 area to 3000 m^2 monitored during this study). The same statement was also observed compared to the single-species meadow of the equatorial island of Principé in which *H. wrightii* 's shoot density was three times higher than Gamboa (Alexandre et al., 2017). Even on the mainland side, in the intermixed seagrass bed of PNBA of Mauritania, *H. wrightii* biomass was slightly denser (SD=376.8 ± 25.8 g/m^2 Vs 310 ± 164 g/m^2) (Chefaoui et al., 2021). However, strong similarities with two polluted bays (Ferradura and Eguas) along the Brazilian coast carried out a few years earlier in the same month (January) were noted such as a subtidal meadow with single species (*H. wrightii*) in a tiny size growing in fine sandy sediment, a quite equivalent values of BGDW, ABDW biomasses, heterogeneous habitat and finally an unremarkable flora and fauna (Creed, 1997; Sordo et al., 2011).

Being at its southernmost distribution limit in Brazilian in the western Atlantic (Creed, 1997), and therefore more vulnerable, and based on the low seagrass abundance (DW) and biometrics (LL, LW, RD, internode) in comparison to Bétenty, were enough conclude that the mainland had healthier seagrass meadow than Gamboa bay (Bertelli et al., 2020; Lapointe et al., 2020) although the *H. wrightii'* shoot density between the two was not significantly different.

5.6   Ecosystem services findings concerning the species distribution difference

Seagrass beds provide important ecosystem services to the marine environment and direct benefit stakeholders (Maria Potouroglou et al., 2017). The primary provisioning role of seagrass meadows was well-known to local fishermen in both areas especially those on the mainland side where fishermen were aware of other functions including regulation and culture services (UNEP, 2020).

A variety of valuable species (Guidetti & Bussotti, 2000) and infaunal organisms were observed in both meadows used as nursery grounds, habitat, and refuge from strong currents thanks to the seagrass' buffering capacity (snails and echinoderms) (Travassos, 2012). Among the organisms mentioned were grazers, including echinoderms (prevalent in Bétenty) and sea turtles, which feed directly on leaves or algae (Patrício et al., 2022; Wallner-Hahn et al., 2022) especially in this tropical Atlantic region (Short et al., 2007). Fishermen's responses, therefore, raised the idea of geographical connectivity between the two meadows resulting from zoochore reproduction mode through the green turtles (*Chelonia mydas*) being the biotic vector (Tol et al., 2017).

This mega-herbivore makes seasonal migrations (corridors) from Mauritania to the Bijagós Islands (Guinea-Bissau) and Cape Verde (nesting sites) along the Saloum Delta meadow (through the Bétenty feeding grounds) (Patrício et al., 2022) dispersing viable seagrass seeds over approximately 650 km dispersal distance (Tol et al., 2017). Though the small survival shoots percentage, the fast-growing species *H. wrightii* can form patches (Burkholder et al., 2007). Thus, this biotic factor could drive the only presence of *H. wrightii* (Kenworthy et al., 2018) in Gamboa Bay at the expense of *C. nodosa* and *Z.* nodosa which need suitable conditions at their geographical distribution limit in Bétenty.

5.7 Threats findings

In both locations, seagrass meadows faced visible threats related to the surrounding ongoing anthropogenic activities. In general, one of the main impacts noted along the West African coast was mainly due to the unknown status of the ecosystem to some local stakeholders (Nordlund et al., 2016), in addition to the lack of legal protection, policies or spatial plans (Griffiths et al., 2020).

In Bétenty the main sources of income depend on fishing and agriculture (DPN, 2021). Therefore, seagrass beds were undergoing unsustainable fishing practices caused by the dredging nets. In addition, during the mussel harvesting season by the women (presence of empty shells during sediment sieving) (DPN, 2021) the continuous turning of the substrate was quoted by fishermen as described by Turner et al. (2006). Finally, the propellers of passing artisanal pirogues heading out to sea (through Diombos mouth) at low water levels put daily pressure on this habitat (Orth et al., 2006; Turner & Schwarz, 2006). These disturbances have direct impacts on seagrasses, such as the uprooting of shoots and leaves mowing (Potouroglou & Vegh, 2018; Waycott et al., 2006)..

