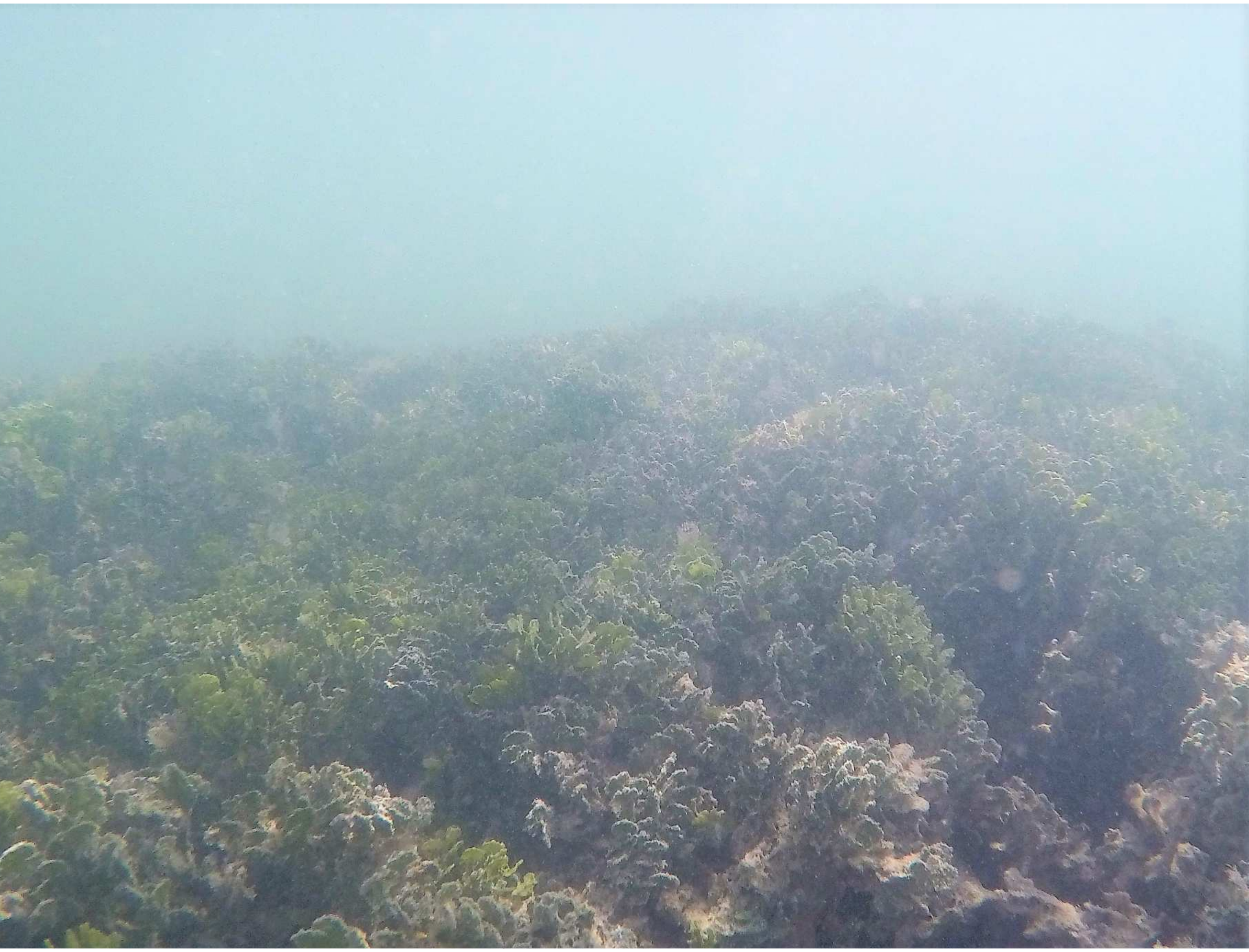
Investigating the endogenous sediment infilling by the calcareous macroalgae *Halimeda opuntia* in Lac Bay, Bonaire



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Investigating the endogenous sediment infilling by the calcareous macroalgae *Halimeda opuntia* in Lac Bay, Bonaire

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Abstract

Coastal lagoon ecosystems are transitional waters, that provide important ecological features and deliver valuable services to mankind. However, due to their geographical positioning at the coast line and physical restrictions, coastal lagoon ecosystems are highly vulnerable towards environmental and anthropogenic stressors. Terrestrial sediment influx and the endogenous sediment production can lead to the infilling of a lagoon. In this study the calcareous algae *Halimeda* species was investigated and the $CaCO_3$ sediment contribution of *Halimeda opuntia* on the endogenous infilling of Lac Bay, Bonaire determined. Therefore, the distribution and coverage of the two species *Halimeda incrassata* and *Halimeda opuntia* in Lac Bay has been mapped; *H. opuntia* species growth pattern and regrowth capacity analysed; their new growth and biomass production identified, and their calcium carbonate content established. Eventually the contribution of *Halimeda opuntia* sediment on to the endogenous infilling of Lac Bay was determined. The data collection took place at 49 grid points and experiments were conducted at five research locations, that showed different *H. opuntia* growth characteristics. At half of the visited grid points *Halimeda* spp*.* were present, whereby *H. incrassata* populated slightly more grids than *H. opuntia.* The coverage of *H. opuntia* was highest in north-east and south-west, *H. incrassata* showed the highest coverage in the north-west and south west. After a manual removal, *H. opuntia* did not show any regrowth within a two-month period. The alizarin Red -S staining experiment on *H. opuntia* specimen showed no significant differences in new growth (gram) between research location 1-4. However, a significant difference in new growth (number of segments) was found between Location1 and Location4. The alizarin Red -S staining experiment on *H. incrassata* was limited, but showed, that the method is suitable to determine new growth for *H. incrassata* species. The treated *H. opuntia* individuals showed a high $CaCO_3$ content. At the research locations (1-4) no significant differences were found between the calcareous sediment production per *H. opuntia* individual. The average annuals $CaCO_3$ sediment production of a *H. opuntia* specimen was 21g*. H. opuntia* species presented different growth characteristics between the four research locations, which were quantified in the grammage of *H. opuntia* (Kg/ m^2). Location1 showed hereby the highest *H. opuntia* grammage and Location4 the lowest. Throughout Lac Bay, *H. opuntia* species presented different growth characteristics, which were linked to the coverage for each grid, to estimate the biomass and calcareous sediment production per grid. The biomass production per grid varied between 708 kg/year and 3.438.440 kg/year and the calcareous sediment production per grid varied between 701 kg/year and 3.094.596 kg/year. For an area of 580 ha (49 Grids)*,* this translates to a biomass production of 5.010.181 kg/year and a calcareous sediment production of 4.509.163 kg/year. The production per kg/m^2 was converted into a calcareous sediment production per g/m^3 and resulted in a *H. opuntia* induced depth change per grid from 0.0019 mm /Year/m^2 to 8.801 mm /Year/m^2. In Lac Bay, the average calcareous sediment increase is 0.261 mm/Year/m^2. *H. opuntia* sediment does not contribute a significant amount of calcareous sediment to the infilling of Lac Bay. However, locally the calcareous sediment production of *H. opuntia* could become an issue and requires an adapted management approach. However, further investigations on *Halimeda* spp. in Lac Bay should be conducted, to improve the lack of *Halimeda* spp*.* data.

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1 Introduction

Coastal lagoons provide crucial ecological features (Basset, Elliott, West & Wilson, 2013) and deliver valuable services like coastal protection, tourism and fisheries to mankind (Anthony et al., 2009). Yet do coastal lagoons face significant disturbances caused by a variety of different stressors (Anthony et al., 2009).

Lagoons are inland bodies of seawater, which are enclosed by a barrier, though connected to the open ocean through inlets (Kjerfve & Magill 1989). They function as transitional coastal areas, connecting freshwater and marine habitats (Anthony et al., 2009). Various biotopes can be present in a lagoon, which includes channels, zones with sand and rubble, or areas with exposed sediment (Nagelkerken, Dorenbosch, Verberk, Cocheret de la Morinière & van der Velde, 2000). Besides, lagoons often feature productive habitat types, like seagrass beds, mangrove forests, patch reefs and algal meadows (Anthony et al., 2009; Hogarth, 2015), which contribute to the high level of biodiversity found in shallow water lagoons. Abiotic factors like the temperature and salinity are strongly variable in these ecosystems. Due to the restricted water flow, major issues in coastal lagoons, are eutrophication, the quality of water and sedimentation (Basset, Elliott, West & Wilson, 2013). The formation and maintenance of coastal lagoons is caused by the continuous process of sediment transportation, which can eventually cause their in-filling (Anthony et al., 2009). Furthermore, does their geographical position at the costal frontline, make them especially vulnerable towards climate change effects. Another major stressor is the increasing anthropogenic impact on coastal lagoons (Basset, Elliott, West & Wilson, 2013). The high variability of the lagoon environment may lead to issues concerning their management and monitoring (Anthony et al., 2009). However, especially in regard to global climate change, coastal lagoon management and knowledge enhancement are inevitable to sustain these crucial ecosystems (Anthony et al., 2009; Basset, Elliott, West & Wilson, 2013).

In the Dutch Caribbean, the greatest shallow water, semi enclosed coastal lagoon is found on the island of Bonaire (Engel, 2017). The lagoon is called ´Lac Bay´ and is located at the south eastern side of Bonaire (Debrot, Meesters & Slijkerman, 2010; Engel, 2017; STINAPA, 2018b). Lac Bay hosts crucial habitat types like the mangrove forest (Debrot et al., 2012), which is the largest mangrove forest on Bonaire (Debrot et al., 2010). Mangroves serve inter alia as important reef fish nurseries and feeding grounds (Engel, 2017) and host a high level of biodiversity (Christianen et al., 2018). Another ecosystem which is highly biodiverse, though often underestimated, is the seagrass bed. Like mangrove forests, they are also in decline, 7% of the global seagrass fields are already lost (Hogarth, 2015). In Lac Bay, seagrass fields form the greatest seagrass ecosystem of the island (Debrot et al., 2012). In the open lagoon, these seagrass beds span over an area of approx. 200 hectares and are mainly populated by the native *T. testudinum* and *Syringodium filiforme* (Christianen et al., 2018). Besides these, two other species of seagrass, *Diplanthera wrightii* and *Ruppia maritime* are present in Lac Bay (Davaasuren & Meesters, 2011). Along with the native seagrass species, the invasive *H. stipulacea* has shown an expansion in the recent years (Debrot et al., 2012).

Next to the seagrass meadows, beds of the macroalgae *Halimeda* spp*.,* including *Halimeda opuntia* and *Halimeda incrassata* cover the seafloor (Davaasuren & Meesters,2011; Christianen et al., 2018). This coral reef and lagoonal sand coloniser is a green macroalgae with a calcifiying nature (Drew, 1983; Perry, Morgan & Salter, 2016). In the Caribbean, *Halimeda opuntia* is a significant feature of the shallow water (Fricke, Titlyanova, Teichberg, Nugues & Bischof, 2018). However, *Halimeda opuntia* is not native in the western Atlantic and has been introduced to the Caribbean, as part of the biota in the ballast water of `early inter-oceanic shipping´ (Verbruggen et al., 2009). Throughout the Caribbean reefs, algae species population have risen after the sea urchin, *Diadema Antillarum* mass mortality period from 1983-1984. Hereby especially the biomass of *Halimeda* spp. increased significantly (Vroom et al., 2003). *Halimeda* spp. segments consist of calcium carbonate ($CaCO_3$) deposits, which gradually calcify (Perry et al., 2016). Sedimentation takes place, when the carbonated segments of *Halimeda* break off (Perez, Phinn, Roelfsema, Shaw, Johnston & Iguel, 2018). Eventually the whole thallus can be assimilated to the sediment around the algae, when it dies off (Perry et al., 2016). The potential carbonate production of a dense *Halimeda* meadow can reach up to 2 $kgCaCO_3\,m^{-2}\,y^1$ (Adams, 2008; Perry et al., 2016). In the recent years, *Halimeda* spp. have become increasingly abundant in Lac Bay (Debrot, Meesters & Slijkerman, 2010). They dominate the shallow waters from the edges of the mangroves to the central bay, called “Bay border” (Hylkema et al., 2014). The depth of these *Halimeda* beds lowers from 1.7 to 2.0m (Hylkema et al., 2014). Research by Wagenaar-Hummelink & Roos (1969) and Lott (2001) highlights, that great parts of Lac Bay are covered by thick *Halimeda* matts, which have a high coverage of calcareous *Halimeda* sand (Debrot, Meesters & Slijkerman, 2010).

The different habitat types in Lac Bay, especially the mangroves and seagrass fields function as feeding grounds and nurseries for countless invertebrate species and marine fish (Debrot, Meesters & Slijkerman, 2010; Slijkerman et al., 2011; Debrot et al., 2012; Hylkema et al., 2014; Engel, 2017). Furthermore, are the seagrass fields critical feeding grounds for endangered sea turtles like the green sea turtle (*Chelonia mydas*) (DCNA, 2014a; STINAPA, 2018d). All year around, the green turtles from the Caribbean region visit Lac Bay to graze. Therefore, the local density of green turtles in Lac Bay is comparably high (Debrot et al., 2012; Christianen et al., 2018). The status of the Lac Bay habitat with its seagrass fields is therefore vital for the protection of the endangered green sea turtles (STINAPA, 2018d). The lagoon is also a crucial habitat for the endangered caribbean queen conch (*Strombus gigas*) (Debrot, Meesters & Slijkerman, 2010; Debrot et al., 2012; Engel, 2017; Christianen et al., 2018). Besides, Lac Bay is also a designated bird area, with important breeding and foraging areas for sea- and shorebirds (DCNA, 2014a), in particular for the population of herons, terns and the Caribbean flamingo (Engel, 2017).

Besides the ecological importance of Lac Bay, there is a great economical interest in the area. Various businesses are operating in the bay and people are present at any time, day or night. Recreational activities taking place in the Lac Bay area are, fishing, hiking, guided kayak tours through the mangroves, scuba diving, boat tours, horseback riding, windsurfing and recreational beach activities at Sorobon and Cai. In the south-west, at Sorobon a hotel and several restaurants are located (Debrot et al., 2010; Davaasuren & Meesters, 2011) Lac Bay experiences a rapid expansion of the recreational sector, with no growth limit to be seen (Debrot et al., 2010). Lac Bay is part of the *Bonaire National Marine Park* (BNMP), which is managed by Stichting Nationale Parken Bonaire (STINAPA) (STINAPA, 2018a). Besides the status of Lac Bay as an important wetland ecosystem since 1980 (Debrot, Meesters & Slijkerman, 2010; Engel, 2017; STINAPA, 2018b), as Ramsar site (no. 199) (DCNA, 2014a), there are several more legal frameworks in place, for the protection of Lac Bay (Slijkerman, Peachey, Hausmann & Meesters, 2011).

Concerns about the status of Lac Bay are rising. Observations of Lac Bay show, a long-term decline. Different stressors impact the balance of the entire ecosystem (Debrot etal., 2010).
Direct anthropogenic pressure on Lac Bay is high, due to a greater recreational interest in the bay and the increase in cruise ship tourism. People often physically damage the seagrass fields and corals. This direct anthropogenic impact can harm endangered wildlife (Debrot et al., 2010). Furthermore, can their activities in the water lead to the enrichment with nutrients, which stimulates the bloom of harmful algal and results in a threat to coral species (Debrot etal., 2010). Eutrophication affects many lagoons in the Caribbean region, which sea grass beds and coral reefs have been degraded. Eutrophication can also degrade important fish habitats and therefore negatively impact local fisheries (Slijkerman, et al., 2011). A study conducted by Slijkerman et al. (2011) reveals, that

there are ` notable signs´ of eutrophication in Lac Bay. Eutrophication also increases the abundance of *Halimeda* species, which can therefore act as an indicator species for eutrophication in an ecosystem (Debrot et al., 2010). As findings of Fricke et al. (2018) indicate, growth rates and the pigment concentration increase by an enrichment with nutrients. This enables *H. opuntia* to populate deeper areas with less light penetration (Fricke et al.,2018).

The external infilling of Lac Bay by terrigenous sediment runoff, is caused by overgrazing (Debrot etal., 2010). This sediment infilling process has already led to a continuous decline of Lac Bay (Debrot et al., 2012). Furthermore, does the production of endogenous sediment in Lac Bay certainly contributes to the infilling of Lac Bay. Besides the mangroves, also seagrass fields and algae mats play a vital role in the infilling process. Especially the sediment of *Halimeda* species, make up a great part of the present sand (Debrot, Meesters & Slijkerman, 2010; Debrot et al., 2012). In the eutrophic Spanish water of Curacao, *H. incrassata and H. opuntia* have caused an infilling in isolated parts (Debrot et al., 2012). In Lac Bay, the channels, which have been cleared by fisherman are now encroached by *Halimeda* species and mangroves. This clocking gives no chance for a proper mixing of the water in the backwaters (Debrot et al., 2010). Furthermore, is the fish diversity and abundance in the bays open sub habitats threatened by the expansion of mangroves into the bay. This continual expansion forms so called "mangrove pools" and drastically lowers the fish diversity and abundance (Hylkema et al., 2014). Furthermore, the lack of water circulation threatens the mangrove survival (Debrot et al., 2010)**.** For a semi enclosed lagoon like Lac Bay, an expansion towards the sea is impossible (Hylkema et al.,2014). The infilling of Lac Bay is complex and caused by different processes, which Debrot et al. (2012) suggests, need further investigations.

Research on *Halimeda* species in Lac Bay is limited. However, *Halimeda* spp. have been described as part of other studies in Lac Bay. Debrot et al. (2012) for example mentions the role of *Halimeda* spp. in Lac Bay. Hylkema et al. (2014) delivers valuable information on the occurrence of *Halimeda* in "*Thalassia/Halimeda* habitat types". In Debrot et al. (2010) it is described, that an increase in *Halimeda* species has been noticed by some researches. This recognition and the knowledge on the sediment contribution of *Halimeda* has probably led to the interest in a *Halimeda* investigation, which enables this research. A baseline study has been laid by Timmermans (2018) a former student of Wageningen UR, who conducted a field research on the infilling of Lac bay by *Halimeda incrassata*. Therefore, this research's objective is to provide the missing data on the contribution of *Halimeda opuntia* on the infilling of Lac Bay and investigates the management approaches in place. Data on *Halimeda opuntia* growth characteristics and sedimentation will widen the knowledge on *Halimeda* spp. and lay a foundation for STINAPA to find suitable management approaches. If necessary, regulative controlling mechanisms, which tackle the sedimentation by *Halimeda spp.* of Lac Bay need to be established.

1.1 Problem description

Observations in Lac Bay show, that *Halimeda* spp*.* have become increasingly abundant in the recent years (Debrot et al., 2010). However, local research on the non-native species *Halimeda incrassata* and *Halimeda opuntia* (Verbruggen et al., 2009) is limited. This leads to a lack of data, concerning the distribution, growth characteristics and resulting calcareous sediment production by *Halimeda* species*.* Former research conducted on *Halimeda incrassata* by Timmermans (2018) gives a picture on the species contribution of $CaCO_3$ sediment. Though, due to the exclusion of *Halimeda opuntia,* the overall impact of *Halimeda spp.* on the endogenous infilling of Lac Bay remains vague. It is necessary to identify potential threats to Lac Bay, in order to sustain the unique ecosystem services and if necessary adapt current management approaches.

1.2 Problem statement

The calcareous macroalgae species *Halimeda incrassata* and *Halimeda opuntia* in Lac Bay are scarcely investigated*.* This results in a lack of knowledge concerning *Halimeda opuntia* species sedimentation processes and their contribution to the endogenous sediment infilling of Lac Bay.

1.3 Research aim

This study aims, to investigate the calcareous macroalgae species *Halimeda incrassata* and *Halimeda opuntia* in Lac Bay. The focus lies herby on the sedimentation process of *Halimeda opuntia* and the contribution on the endogenous infilling of Lac Bay. Furthermore, does this research aims, to investigate how *Halimeda* species in Lac Bay could be managed.

1.4 Research questions

The research aim results in the following main research questions, which will be answered by incorporating the sub research questions.

1. What is the total sediment production by *Halimeda opuntia* in Lac Bay and what contribution to the endogenous sediment infilling does it represent*?*

   a. What is the spatial distribution of *Halimeda opuntia* and *Halimeda incrassata* in Lac Bay?
   b. What is the coverage of *Halimeda opuntia* and *Halimeda incrassata* throughout Lac Bay?
   c. What are the growth characteristics of *Halimeda opuntia* in Lac Bay and do they differ between locations?
   d. How much biomass is produced by *Halimeda opuntia*?
   e. How much calcareous sediment is produced by *Halimeda opuntia*?
   f. How is the depth of Lac Bay influenced by the sediment production of *Halimeda opuntia?*

2. How could *Halimeda* spp. be managed regarding the endogenous infilling of Lac Bay?

   a. How are *Halimeda* spp*.* currently managed in Lac Bay?
   b. How could the *Halimeda* spp. management be adapted?

2 Background information research

This research on *Halimeda* species in Lac Bay is an investigative research conducted on behalf of Sabine Engel (STINAPA) and Dolfi Debrot (Wageningen Marine Research).

2.1 Stakeholders

Relevant stakeholders of this research are either directly or indirectly involved and play an active or passive role. Wageningen marine research (WMR), Van Hall Larenstein University of Applied Sciences (VHL) and Stichting Nationale Parken Bonaire (STINAPA) are directly involved in the research, whereby STINAPA and (WMR) do play an active role. VHL plays a passive role, though is actively represented by the researcher. The following stakeholder are all indirect involved and play a passive role within this research. Wild conscience, Dutch Caribbean Nature Alliance (DCNA), the Netherlands Ministry of Economics Ministry Economic Affairs, Agriculture and Innovation and the Public Entity of Bonaire (DRO). Local fisherman however, are directly involved and play an active role. Further stakeholder with an overall interest in the Lac Bay area are listed in the (Appendix A).

2.1.1 STINAPA

Stichting Nationale Parken Bonaire (STINAPA) is the management organisation of the terrestrial *Washington Slagbaai National Park* (WSNP) and *Bonaire National Marine Park* (BNMP) (STINAPA, 2018a). Lac Bay is also part of the BNMP and an ecological important, biodiverse area (Engel, 2017). In the boundaries of the BNMP STINAPA has several tasks, like the law enforcement, overall maintenance, maintenance of the moorings, monitoring and the conduction of research (STINAPA, 2018b). STINAPA is represented by Sabine Engel, who also holds the supervision role during this research.