On the island side, this urban meadow of Gamboa Bay was mainly exposed to the effects of construction activities (hotels, bridge) leading to land reclamation and bed destruction by the sediment dynamics (denaturation of the soft initial sediment which turned hard and rocky under the bridge)(Rosell-Fieschi & Polifrone, 2014; Yates et al., 2011). Consequently, meadow extent reduction with the complete and irretrievable removal of seagrass from natural habitats in the long term (bare sand near the bridge) (Nguyen et al., 2021). The indirect effect of the pollution from the drained waste pipe, especially in Gamboa Bay (sewage pipe over the meadow) affected the seagrass as reflected by the high epiphytes cover and the low water clarity which together would reduce the needed photosynthesis solar ray incidence (Greve & Binzer, 2004; F. T. Short & Neckles, 1999).

Overall, these threats can have indirect impacts on the livelihoods and environmental well-being of local people (Björk et al., 2008).. Therefore, effective and specific management actions had to be taken according to the situation and provided services at the local scale, rather than relying on general seagrass services information taken from the literature (Nordlund et al., 2016; Santos et al., 2020).

6   Conclusion and recommendations

This study aimed to compare two seagrass communities from the mainland (Bétenty) and the island (Gamboa Bay) of the West African coast according to the differences in abiotic and biotic factors. In the end, three species were found, namely *C. nodosa*, *Z. noltei* and *H. wrightii* forming an intermixed meadow on the mainland side while the island had one species which was in common with the mainland side, *H. wrightii*. DNA barcoding comparison of this common species revealed a common genus between the two sites, Halodule, while the species determination remained inconclusive, and therefore requiring more complex and continuous seagrass genetic studies on the West African coast in general (still rudimentary).

Species distribution within each meadow was different between both sides as well with fine sandy sediment in Gamboa Bay in addition to an uneven (patchy) seagrass reparation where the center of the was more covered than the quasi-unvegetated meadow edges. This fragmented habitat was due to physical exposure to microcurrent (towards the bridge) and sediment instability (rocky shoreward). On Bétenty side, whereas, combination of species' sun-exposed adaptation and sediment nature differences revealed dominance of Z. noltei and H. wrightii on the exposed intertidal sandy-muddy elevated bank while the third species C. nodosa was constantly immersed either in the sandy channel edges (prevailing zone) or inside some water pools in the intertidal bank. Concerning the morphometric measurements, the intermixed meadow of Bétenty was) healthier than Gamboa Bay based on the dense seagrass coverage, high seagrass biomasses, the diverse associated epifauna and the large species (high LL, LW, RD). In addition, this bay the tiny *H. wrightii* faced high epiphytes cover, and low water clarity. Secchi depth measurements showed a clearer water in Bétenty, however, due to the shallow water (secchi disk touched the seafloor in some places) the methodology determining the water transparency using the upright position of the rope method should be adapted. On both sides, physical parameters (temperature, pH, salinity) were within the limits of these tropical species, nutrient concentrations were higher during high water levels and low at low tide hence, resulting in nutrient uptake by the seagrasses (aerial/submerged part) mainly in Gamboa Bay. These local differences in parameters were consistent with the hypothesis that environmental factors determine the distribution limit to the temperate species (*Z. noltei* and *C. nodosa)* which require stable conditions for establishment and growth (clear water/stable substratum, less intense physical exposure) at as being to their southernmost distribution limit on the mainland side. On the other hand, *H. wrightii*, in contrary is a pioneer species in this West African region, and

therefore, possesses high tolerance capacity to disturbance and can rapidly recolonize damaged areas. This research also provided information concerning the ecosystem services provided at local level mainly to fishermen who were more aware of the basic regulation and provisioning seagrass functions than the other functions (cultural and supporting services). Therefore, these stakeholders need additional knowledge on other benefits of seagrass in mitigating climate change: "carbon storage" capacity.

The perceived threats were related to the location of the seagrass bed in relation to the ongoing activities in the vicinity such as poor fishing practices and urban coastal development and pollution requiring adequate and specific management plan. In Gamboa Bay, an environmental impact assessment in urban zone and MPA creation in rural zone. Globally, the results suggest that a more in-depth study by doing regular monitoring of parameters determining seagrass distribution and health and launching of a mapping program by the two countries in order to understand the trends in the coverage.

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Appendix

Appendix A: Different steps of the sediment characterization (sediment type and grain size):

Step 1: Adopted datasheet to calculate the soil retained (g) (according to the available sieve diameter).

Opening Diameter of the sieve (mm)	Weight of Empty Sieve (g)	Weight of Sieve + Soil Retained (g)	Soil Retained (g)
	A	B	C $C_i = B_i - A_i$
2			
0.5			
0.25			
0.125			
0.063			
Pan			
		Total weight:	

Step 2: Excel sheet to determine the sediment nature (%) and the grain size of the soil. (Source :Blott & Pye, 2001)

Sample identity:

Sample type:

Sediment name:

Textural group:

SAMPLE STATISTICS			GRAIN SIZE DISTRIBUTION			
Grain diameter size	µm	Ɵ	Gravel:	%		
MODE 1:			Sand:	%		
MODE 2:			Mud:	%		
MODE 3:						
D10:			Very coarse gravel:	%	Coarse sand:	%
MEDIAN D50:			Coarse gravel	%	Very fine sand:	%
D90:			Coarse gravel	%	Very fine sand:	%
			Medium gravel:	%	Very coarse silt:	%
			Fine gravel:	%	Coarse silt:	%
			Very fine gravel:	%	Medium silt:	%
			Very coarse sand	%	Fine silt:	%
			Medium sand:	%	Very Fine silt:	%
			Fine sand:	%	Clay:	%

Step 3: Grain size classification according to the GRADISTAT program adapted from WentWorld classification

Grain size		Descriptive terminology		
phi	mm/μm	Udden (1914) and Wentworth (1922)	Friedman and Sanders (1978)	GRADISTAT program
−11	2048 mm		Very large boulders	
−10	1024		Large boulders	Very large
−9	512	Cobbles	Medium boulders	Large
−8	256		Small boulders	Medium
−7	128		Large cobbles	Small
−6	64		Small cobbles	Very small
−5	32		Very coarse pebbles	Very coarse
−4	16	Pebbles	Coarse pebbles	Coarse
−3	8		Medium pebbles	Medium
−2	4		Fine pebbles	Fine
−1	2	Granules	Very fine pebbles	Very fine
0	1	Very coarse sand	Very coarse sand	Very coarse
1	500 μm	Coarse sand	Coarse sand	Coarse
2	250	Medium sand	Medium sand	Medium
3	125	Fine sand	Fine sand	Fine
4	63	Very fine sand	Very fine sand	Very fine
5	31		Very coarse silt	Very coarse
6	16	Silt	Coarse silt	Coarse
7	8		Medium silt	Medium
8	4		Fine silt	Fine
9	2	Clay	Very fine silt	Very fine
			Clay	Clay

Boulders (GRADISTAT: Very large through Very small)
Gravel (GRADISTAT: Very coarse through Very fine)
Sand (GRADISTAT: Very coarse through Very fine)
Silt (GRADISTAT: Very coarse through Very fine, Clay)

Appendix B: Conversion steps to obtain 10 NO_3:1PO_4 as a substitute to 16N:1P Redfield ratio.

From Redfield ratio 106C:263H: 110O:16N:1P

- Step 1:

16N:1P corresponds 16 moles of NO_3 to 1 mole of PO_4.

The N atomic mass = 14.007 g/mole,

The P atomic mass = 30.974 g/mole

The O atomic mass = 15.999 g/mole.

- Step 2:

16N:1P ratio in moles translates to 992064 mg NO_3: 94970 mg PO_4.
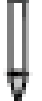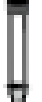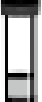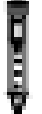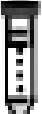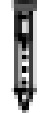
- Step 3:

Dissolving 992064 mg NO_3 and 94970 mg PO_4 in one liter of water.