2.1.2 Research institutes

Wageningen marine research (WMR) is a research institute, dedicated to investigate marine and coastal regions. Their main tasks are the conduction of research and provision of scientific advice (Wageningen University & Research, 2018a). In Lac Bay, WMR holds an advisory task and is included in the conduction of research projects (Wageningen University & Research, 2018b). Inter alia WMR is involved in the project “Ecological restoration Lac and south coast Bonaire “to provide scientific support (Engel, 2017). Furthermore, does WMR connect students with STINAPA and provides internship positions. WMR students are participating in different research projects. Students are also directly involved in the *Halimeda* spp*.* investigations in Lac Bay (Engel, 2017).

Van Hall Larenstein, University of Applied Sciences (VHL) is connected to the research through the Coastal and Marine Management faculty, who provides supervision during the process and evaluates the research and the conducted products at the end. VHL´s interest lies in the successful cooperation between the student and the problem owner (Smit & Stelwagen, 2018b). Students and teachers of VHL are frequently involved in research projects conducted in Lac Bay. The research by Hylkema et al. (2014) for example delivers valuable information on the occurrence of *Halimeda* spp. in Lac Bay.

2.1.3 Local partners

Wild conscience provides scientific assistance in environmental projects (Wildlife Conservation, Science and Education, 2018a). The organisation is partner of STINAPA within the project: ´Ecological restoration Lac and south coast Bonaire´ (Engel, 2017) and operates different research activities in Lac Bay (Wildlife Conservation, Science and Education, 2018b).

Dutch Caribbean Nature Alliance (DCNA) is an NGO dedicated to protect the nature of the Dutch Caribbean islands, including Bonaire (DCNA, 2014b). DCNA publishes the Bio News newsletter about research and conservation projects in the Dutch Caribbean (DCNA, 2014c) and about research projects in Lac Bay (DCNA, 2014c). As part of the "Ecological restoration Lac and south coast Bonaire "project, this research could be an interesting topic for the Bio News. One of the DCNA´s core tasks is the establishment of a GIS (geographical information systems) on Bonaire (DCNA,2014d). Therefore, the GIS maps created during the research could be in DCNA´s interest.

Sea turtle conservation (STCB) is the conservation organisation for the protection of the sea turtle population on Bonaire (STINAPA, 2018d). The status of the Lac Bay habitat with its seagrass fields is vital for the protection of the endangered green sea turtles (STINAPA, 2018d). STCB is a partner of STINAPA in the project `Ecological restoration Lac and south coast Bonaire´ (Engel, 2017).

The Dutch Ministry of Economics Ministry Economic Affairs, Agriculture and Innovation (EL&I). Commissions research in Lac Bay, like for e.g. ` Extent and health of mangroves in Lac Bay, Bonaire using satellite data" by Davasuuren & Meesters (2011). Provides the funding for the for 4-year long project: "Ecological restoration Lac and south coast Bonaire" through the Nature Fund (Engel, 2017).

The Public Entity of Bonaire (DRO) cooperates frequently with other governmental bodies and stakeholders (Openbaar lichaam Bonaire, 2015a). Lac Bay has an important status, because of the area´s cultural history and the great recreational value (Openbaar lichaam Bonaire, 2015b). The department of Spatial Planning and Development (unit Nature and Environment) coordinates the project: `Ecological restoration Lac and south coast Bonaire´ (Engel, 2017).

Fisherman produced charcoal from mangrove trees in the past. Fishing activities in the Lac Bay area have however decreased (Debrot et al., 2012). Nowadays, local fisherman provide knowledge within the project: `Ecological restoration Lac and south coast Bonaire´ (Engel, 2017). The family of G. Soliana, who lives in Lac Bay and owns the boats used by STINAPA is heavily involved in research projects conducted in Lac Bay. During this research the family also assisted in the data gathering, providing the boat and their expertise.

2.1.4 Client and researcher

Client

The clients of this research were STINAPA, represented by Sabine Engel and WMR, represented by Dolfi Debrot. The clients requested an investigation of *Halimeda incrassata* and *Halimeda opuntia* in Lac Bay, with a focus on the calcareous sediment production of *Halimeda opuntia* and its contribution to the endogenous infilling. During the establishment of the research proposal, methods and ideas were discussed with the clients and supervisors. Supervisors of VHL were included in the decision-making process and management part of the proposal. The clients mainly in the methods used during the field research.

Researcher

As a coastal and marine management student conducting this research in the frame of my bachelor thesis, it was my goal to apply the skills I have acquired during my study. Furthermore, I wanted to deepen my knowledge on data collection methods and software, I have been introduced to during my study. My personal interest was to improve my knowledge of tropical calcareous sediment producers outside the Scleractinia family. Furthermore, I wanted to establish a sophisticated knowledge about *Halimeda* spp., coastal lagoons and the Lac Bay ecosystem.

2.2 Relation with other studies

Research on *Halimeda* species in Lac Bay is limited. *Halimeda* spp. have been described as part of other studies in Lac Bay. Debrot et al. (2012) for example mentions the role of *Halimeda* spp. in Lac Bay. Hylkema et al. (2014) delivers valuable information on the occurrence of *Halimeda* in "Thalassia/Halimeda habitat types". In Debrot et al. (2010) it is described, that an increase in *Halimeda* species has been noticed by some researches. This recognition and the knowledge on the sediment contribution of *Halimeda* has probably led to the interest in a *Halimeda* investigation, which enables this research. Furthermore, the research is also indirectly linked to the project "Ecological restoration Lac Bay and south coast, Bonaire", in which frame *Halimeda* species are investigated (Engel, 2017). The first research tackling this topic has been conducted in 2017 by Timmermans (2018) in the framework of a bachelor thesis, which focused on *Halimeda incrassata.* This study laid a foundation for *Halimeda* research in Lac Bay.

Though due to the exclusive focus on *Halimeda incrassata*, the results are not representative for the general status of *Halimeda* species in Lac Bay. Therefore, this research on *Halimeda opuntia* delivers crucial data, to improve the knowledge on the status of *Halimeda* species in Lac Bay. A personal conversation with Sabine Engel reveals, that the data gathered during this research will be used for further research.

2.3 Sustainability

This study is a field research, investigating an ecological topic. Therefore, the environmental (Planet) aspect of the research is strongest. However, the ecological status of Lac Bay has an influence on the socio-economical exploitation. When interventions are necessary, the implementation also impacts the businesses (Profit) and social use (People). This could for e.g. happen through profit loss or investments and through for e.g. adapted utilization regulations.

2.4 Project and policy

This study on *Halimeda species* in Lac Bay is an investigative research and not a direct part of a research project. However, the research is indirectly liked to the "Ecological restoration Lac and south coast Bonaire ". Lac Bay is also part of the BNMP and an ecological important, biodiverse area. However, Lac Bay is also facing serious threats. An attempt to tackle these threats was laid with the project "Ecological restoration Lac Bay and south coast, Bonaire ", which will be concluded in 2019 (Engel, 2017).

The project has been established in 2015 and aims to restore the hydrology of Lac Bay, increase and protect biodiversity and enhance tourism sustainability. Within the project, STINAPA works in close collaboration with the project coordination, the Public Entity of Bonaire. Part of this project is inter alia the investigation of *Halimeda* spp. by students of WMR (Engel, 2017). This research will be the first investigation of *Halimeda opuntia* in Lac Bay. Therefore, STINAPA has a great interest in the data collected during the research. Mainly, to gain a better picture on the *Halimeda spp.* sediment production in Lac Bay.

This research project is a locally bounded research. However, the results can reveal important data on *H. opuntia*, which could be interesting on a global scale, due the limited amount of data available on this species. The results of this research could influence the further measures within the "Ecological restoration Lac and south coast Bonaire ".

3 Material & Methods

This study was conducted from the 01-10-2018 till the 28-03-2019. Field research including the data collection took place from 24-10-2018 till 27-12-2018 in Lac Bay, on the island of Bonaire.

3.1 Research area

The island of Bonaire is located in the south-eastern part of the Caribbean, between Cartagena in Colombia (75° W long., 10.5 N lat.) and the Araya Peninsula in Venezuela (64° W long., 11° N lat.). This area is called ‘Caribbean dry region’ and is dominated by semi-arid areas, with seasonal rainfall below 800 mm to 500 mm per year, with constant trade winds, which creates a dry climate (Davaasuren & Meesters, 2011; Hylkema et al., 2014). Bonaire, which is part of the Dutch Antilles (Leeward Antilles) ABC islands, is a flat coralline island build-up of volcanic deposits and limestone (Eckrich et al., 2010). The Lac Bay lagoon is located at the south eastern side of Bonaire (12°06’N 068°14’W) and covers 700ha (Debrot et al., 2010; Christianen et al., 2018). A coral reef barrier in the south forms the bay, by protecting it from the open ocean, creating a semi-enclosed inland lagoon with different habitat types (Debrot et al., 2010; Slijkerman et al., 2011; Christianen et al., 2018). The connection to the open ocean is bordered by two peninsulas (STINAPA, 2018b). Cai Peninsula, which lies in the north and Sorobon peninsula, which lies in the south (DCNA, 2014a). At the edge of the lagoon, a shallow reef habitat (Debrot et al., 2010) with a maximum depth of 6 meter can be found (Christianen et al., 2018). In the northern part of the bay four different species of mangroves form a forest (Davaasuren & Meesters, 2011). A research conducted by Debrot et al. (2012) showed, that a zonation of Lac Bay can be made, based on the four existing primary habitat types. The discoloured “dark mangrove pools”, so called “blue mangrove pools”, the shallow “bay border “with a dense vegetation and the “central bay” (Debrot et al., 2012).

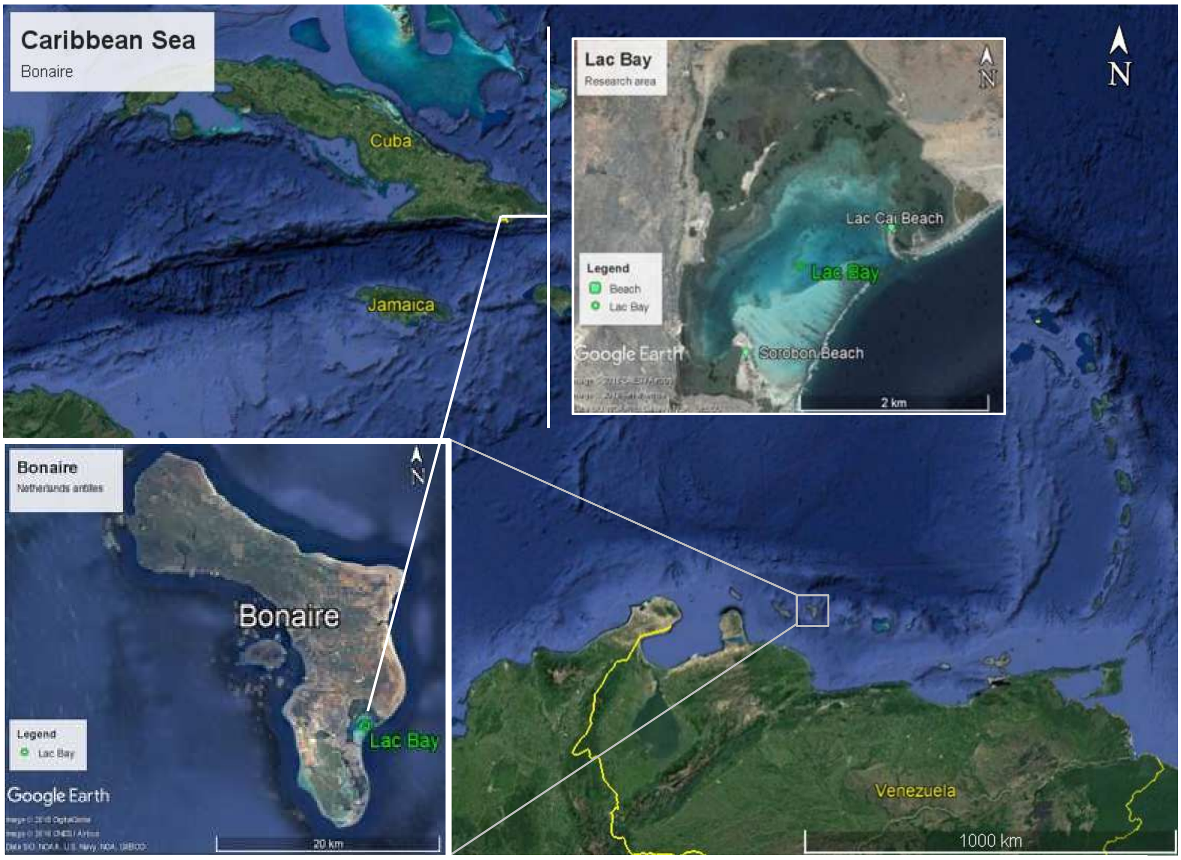


Figure 1: Map of the Caribbean Sea (Google earth Pro, 2015); the island of Bonaire (Google earth Pro, 2016a) and the zoomed in Lac Bay area (Google earth Pro, 2016b).

3.2 Data collection

3.2.1 Distribution and coverage analysis of *Halimeda* spp. in Lac Bay

Between the 14-11-2018 and 28-11-2018 Lac Bay was visited to gather information about the distribution and coverage of *Halimeda* species.

In the frame of this research the target species was *Halimeda opuntia*. Though, in consultation with STINAPA it was decided to include *Halimeda incrassata* into the monitoring. Therefore, 49 grid points have been visited, which GPS coordinates were predetermined by STINAPA and used in former research of Prent (2013) (*Appendix B*). These sampling points resemble a grid, which grid points were placed 400 meters N/S and 300 meters NW/SE apart from each other (Prent, 2013). Former designation of the sampling points was however changed to a more applicable numbering (A-K;0-6). GPS coordinates and former numbering of the sampling points can be found in the *Appendix B*.

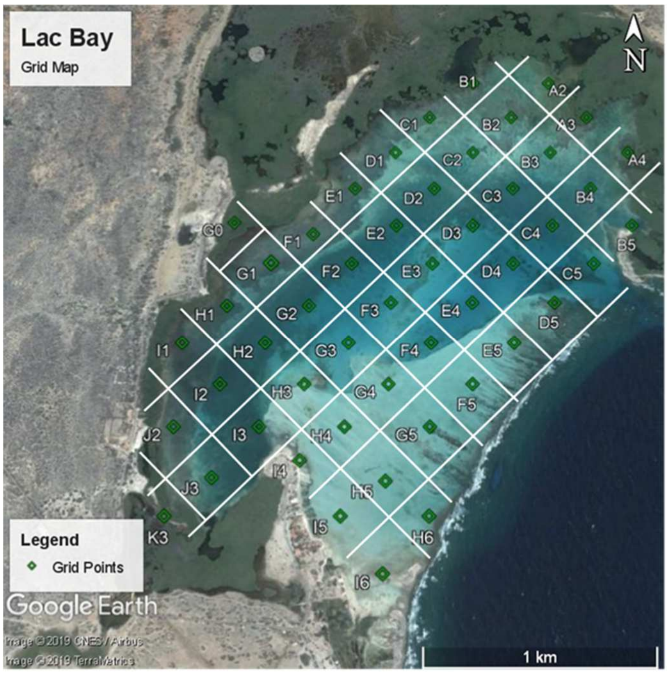


Figure 8: Map of Lac Bay (Google earth Pro, 2016b), showing the grid points (north-south; A2-K3) and an indication of the Grid-layer in white.

GPS coordinates of the grid points were approached by using the Garmin eTrex GPS. At arrival, the skipper released a buoy for location marking. Afterwards one researcher entered the water to check for depth and visibility. At shallow spots (0.30m-1m) with good visibility, measurements were taken snorkelling. Deeper spots were sampled in Scuba gear. Researchers carried a PVC frame, an underwater slate and a camera. At the buoy the researchers descended and followed the rope until the sea bottom was researched. Each grid was randomly measured for 6 times (both researchers took 3 measurements each). The first measurement was taken next to the GPS coordinate. Each measurement was taken with a 1m^2 PVC frame, which is divided in 100 squares (10cm^2). After placement, every square within the PVC frame was inspected visually and if covered (minimum one specimen of *H. opuntia* or *H. incrassata*) squares were counted species specific and the % of coverage (1-100) was noted. Furthermore, the depth at each grid point was measured with a Suunto D4i Novo and pictures of the site and PVC frame measurements were taken with a Go Pro4, for later reference.

3.2.2 Research locations of *Halimeda* spp. investigation

3.2.2.1 Site selection

In the period between the 05-11-2018 till 08-11-2018 Lac Bay has been assessed for the appearance and growth characteristics of *Halimeda opuntia*. Locations with *Halimeda* spp. presence were visited by considering the knowledge about their appearance in the shallow waters, from the edges of the mangroves to the central bay, called "Bay border" (Debrot et al.,2012; Hylkema et al., 2014).

First, sites were visited by boat and inspected visually using snorkelling gear. If *H. opuntia* was present, morphological features were inspected, and pictures taken. The goal was, to find locations where *H. opuntia* presents different growth characterises. Furthermore, locations needed to be distributed throughout the bay. The last considered criterion was related to the data collection, which needed to be executed by kayak or by shore, using exclusively snorkelling gear. This guaranteed a convenient sampling design (Explorable, 2019). Location 1-4 *(Figure 9)* meet these criteria and were therefore chosen as research locations. At these locations all experimental setups took place. Location4 functioned as a research location for *H. opuntia* and *H. incrassata*. Location 5 also complied with the conditions for a convenient sampling design (Explorable, 2019) and showed a dominance of *H. incrassata*. Therefore, this location was chosen for the *H. incrassata* investigation.

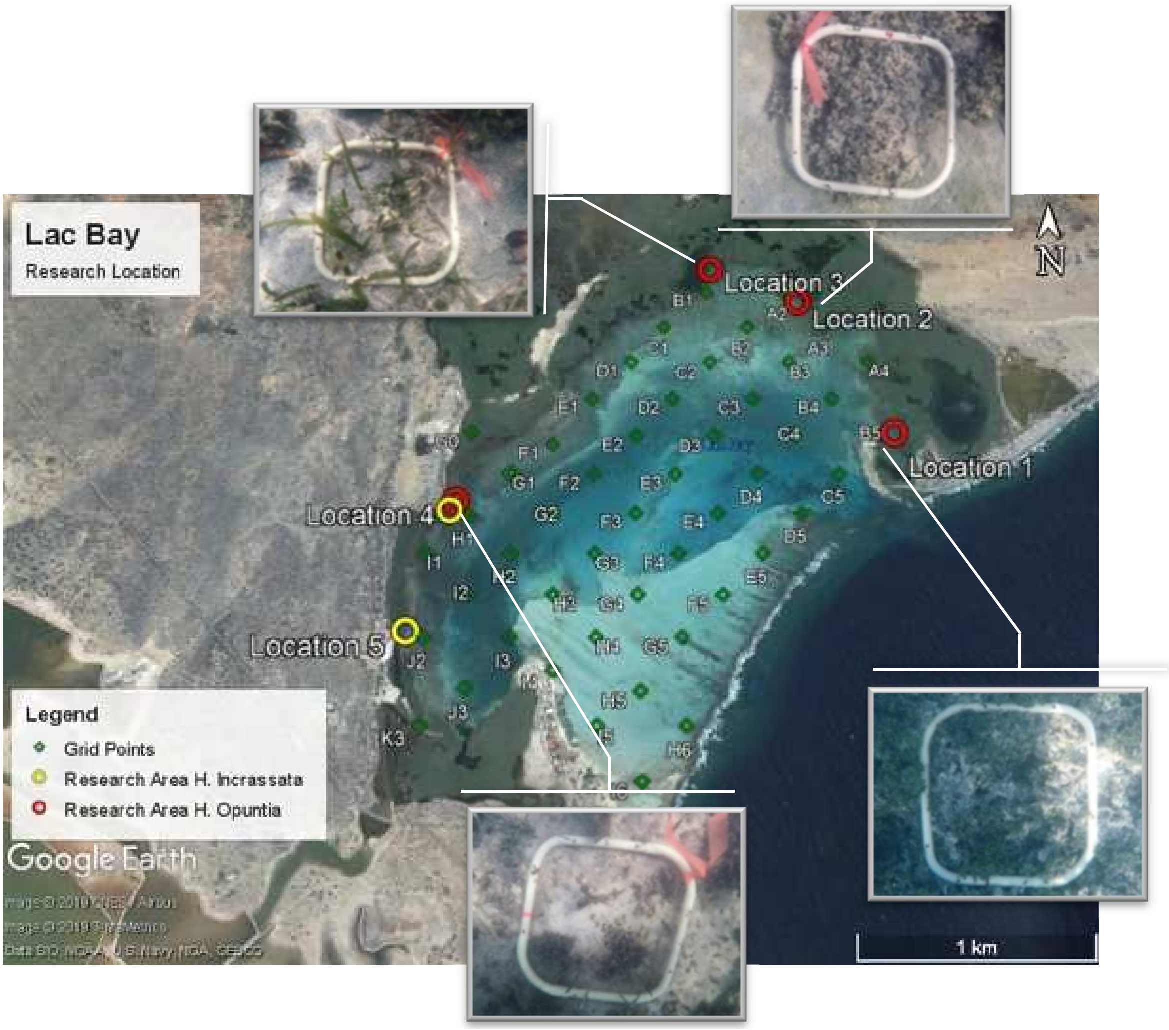


Figure 9: Map of Lac Bay (Google earth Pro, 2016b), including the research locations for *H. incrassata* and *opuntia.* Representative pictures of *H. opuntia* growth characteristics are given for Location1. November 05, 2018. Location2. November 05, 2018. Location3, November 07, 2018 and Location4. November 08, 2018.

3.2.2.2 Site description

Location 1 (12° 6'18.44"N | 68°13'15.71"W) is located near Cai beach, in a wider channel, which leads to the back mangroves. The spot can be reached snorkelling from shore, which makes monitoring convenient. The average depth at this location is between 0.70m – 0.50m with mostly poor visibility and a noticeable thermocline. *H. opuntia* mounds with a strong horizontal and vertical growth dominate the underwater landscape and in places, can even reach the surface. *H. opuntia* segments are heavily overgrown with epiphytes.

Location 2 (12° 6'42.23"N | 68°13'29.31"W) is located at the mangrove edges in the north-eastern part of Lac Bay. The spot is reachable with the kayak, optionally with a boat. The average depth at this location is between 1.20m – 1.00m with mostly good visibility and a considerably constant water temperature of (29C). Thick, vertical growing mounds of *Halimeda opuntia* can be found, which are heavily overgrown with epiphytes. The mounds are surrounded by sand and little other vegetation.