1mg = 1.10^{-6} L

Ration simplification gives approximately 10 ppm NO_3: 1 ppm PO_4.

Appendix C: DNA extraction and elution steps (source: (Macherey-Nagel, 2014)

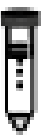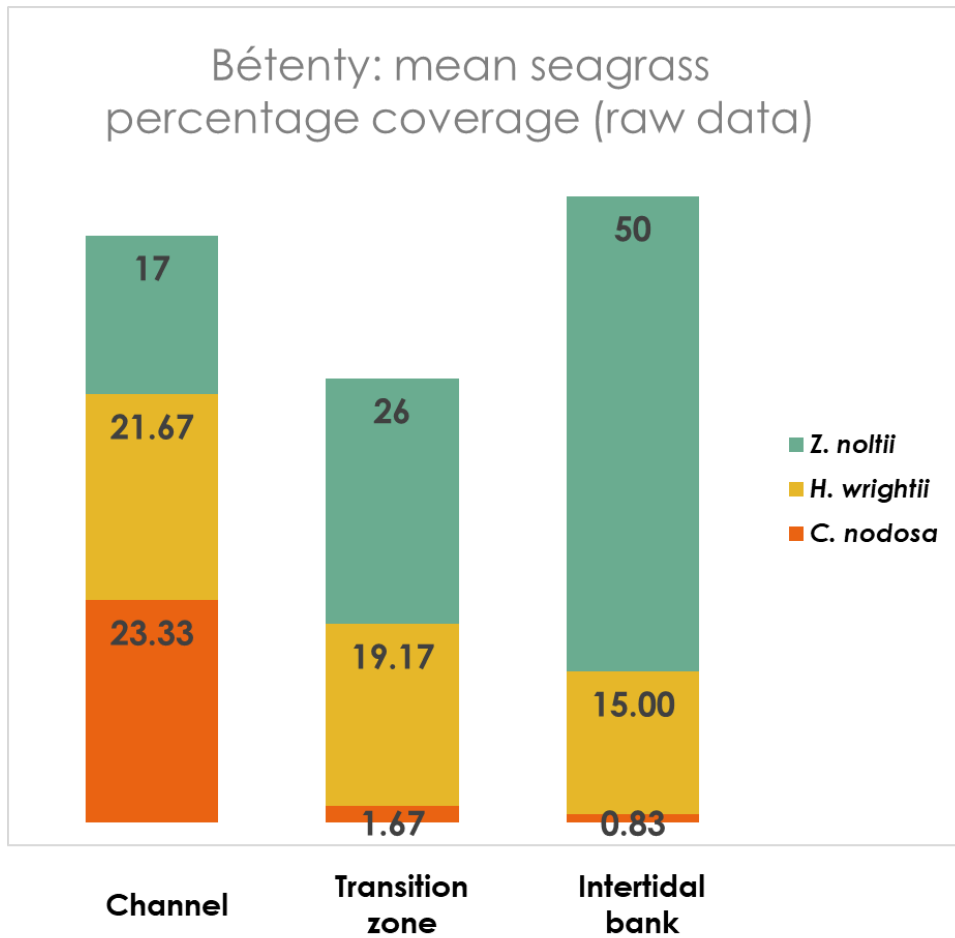| NucleoSpin® Plant II	Mini	Midi	Maxi
1 Homogenize samples	100 mg	400 mg	1500 mg
2 Cell lysis	400 µL PL1 10 µL RNase A 65°C, 10 min *ALTERNATIVELY* 300 µL PL2 10 µL RNase A 65°C, 10 min 75 µL PL3 on ice, 5 min	1.7 mL PL1 25 µL RNase A 65°C, 15 min *ALTERNATIVELY* 1.5 mL PL2 25 µL RNase A 65°C, 15 min 200 µL PL3 on ice, 5 min	6 mL PL1 100 µL RNase A 65°C, 20 min *ALTERNATIVELY* 5.3 mL PL2 100 µL RNase A 65°C, 20 min 700 µL PL3 on ice, 5 min
3 Filtration / Clarification of lysate	≈11,000 x g, 2 min	4,500 x g, 10 min	4,500 x g, 10 min
4 Adjust DNA binding conditions	450 µL PC	2.3 mL PC	10 mL PC
5 Bind DNA	≈11,000 x g, 1 min	4,500 x g, 2 min	4,500 x g, 2 min
6 Wash and dry silica membrane	1ˢᵗ 400 µL PW1 ≈11,000 x g, 1 min 2ⁿᵈ 700 µL PW2 ≈11,000 x g, 1 min 3ʳᵈ 200 µL PW2 ≈11,000 x g, 2 min	1ˢᵗ 1 mL PW1 4,500 x g, 2 min 2ⁿᵈ 3 mL PW2 4,500 x g, 2 min 3ʳᵈ 1 mL PW2 4,500 x g, 10 min	1ˢᵗ 4 mL PW1 4,500 x g, 2 min 2ⁿᵈ 10 mL PW2 4,500 x g, 2 min 3ʳᵈ 2 mL PW2 4,500 x g, 10 min
7 Elute DNA	50 µL PE 65°C, 5 min ≈11,000 x g, 1 min Repeat elution step	200 µL PE 65°C, 5 min 4,500 x g, 2 min Repeat elution step	1000 µL PE 65°C, 5 min 4,500 x g, 2 min Repeat elution step

Appendix D: Difference in species distribution within the Bétenty meadow on the mainland side.

Bétenty: mean seagrass percentage coverage (raw data)

	Channel	Transition zone	Intertidal bank
Z. noltii	17	26	50
H. wrightii	21.67	19.17	15.00
C. nodosa	23.33	1.67	0.83

These values were obtained from the raw data before applying statistical tests to compare the two study areas (mainland vs. island).

Thus, *C. nodosa* predominated in the submerged zone of Bétenty and was less abundant on the exposed high bank, where *Z. noltei* and *H. wrightii* dominated.

Appendix E: List of the epifauna associated with seagrass beds

- In Bétenty

Species	Elevation gradient			Group
	Lower (channel edge)	**Middle (transition zone)**	**Upper (intertidal bank)**	
Tagelus adansonii (Bosc, 1801)	3	1	2	Mollusk Bivalvia
Pteria colymbus (Röding, 1798)	10	3	5	Mollusk Bivalvia
Senilia senilis (Linnaeus, 1758)	11	5	5	Mollusk Bivalvia
Pachymelania fusca (Gmelin, 1791)	25	15	9	Mollusk Gastropod
Bolinus cornutus (Linnaeus, 1758)	5	3	0	Mollusk Gastropod
Persicula cornea (Lamarck, 1822)	5	3	0	Mollusk Gastropod
Persicula persicula (Linnaeus, 1758)	7	3	2	Mollusk Gastropod
Natica adansoni Blainville, 1825	0	1	1	Mollusk Gastropod
Turritella ungulina (Linnaeus, 1758)	3	0	3	Mollusk Gastropod
Conus pulcher [Lightfoot], 1786	0	2	0	Mollusk Gastropod
Pinctada radiata (Leach, 1814)	0	0	3	Mollusk Bivalvia
Diplodonta diaphana (Gmelin, 1791)	1	1	0	Mollusk Bivalvia
Cerithium atratum (Born, 1778)	2	0	0	Mollusk Gastropod
Prunum amygdalum (Kiener, 1841)	2	0	0	Mollusk Gastropod
Clibanarius africanus Aurivillus, 1898	2	1	1	Arthropod crustacea
Bulla striata Bruguière, 1792	3	0	0	Mollusk Gastropod
Pagurus bernhardus (Linnaeus, 1758)	2	0	1	Arthropod crustacea
Thais sp	6	2	1	Mollusk Gastropod
Marginella sp	3	1	2	Mollusk Gastropod
Callinectes sp	1	0	0	Arthropod crustacea
Diademma sp	3	15	10	Echinoderm
Shrimp (whitish colour)	0	0	1	Arthropod crustacea
Total	94	56	46	