Location 3 (12° 6'48.33"N | 68°13'43.70"W) is located in the northern part of Lac Bay at the mangrove edges. The spot is reachable with the kayak, optionally with a boat. The average depth at this location is between 0.90m- 0.80 m with a good visibility. *Thalassia* is dominant. Mainly individual, horizontal growing *H. opuntia*, close to mangroves *H. opuntia* mounds can be found.

Location 4 (12° 6'7.03"N | 68°14'25.74"W) is located in the mid-western part of Lac Bay and is called ´Punta Kalbas´.The location is reachable snorkelling from the entrance close to the street which leads to ´Foodies restaurant´. The depth is between 0.80m – 0.70m and the visibility is poor. *H. incrassata* is present. *H. opuntia* individuals show a separated and horizontal growth and are covered with epiphytes.

Location 5 (12° 5'46.43"N | 68°14'32.64"W) is located in front of the ´Foodies restaurant´ in the south-west of Lac Bay and is reachable snorkelling from shore. The average depth at this location is between 0.20m – 0.30m with mostly poor visibility. Occasionally *H. opuntia* growth. Mainly *H. incrassata* and different algae.

3.2.2.3 Growth characteristics of *H. opuntia*

Per location (1-4), the growth pattern of *H. opuntia* was analysed and growth characteristics classified. Characteristics were analysed by visual inspection of the pictures taken in *Chapter 3.3.2.* Therefore, the following classification table was produced, which shows the distinctive growth pattern per location (1-4), includes a reference picture and the growth characteristics.

Table 2: *H. opuntia* growth characteristics per research location

Location 1-4	**Reference picture**	**Characteristics**
Location1	 Figure 10: Representative square of *H. opuntia* growth at Location1. November 05, 2018.	*Species composition:* *H. opuntia* ☑ *H. incrassata* ☐ other ☐ *Vertical growth:* < 10cm ☐ > 10cm < 30cm ☐ > 30cm ☑ *Growth pattern:* Massive ☑ Sprawling ☐ Limited ☐ *Patchiness:* Comprehensive ☑ Groups ☐ Single ☐

<table>
<tr>
<td>Location2</td>
<td>
Figure 11: Representative square of H. opuntia growth at Location2. November 05, 2018.</td>
<td>Species composition:
H. opuntia ☑
H. incrassata ☑
other ☐
Vertical growth:
< 10cm ☐
> 10cm < 30cm ☑
> 30cm ☑
Growth pattern:
Massive ☑
Sprawling ☐
Limited ☐
Patchiness:
Comprehensive ☑
Groups ☐
Single ☐</td>
</tr>
<tr>
<td>Location3</td>
<td>
Figure 12: Representative square of H. opuntia growth at Location3. November 07, 2018.</td>
<td>Species composition:
H. opuntia ☑
H. incrassata ☑
other ☑
Vertical growth:
< 10cm ☐
> 10cm < 30cm ☑
> 30cm ☐
Growth pattern:
Massive ☐
Sprawling ☑
Limited ☐
Patchiness:
Comprehensive ☐
Groups ☑
Single ☑</td>
</tr>
<tr>
<td>Location4</td>
<td>
Figure 13: Representative square of H. opuntia growth at Location4. November 08, 2018.</td>
<td>Species composition:
H. opuntia ☑
H. incrassata ☑
other ☑
Vertical growth:
< 10cm ☑
> 10cm < 30cm ☐
> 30cm ☐
Growth pattern:
Massive ☐
Sprawling ☐
Limited ☑
Patchiness:
Comprehensive ☐
Groups ☑
Single ☑</td>
</tr>
</table>

3.3 Target species

3.3.1 Halimeda species

In Lac Bay, two *Halimeda* spp. have been described so far *H. opuntia* and *H. incrassata* (Debrot et al., 2012). During this research, primarily the target species *H. opuntia* was investigated. In addition, *H. incrassata* was investigated in a very limited extend. For a better understanding of the crucial differences between these two species, a descriptive table was produced, which is given below.

Table 1: H. opuntia and H. incrassata growth description.

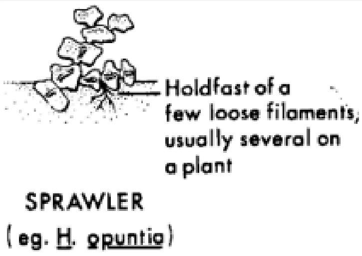
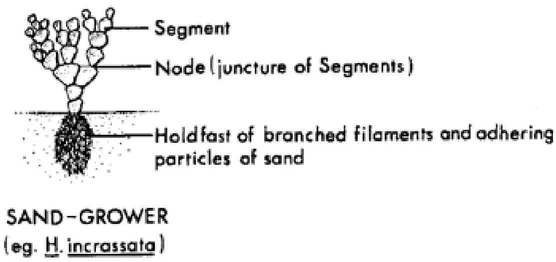
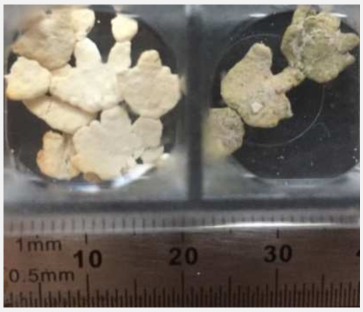
	Halimeda opuntia	*Halimeda incrassata*
Growth	Size can range to >1m (Hillis-Colinvaux, 1980).	Erect growth, up to 10-15 cm in height (Perry et al., 2016).
Growth Characteristics	 Figure 2: *H. opuntia* photographed during the distribution/ coverage sampling in Lac Bay. November 15, 2018 *H. opuntia* grows in masses, which look "shrub like" (Adams, 2008) and appear" straggly and cushion shaped (Hillis - Colinvaux,1980). *H. opuntia* forms "mounds" (Debrot et al., 2012) and is a sprawling ´species (Drew & Abel, 1988).	 Figure 3: *H. incrassata* photographed during the distribution/ coverage sampling in Lac Bay. November 15, 2018 *H. incrassata* separated growth pattern; individuals clearly identifiable (Hillis-Colinvaux, 1980).
Thallus & Holdfast		

	Figure 4: *H. opuntia* illustration, showing the loose, not distinguishable holdfast (Hillis-Colinvaux, 1980). Over a short distance, filaments can fuse in pairs. Thallus is extensive and proliferating (Hillis-Colinvaux, 1980). Multiple holdfasts, which causes a fragmented thallus (Drew & Abel, 1988).	Figure 5: *H. incrassata* illustration, showing the single, clearly distinguishable holdfast (Hillis-Colinvaux, 1980). Filaments growth in a single unit. Holdfast grows bulbously, with an Ø length of > 1cm (Hillis-Colinvaux, 1980).
Segments	 Figure 6: *H. opuntia* calcified segments (left); dried segments (right), collected during this research. December 25, 2018. Most *H. opuntia* segments analysed during this research comply with *Figure 6.* However, the size and shape of *H. opuntia* segments can vary widely (Hillis-Colinvaux, 1980). Segments can appear relatively small (Adams, 2008). Rhizoid develops from segments (Drew & Abel, 1988).	 Figure 7: *H. incrassata* calcified segments (left); dried segments (right), collected during this research. December 25, 2018. Most *H. incrassata* segments analysed during this research comply with *Figure 7*. Segments are flat and shaped like diamonds, or roundish (Alós et al.,2018). However, the size and shape of *H. incrassata* segments can vary widely (Hillis-Colinvaux, 1980). Segments can be up to 10mm in diameter Perry et al., 2016).
Sediment	Prefers hard substrate, on which it grows, or grows around (Hillis-Colinvaux, 1980). *Opuntia* species are lithophytes (Drew & Abel, 1988). Occasionally, the species can be found on sandy substrate (Hillis-Colinvaux, 1980).	Sand-dwelling species, which can be found in soft substrate type like gravel, sand and silt (Hillis-Colinvaux, 1980, Perry et al., 2016).

For the description of thick *H. opuntia* assemblages the terminology "*H. opuntia* mounds" (Debrot et al., 2012) will be used within this report. Assemblages of *H. incrassata* will be referred to as "*H. incrassata* meadows" (Debrot et al., 2012). Due to the sprawling nature of *H. opuntia* (Drew, 1983), the undistinguishable thallus and multiple holdfasts, it is difficult to identify an individual *H. opuntia* specimen. No literature about the morphological determination of an individual *H. opuntia* could be found. Therefore, the following terminology was established to describe *H. opuntia* "individuals". Specimen need to appear like individuals and show a selective and separated growth pattern. Furthermore, does the filamentous holdfast needs to growth from a single area on the specimen. The described terminology will be used throughout this report.

3.3.2 Investigation of *Halimeda* spp.

3.3.2.1 Regrowth experiment *H. opuntia*

In the frame of this *H. opuntia* growth investigation, the following method was applied to analyse the regrowth of *H. opuntia* after a complete manual removal. This method has been applied by Conklin & Smith (2005) for the evaluation of a manual algae removal to reduce their biomass. The regrowth experiment is especially interesting in terms of possible intervening measurements to restrict *Halimeda* spp. growth and expansion in Lac Bay.

Regrowth experiments took place at each of the 4 research locations, which can be seen in *(Figure 9)*. Per location (1-4) five (25cm x 25cm) 0.0625m^{2}PVC squares were randomly placed (Schiel & Lilley, 2011) at spots with *H. opuntia* coverage. The approach of the removal was to be as least invasive as possible and solely included *Halimeda* species. Attempts were made to choose patches with exclusively *H. opuntia* growth, though if present in a square *H. incrassata* was removed as well (Location 2&3). Besides from that, other algae and seagrass species like *Thalassia* remained untouched. When a suitable spot was found, PVC frames (numbered and marked with flagging tape) were securely anchored to the sediment with metal sticks. Afterwards a picture of each square was taken. A representative picture per location can be seen in *Figure 9*. The *H. opuntia* and *H. incrassata* square content was gently cleared by hand and placed in a plastic bag.

The monitoring started with the first measurement cycle (C_1), day 7 after placement (12-11-2018/ 13-11-2018/ 14-11-2018) and continued until 14 days (C_2), 21days (C_3), 28 days(C_4), 35 days (C_5), 42 days (C_6) and 49 days (C_7) (24-12-2018/ 25-15-2018/ 26-12-2018) after placement. At every monitoring moment, pictures of each square (1-5) were taken for later analysis. Furthermore, the PVC frame was gently cleaned by hand, keeping disturbance to a minimum.

3.3.2.2 New growth *Halimeda* spp.

Alizarin Red-S staining method

The alizarin Red-S staining method has been applied in former research by Timmerman (2018) on *H. incrassata.* However, the method was malfunctioning and did not show the expected results. During this research, primarily *H. opuntia* species were investigated. Though, it was decided to include *H. incrassata* in the try and error phase of the alizarin Red-S set up, to evaluate if and how the method could function for *H. incrassata* species.

The new growth of *Halimeda* spp. was measured during a 7-day period at each research location (1-5). To determine the new growth, the alizarin Red-S staining method was applied. Alizarin Red-S (alizarin Red-S monosodium salt) is a stain, that colours calcium carbonate ($CaCO_3$) pink (Vroom et al.,2003). In calcareous algae, this method is commonly used to determine their growth rate (Vroom et al.,2003) and shows little to no disadvantages. Studies reveal, that the alizarin Red-S treatment has nearly no effect on the growth of *Halimeda* species (Castro-Sanguino, Lovelock & Mumby, 2016). Furthermore, do the treated samples not experience a difference in grazing pressure (Vroom et al., 2003; Smith et al., 2004; Castro-Sanguino et al., 2016).

First, the try and error phase was initiated, were different set ups were tested on *H. incrassata and H. opuntia*. The studies conducted by Hudson (1985), Multer (1988), Vroom et al. (2003), Smith et al. (2004), Perry et al. (2016) and Castro-Sanguino et al. (2016) were used as information resources and lead to the final set up, which will be described in the following.

Onshore, a plastic bag (160mm width) was prepared with the alizarin Red-S powder, which was sealed off in a corner of the bag with a rubber band. Afterwards the plastic bag was put around a PVC coupling slip (125mm) and the bottom of the bag levelled with the top of the tube. To seal off the bag, a clamp hose, was screwed tight at the weld in the middle of the pipe. For each setup a metal peg with a zip tie and flagging tape containing the sample number and input date was prepared.

Figure: 14 Alizarin Red-S staining set up at Location3. December 03, 2018.

The amount of alizarin Red-S powder used by the considered research papers mentioned above, varies extremely, which can be seen in the following: Payri, (1988): 5g/L; Multer (1988): 0,19g /L; Hudson (1985): 2,96g/L; Vroom et al. (2003): 0,003% final solution. Therefore, different concentrations were tested during the try and error phase. The final concentration was 0,5-1g of alizarin Red-S powder per setup (650-1000ml of seater), which is closest to the amount used by Hudson (1985). At location (1-4), visibly healthy individuals of *H. opuntia* (N=80) with no to little epiphytes were selected and gently cleaned off sediment and debris by hand. *H. incrassata* (N=20) was investigated at Location4 &5. Afterwards the prepared plastic pipe was placed over the sample and hammered in the sediment until the marking line (weld) was not visible anymore. When the setup was secured, the rubber band containing the alizarin Red-S powder was released and mixed with the seawater, until the entire bag was equally coloured red. For later identification of the sample, the prepared metal peg was placed in proximity of the setup. 24 hours later, the PVC pipe setup was removed, leaving the stained *Halimeda* sample, which was then marked with the metal peg (by anchoring the metal peg in the middle of the stained specimen). The state of the research setup was checked frequently and after 7 days the samples (Vroom et al., 2003), including the associated tagging was collected. Hereby samples were gently cleaned of sediment and put into a plastic bag, which was flushed out at the surface. Afterwards samples were brought to the laboratory, where they were rinsed with fresh water and cleaned (of potential epiphytes, other algae and sediment) drained on a paper towel and weighted. To erase the natural pigmentation of the segments, samples were treated with a solution consisting of 50% distilled water and 50% commercial bleach (5% Natriumhypochlorite) (Vroom et al. ,2003; Perry et al., 2016). After 5-10 minutes samples were removed, rinsed with fresh water (Vroom et al. ,2003) and put onto labelled aluminium plates.

The bleaching process revealed the unstained, white segments (new growth) above the stained, pink segments (old growth) (Hudson, 1985; Multer, 1988; Vroom et al. ,2003). Individuals were dried in the sun for 8h and residual moisture was removed, by drying specimen in a rice cooker, until a constant dry weight was reached (Hudson, 1985). Treated *H. opuntia* and *H. incrassata* samples were analysed in the laboratory. First, the maximum height, maximum width and diameter was measured. Afterwards, the new grown, white segments were separated from the stained segments (old growth), counted and weighted (Multer, 1988).

Figure 15: Stained *H. species* with coloured segments (old growth) and white segments (new growth). Left: *H. opuntia*; Middle: *H. incrassata* Right: *H. incrassata* with separated new growth. December 25, 2018.

Weight increase of *H. opuntia*

The following method was applied in consultation with Sabine Engel (STINAPA) as an alternative research method to the alizarin Red-S staining method, which was malfunctioning during former testing in 2017 (Timmermans, 2018).

For the selection of the research location *H. opuntia* species needed to show a fragmented growth. Location4 complied to this characteristic and was determined as the sampling location. At place, *H. opuntia* specimen were randomly collected while snorkelling, using a diving mesh bag. Subsequently the bag was brought to the shallow, considerably clean water of Location5, where the specimen (N= 50) were gently cleaned and transferred to a bucket with sea water. Afterwards the samples were brought to the laboratory, where each specimen was weighted (wet weight) and labelled (with a flagging tape and zip tie containing the sampling number). After this, samples were transported in the waterfilled bucket and brought back to Location4, where they were separately anchored to a 1m^2 PVC frame, using metal sticks. After a period of 7 days, samples were collected and brought to the laboratory, where they were rinsed and weighted again (wet weight).

3.3.3 Grammage of *H. opuntia*

For the new growth analysis of *Halimeda* spp. the alizarin Red-S staining method was applied. However, *H. opuntia* growth characteristics needed to fit the experimental set up, which limited the selection of specimen. *H. opuntia* needed to show certain growth characteristics concerning their height and width, which needed to fit the tube properties (< 15cm height and width). The selected specimen did therefore not reflect the actual growth characteristics observed at each location *(s. Chapter 3.3.2.3).* To overcome these limitations and present a more accurate estimation of the *H. opuntia* biomass and calcareous sediment production, the grammage of *H. opuntia* per location needed to be included.

To establish the grammage of *H. opuntia*, measurements were taken in *Chapter 3.4.1*. The content of the five 0.0625m^2PVC squares collected during the regrowth experiment was brought to the lab, where it was sorted and rinsed with fresh water to cleanse of sediment and organic material.

Afterwards, samples were put on aluminium plates, which were labelled with the associated tagging and placed into the sun for a period of 7days. The *Halimeda* spp. samples were weighted and re-dried until a constant weight was reached. Furthermore, the average dry weight (kg) of the 5 squares was calculated per location (1-4). This average dry weight represented the grammage of *H. opuntia* per 0.0625m^{2}and was fitted to a surface of 1m^2.

3.3.4 Biomass production of *Halimeda* spp.

To calculate the annual biomass production per location (1-4), the new growth of *H. opuntia* individuals (N=80) was estimated for 356 days and put into relation with the grammage of *H. opuntia* per 1m^2, per location (1-4). *H. incrassata* samples from Location4&5 were analysed together (N=20).

3.3.5 $CaCO_3$ content *Halimeda* spp.

To determine the $CaCO_3$ content of *Halimeda* spp. in Lac Bay, *H. opuntia* specimen (N= 50) were randomly collected at the Locations (1-4). For comparability reasons however, specimen with a similar size range as the alizarin Red-S samples were chosen. Specimen were brought to the lab, where they were cleaned with fresh water. Afterwards they were weighted and measured (height; width; diameter). Furthermore, their number of segments was determined. Dry weight

For the decalcification process, specimen (N=50) were put in separate plastic boxes filled with a 5% HCL/ distilled water solution (Vroom et al., 2003; Smith et al., 2004). The reaction started during placement (bubbles) and stopped, when no reaction with the carbonate (bubbles) was detectable (Vroom et al., 2003; Perry et al., 2016). Furthermore, samples were lifted out of the solution for 3 times, to ensure the reaction had ceased (Vroom et al., 2003). Afterwards individuals were put in distilled water, marked with their labelling and put in the oven for 24 hours on 65 ◦C degrees, till a constant dry weight was reached (Vroom et al., 2003). This was achieved by weighting the individuals after 24 hours, starting another drying sequence for 15 minutes, re-weight and repeat, if there was a change in weight (weight loss). The constant dry weight of the samples was used, to determine the biomass of the algae. The difference between the dry weight measured before the HCL treatment and after the process, was the actual calcium carbonate content of the algae (Perry et al., 2016).

3.3.6 $CaCO_3$ sediment production *Halimeda* spp.

The ø $CaCO_3$ sediment production per research location was established by incorporating the annual biomass production per research location, average $CaCO_3$ content of *H. opuntia* and grammage per research location.

3.3.7 Sediment displacement at *Halimeda* spp. research locations

Figure 16: Sediment trap, anchored with marbles at Location3. December 10, 2018.

In the period between the 30-11-2018 till 25-12-2018 sediment traps were installed at each research location (1-4), to analyse the sediment displacement of supposedly *Halimeda* spp. sediment. Observations at sight showed, that the sediment partly consisted of broken *Halimeda* spp. segments. However, no actual sediment analysis took place. The traps were placed next to one of the 5 installed squares at each research location, in close distance to *H. opuntia* specimen. The plastic containers, which functioned as flat surface sediment traps (Nolte et al.,2013), were burdened with glass marbles. At locations with a stronger current (location 3&4) they were anchored with metal pegs. Next to each trap a metal peg was placed, which was marked with a flagging tape for easier detection. At each location (1-4) two boxes were installed, one for a period of 7 days, the other for 14 days. At the collection day, traps were removed by enclosing the anchored box with a lid. In the lab, the common procedure for sediment treatment was applied, by first sieving, washing and drying the sediment (Nolte et al., 2013). Therefore, the content of the container was released, by filtering the water through a fine kitchen sieve with a mesh size of 0.5 mm. Remaining sediment in the container was rinsed with fresh water and released into the sieve. Afterwards organic material (>5mm) was sorted out, the sediment was placed on aluminium plates and dried in sun for 48 hours. To measure the dry weight, each sample was put in a rice cooker and re-weighted, until a constant dry weight was reached.

3.4 Investigating *Halimeda* spp. in Lac Bay

3.4.1 Growth characteristics *H. opuntia*

H. opuntia does not show uniform growth characteristics throughout Lac Bay. Therefore, resembling growth types were chosen and represented through the four research locations. The different growth characteristics (*Chapter 3.3.2.3.)* present at the four locations, were translated into a "growth key" (K_{1-4}). This growth key incorporated the distinctive species composition, vertical growth, growth pattern and patchiness per location. The growth key was then applied to the grid measurements. Hereby, pictures (6 per grid) of the coverage measurements were analysed per grid point (A2-K3) and associated with an appropriate growth key (K_{1-4}). If *Halimeda* spp. were not present, the gird point was classified with (0).

3.4.2 Biomass production of *Halimeda* spp. in Lac Bay

The annual biomass production of *H. opuntia* was established by fitting the appropriate growth key biomass value to the average coverage of *H. opuntia* per grid. Finally, the biomass production per grid was summed, to establish the overall biomass production for Lac Bay. Associated calculations can be found in *chapter 3.5.1*

3.4.3 Calcareous sediment production of *Halimeda* spp. in Lac Bay

The annual calcareous sediment production of *H. opuntia* was established by fitting the appropriate growth key $CaCO_3$ sediment value to the coverage of *H. opuntia* per grid. Finally, the $CaCO_3$ sediment production per grid was summed, to establish the overall $CaCO_3$ sediment production for Lac Bay. Associated calculations can be found in *chapter 3.5.1.*

3.4.4 Extend of endogenous infilling by *H. opuntia* sediment

For the analysis of the *H. opuntia* sediment contribution to the endogenous infilling of Lac Bay, depth measurements were taken at each grid point during the distribution and coverage analysis. To establish the depth change induced by the $CaCO_3$ sediment production, the $CaCO_3$ sediment produced in kg/m^2 needed to be converted into g/m^3.