- In GB Bay

| Species	Elevation gradient			Group
	Lower (near the bridge)	**Middle (center meadow)**	**Upper (rocky shore)**	**Group**
Aplysia dactylomela Rang, 1828	3	6	2	Mollusk gastropod
Diademma sp	0	1	3	Echinoderm
Turritella bicingulata Lamarck, 1822	0	3	0	Mollusk Gastropod
Mactra glabrata Linnaeus, 1767	0	1	0	Mollusk Bivalvia
Total	3	11	5	

Appendix F: Percentage of respondents according to age and years of experience

Among the 25% of respondents aged [18-30[, 8% were female.

Among the 38% of respondents aged [30-43[, 10% were female.

Among the 22% of respondents aged [43-56[, 0.5% were female.

All females had an experience of [5 to >=10 years [.
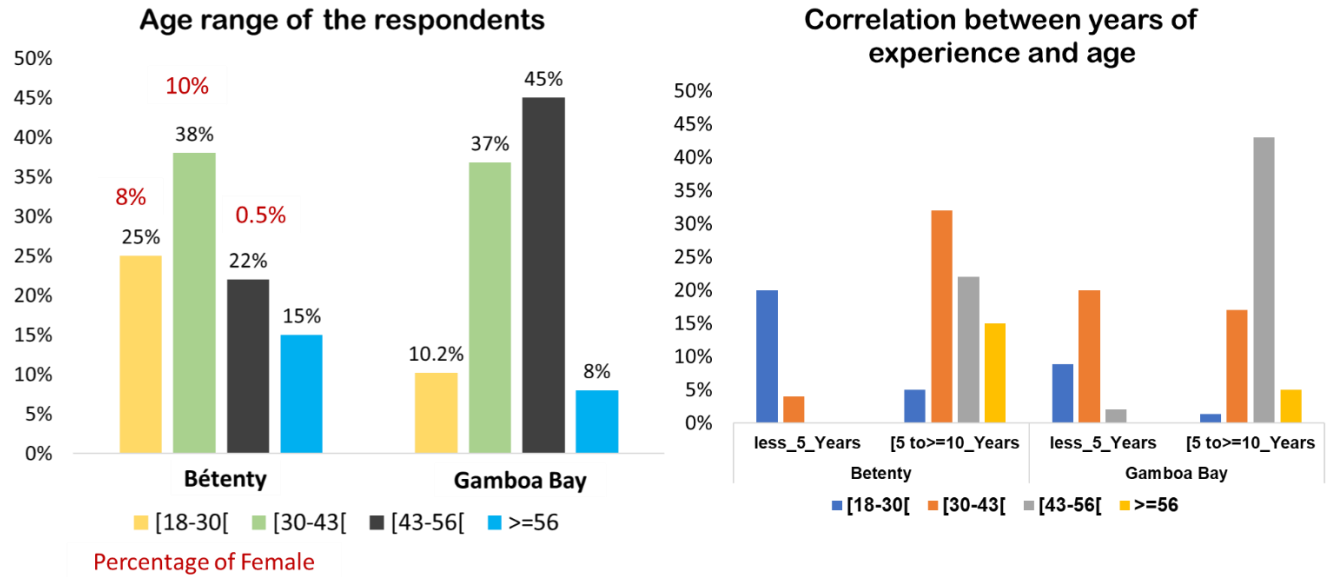
Appendix G: Questionnaire

<u>Personal questions</u>

Full Name:

Age:

-Years of experience:

<u>Questions related to the seagrass meadow</u>

1) Do you recognize seagrass beds? (Show a picture to support this question)

2) What roles do seagrass beds play?

- ❖ For marine life forms (support)

- ❖ For the marine environment (regulation)

- ❖ For culture (entertainment)

- ❖ For humans (supply)

3) What do you know about the threats to seagrass beds?

- ❖ Are they caused by human activities? If so, please list them

- ❖ Are they of natural origin? If so, please list them