Hereby, the density of the $CaCO_3$ sediment needed to be established. No actual sediment analysis took place and due to the fact, that the $CaCO_3$ in *Halimeda spp.* is deposited in form of aragonite (Hillis-Colinvaux, 1980), the density of aragonite was used as the *Halimeda spp.* sediment density. The density of aragonite is 2.93 g/m^3 (Barthelmy, 2014).

3.4.5 Management of *Halimeda* spp. in Lac Bay

To answer the second research question on *Halimeda* spp. management in regard to the endogenous infilling of Lac Bay, a literature study was conducted. Therefore, information on the current management of *Halimeda* spp. in Lac Bay were gathered. Furthermore, possible adaptations on the current *Halimeda* spp. management were investigated.

3.5 Data Analysis

In the framework of this research, the collected data was stored and handled with Microsoft Excel 365.

3.5.1 Calculations

To answer the research question about *H. opuntia* in Lac Bay, the following intermediate calculations were applied.

3.5.1.1 Biomass production of *Halimeda spp.* per location

The following calculation was used, to calculate the biomass production of *H. opuntia* species (N=20) per location (1-4).

$$m_{Bm} = \underbrace{\frac{\emptyset m_{ng}}{\emptyset m_{dry} - \emptyset m_{ng}}}_{\boxed{\text{New growth per initial weight}}} \times \underbrace{\frac{T_{year}}{t_{growth}}}_{\boxed{\text{Projecting to 365 days}}} \times M_{Sq}$$

$m_{Bm} \rightarrow$ *biomass production per location*

$m_{ng} \rightarrow$ *dry weight of new growth per location*

$m_{dry} \rightarrow$ *dry weight per location*

$T_{year} \rightarrow$ *new growth calculated per year*

$t_{growth} \rightarrow$ *new growth period*

$M_{Sq} \rightarrow$ *measured grammage* $\left(\frac{kg}{1m^2}\right)$ *per location*

3.5.1.2 $CaCO_3$ content *Halimeda spp.*

The following calculation was used, to calculate the relative calcium carbonate content of *H. opuntia* species (N=50).

$$c = \frac{\underbrace{\left(\frac{\sum_1^{n'} m'_{dry}}{n'}\right)}_{\boxed{\text{Ø dry weight}}}}{\underbrace{\left(\frac{\sum_1^{n'} m'_{CC}}{n'}\right)}_{\boxed{\text{Ø weight of } CaCO_3}}}$$

$c \rightarrow$ *ø calcium carbonat per ø dry weight* [%]

$m'_{dry} \rightarrow$ *dry weight (carbonat sampels)*

$m'_{CC} \rightarrow$ *weight of calcium carbonat*

$n' \rightarrow$ *number of carbonat samples*

3.5.1.3 Calcareous sediment production *H. opuntia* per location

Subsequent formula was applied, to calculate the average calcium carbonate content of the new growth *(see Chapter 3.4.3)* per Location (1-4), per 365 days.

$$m_{CC} = \underbrace{\frac{\sum_1^n m_{ng}}{n}}_{\boxed{\text{Ø dry weight of new growth}}} \times \underbrace{\frac{T_{year}}{t_{growth}}}_{\boxed{\text{Projecting to 365 days}}} \times c$$

$m_{cc} \rightarrow weight\ of\ calcium\ carbonat\ per\ location$

$m_{ng} \rightarrow dry\ weight\ of\ new\ growth\ per\ location$

$T_{year} \rightarrow new\ growth\ calculated\ per\ year$

$t_{growth} \rightarrow new\ growth\ period$

$n \rightarrow number\ of\ samples\ per\ location$

$c \rightarrow ø\ calcium\ carbonat\ per\ ø\ dry\ weight\ [\%]$

3.5.1.4 Biomass production *H. opuntia* in Lac Bay

The next formula was used, to calculate the biomass production of *H. opuntia* per grid, fitted to the growth characteristics ($K_{1\text{-}4}$) of location (1-4) *(see Chapter 3.4.1.2)* for the growth period of 365 days.

$$m_{Grid} = \underbrace{\frac{Øm_{ng}}{Øm_{dry} - Øm_{ng}}}_{\boxed{\text{New growth per initial weight}}} \times \underbrace{\frac{T_{year}}{t_{growth}}}_{\boxed{\text{Projecting to 365 days}}} \times \overbrace{M_{Sq} \times a_{Grid}}^{\boxed{\text{Projecting grammage to grid size}}}$$

$m_{Grid} \rightarrow biomass\ production\ per\ grid$

$m_{ng} \rightarrow dry\ weight\ of\ new\ growth\ per\ location$

$m_{dry} \rightarrow dry\ weight\ per\ location$

$m_{Sq} \rightarrow\ measured\ grammage\ \left(\frac{kg}{1m^2}\right) per\ location$

$a_{Grid} \rightarrow\ size\ of\ each\ grid;\ 300\ m\ \times 400\ m = 120'000\ m^2$

$T_{year} \rightarrow new\ growth\ calculated\ per\ year$

$t_{growth} \rightarrow new\ growth\ period [days]$

3.5.1.5 Calcareous sediment production *H. opuntia* in Lac Bay

Fitted calcareous sediment production ($K_{1\text{-}4}$) per grid, per 365 days.

$$m_{CC} = m_{Grid} \times c$$

$m_{cc} \rightarrow weight\ of\ calcium\ carbonat\ pergrid$

$m_{Grid} \rightarrow biomass\ production\ per\ grid$

$c \rightarrow ø\ calcium\ carbonat\ \%\ \ per\ dry\ weight$

The following calculation was used, to calculate the calcareous sediment production per grid, with the fitted growth characteristic ($K_{1\text{-}4}$).

$$m_{Sed,n} = c_n \times K_i$$

$n\ \rightarrow specific\ Gridpoint$

$K_i\ \rightarrow key\ 1 \ldots 4, representing\ 100\%\ coverage$

$c_n\ \rightarrow coverage\ of\ Grid$

$m_{Sed,n}\ \rightarrow sediment\ production\ of\ Grid_n$

The final calculation was applied, to translate the calcareous sediment production in gram into the volume produced per grid.

$$V_{Sed,n} = m_{Sed,n} \times d_{CaCO_3}$$

$V_{Sed,n}\ \rightarrow volume\ of sediment\ production\ per\ grid_n$

$m_{Sed,n}\ \rightarrow sediment\ production\ of\ grid_n$

$d_{CaCO_3}\ \rightarrow density\ of\ CaCO_3\ (Aragonit) =\ 2{,}93\,\frac{g}{cm^3}$

3.5.2 Mapping

All informative maps within this report have been created, by using Google Earth Pro 7.3. (Google earth, 2018). GPS coordinates of the grid points visited during the distribution and coverage mapping, were stored in the Garmin basecamp environment of the Garmin eTrex GPS and transferred to Microsoft Excel 365. Further geoprocessing was performed using the newest version (3.4) of QGIS, which has been released in January 2019 (QGIS Development Team, 2018).

The distribution and coverage map of *H. opuntia* and *H. incrassata* were produced using the data collected in *Chapter 3.3.1.* Per grid point the average coverage was calculated and displayed using an interpolation (IDW function). During the data collection, the depth per grid point was measured and is displaced in a depth map.

To produce the calcareous sediment map of Lac Bay, the production per research Location (1-4) was calculated and the growth pattern of these sites was fit, to the growth pattern of the sampled grid points. Therefore, pictures of the grid point measurements were analysed and categorized to a growth pattern of the research locations (1-4). Furthermore, the coverage per grid plus the growth pattern was considered.

3.5.3 Statistical analysis

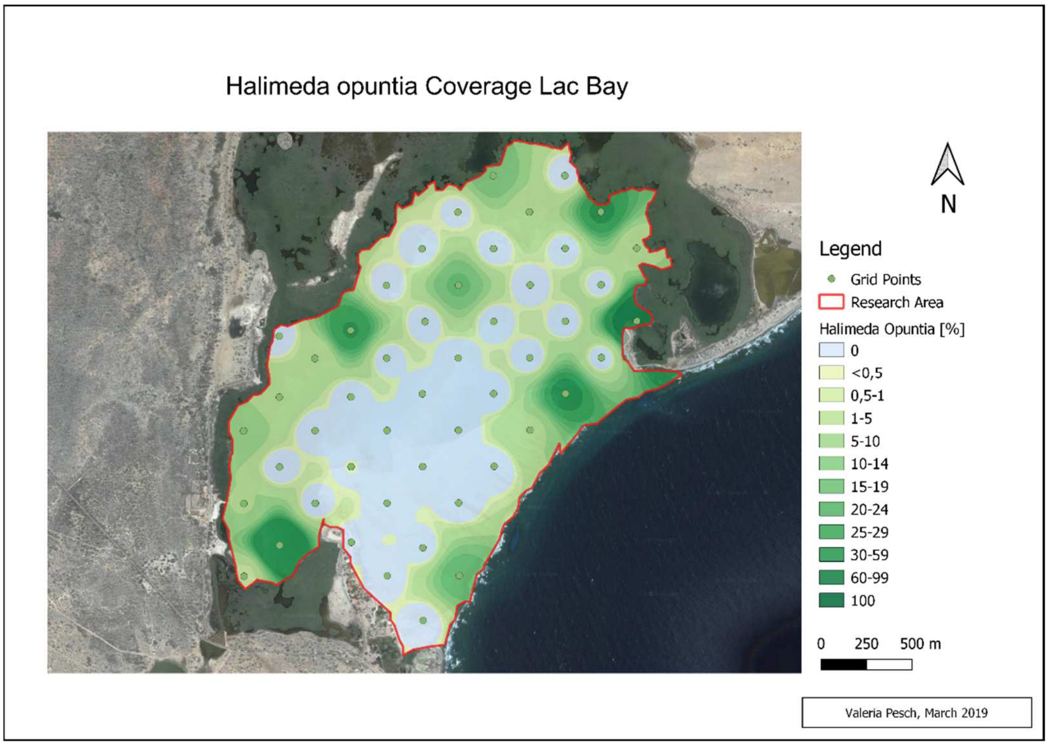
For the statistical analysis and visualisation of the gathered data, 'R-Studio' was chosen. Providing the advantages of a free Software, suited for statistical testing and graphical visualisation (R Foundation, 2017).

First, residual plots were used to visually check for a significant discrepancy of normality and homoscedasticity or tested with the associated Shapiro-Wilk's test (discrepancy of normality) and Levene's test (homoscedasticity). The data was non-parametric, therefore differences between the growth characteristics and sediment production of the four research locations were tested using a Kruskal-Wallis test. Results were regarded as statistically significant when ($p<0.05$). If Kruskal-Wallis test results were considered significant, a post-hoc-Test (Bonferroni correction) was applied to detect unexpected differences between individual research locations.

4 Results

4.1 Distribution and coverage analysis *Halimeda* spp. in Lac Bay

For the analysis of the distribution and coverage of *H. opuntia* and *H. incrassata*, maps for each species were produced by using QGIS. The colour scheme applied in the maps indicates the density from 0% coverage (light green), to moderate (green), to a high coverage (dark green) which can be seen below *(Figure 17)*.



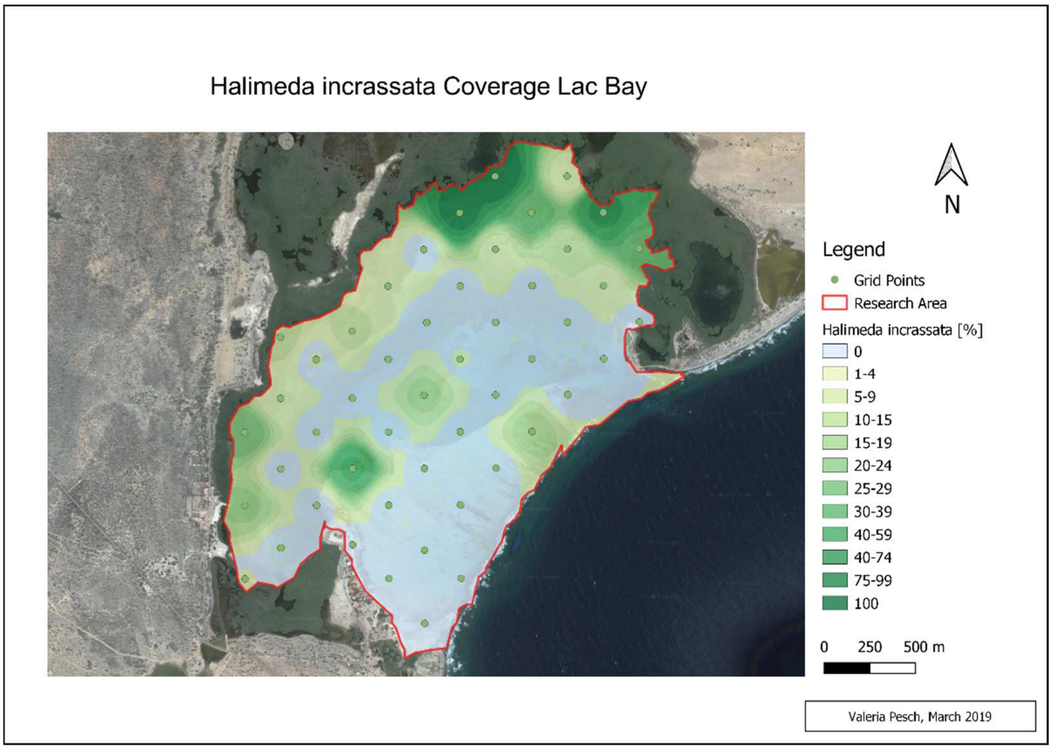


Figure 17: Distribution and coverage of *H. opuntia* (top) and *H. incrassata* (bottom).

Distribution

The *H. opuntia* map shows, that the species populates multiple grids around the mangrove edges in the north, especially north-east and in the south-western part of Lac Bay. Furthermore, *H. opuntia* grows on the coral reef at the shoreline. *H. incrassata* is present at multiple grids in the north, especially north- west and south-western part, along the mangroves.

Coverage

Out of the 49 grids (100%) visited during this research, 26 (53.1%) grids showed a presence of *Halimeda spp.* 18 grids (36.7%) displayed exclusively *H. opuntia* growth and 22 (44.9%) grids were solely populated by *H. incrassata*. 14 grids (28.6%) showed a presence of both species. The Coverage measurements (6 measurements per grid) revealed, that the average coverage of *Halimeda* spp. in Lac Bay is 14.8%. *H. opuntia* has hereby a coverage of ø 6.2% per grid and *H. incrassata* covers ø 8.6% per grid. The highest coverage of *H. opuntia* was found in the north-east and south. *H. incrassata* mounds were present at multiple grids in the north-east. A table of the coverage per grid can be found in the *Appendix D.*

4.2 Investigation of *Halimeda* spp.

4.2.1 Regrowth experiment *H. opuntia*

The first monitoring cycle(C) started 7 days(C_1) after placement and showed no new growth at any Location (1-4). Further pictures were taken after 14 days (C_2), 21 days (C_3), 28 days (C_4), 35 days (C_5), 42 days (C_6) and 49 days (C_7) marking the last investigation. Location 1-4 did not show signs of new growth of *H. opuntia* at any monitoring point.

It was however noticeable, that all squares (1-5) at Location1 experienced a high level of ingrowth pressure from the surrounding *H. opuntia* patches. Squares (1-5) at Location2 which were also positioned next to a *H. opuntia* patch, experienced the same kind of ingrowth. After the *H. opuntia* removal at Location3, *Thalassia* spp. were still dominant and no other species of seagrass or algae could be detected in the squares. Though, there was some ingrowth at square 4, which was located at the mangrove's roots, where *H. opuntia* thrived. Location4 showed no *H. opuntia* ingrowth. However, other algae species populated some squares.

4.2.2 New growth measurements *Halimeda* spp.

4.2.2.1 Alizarin Red-S staining method

***H. opuntia* measurements**

The analysis of the *H. opuntia* specimen showed, that the treated samples (N=80) height was ø 7.67 cm and width ø 6.45 cm.

The average dry weight of the treated samples *(see Chapter 3.4.3)* was highest at Location2 (12.30 ± 6.92 g), followed by Location4 (11.33 ± 9.24 g) and Location1 (10.07 ± 9.82 g). The lowest average dry weight was measured at Location3 (8.80 ± 4.10 g). The Kruskal-Wallis test revealed however, that differences in dry weight between the Locations (1-4) were not significant ($H(3) = 4.5485$, $p= 0.208$).

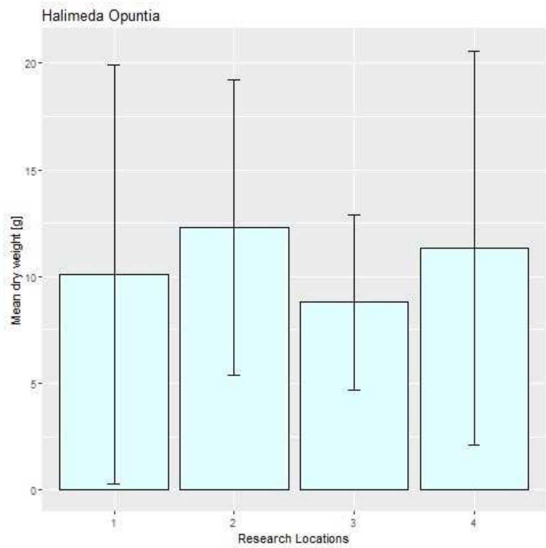


Figure 18: *H. opuntia* average dry weight ($g\ yr^{-1}$) (± SE) per each location (1-4).

***H. opuntia* new growth in weight**

The average new growth (weight in grams) of *H. opuntia* /location/year showed, that Location1 (32.15 ± 32.72 $g\ yr^{-1}$) had the highest average new growth, followed by Location4 (29.21 ± 21.84 $g\ yr^{-1}$). At Location2 (22.09 ± 18.22 $g\ yr^{-1}$) less new growth was observed and Location3 (20.36 ± 15.01 $g\ yr^{-1}$) showed the lowest measured new growth *(Figure 19).* The Kruskal-Wallis test revealed however, that the new growth between the Locations (1-4) did not differ significantly ($H(3) = 2.7863$, $p= 0.4258$).

***H. opuntia* new growth in segments**

The average new growth in number of segments (seg.) of *Halimeda opuntia* Location (1-4) yr^{-1} showed, that Location1 (4507.75 ± 2763.26 seg. yr^{-1}) had the highest average new growth per individual, followed by Location2 (3355.39 ± 2519.29 seg. yr^{-1}). Location3 (2870.46 ± 2276.97 seg. yr^{-1}) showed less new grown segments and the lowest measured new growth was observed at Location4 (2448.11 ± 1402.26 seg. yr^{-1}), see *Figure 19.* The Kruskal-Wallis test revealed, that the new growth in number of segments between the Locations 1-4 differs significantly ($H(3) = 9.409$, $p= 0.02432$). Significant differences were found between Location1 and Location4 ($M = 34477.929$, $SE= 2401.163$, $p= 0.035$).

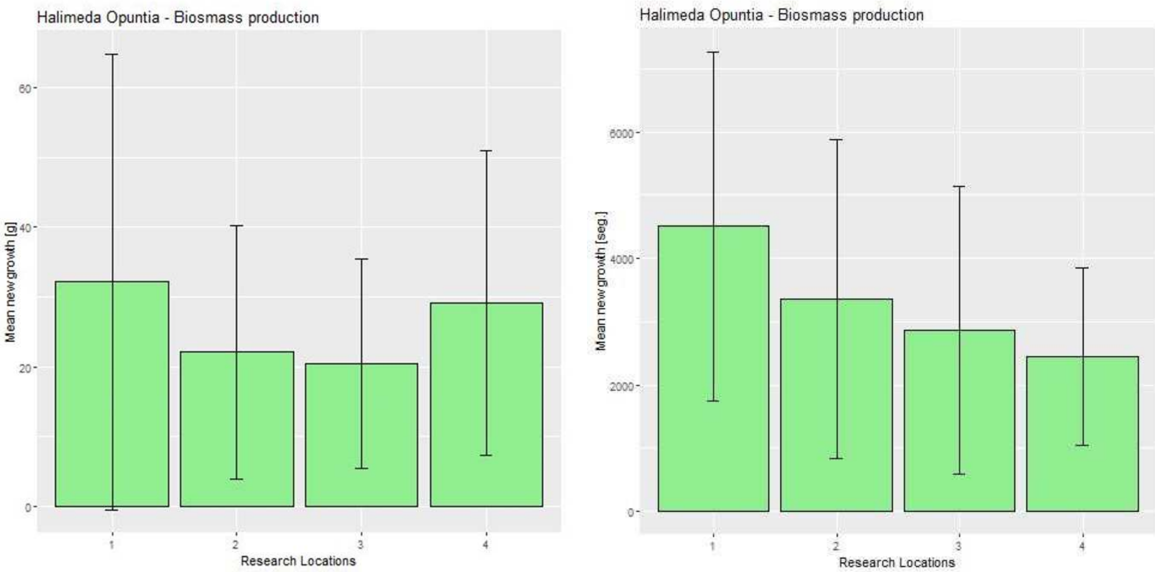


Figure 19: Left: Mean biomass production (g yr^{-1}) (± SE), of *H. opuntia* per Location (1-4). Right: Mean biomass production (seg. yr^{-1}) (± SE), of *H. opuntia* per Location (1-4).

***H. incrassata measurements and* new growth**

H. incrassata was investigated very limited and therefore, samples from locations (4&5) were analysed together (N=20). The growth measurements showed, that *H. incrassata* individuals were ø 6.7 cm high and had minimum width range from minimum ø 0.7 cm to a maximum of ø 4.8 cm. The dry weight of the samples was ø 3.62 g and they consisted of ø 229 segments. Furthermore, *H. incrassata* showed a new growth of ø 0.07g or ø 39 segments in a growth period of 7d. Adapted growth calculations showed an average new growth of (6.10 ± 4.65 g yr^{-1}) and (2747.93 ± 1781.87 seg. yr^{-1}).

4.2.2.2 Weight increase of *H. opuntia*

The data gathered during this research set up showed, that the method is unsuitable for the regrowth measurements of *H. opuntia* species. Results showed, that most samples decreased in weight and fell apart. Therefore, the data will be excluded from the results and not presented in this report.

4.2.3 Grammage *H. opuntia*

The dry weight of the *H. opuntia* squares adapted to a $1m^2$ was highest at Location1 with ø 9.30 kg, followed by Location2 with ø 4.80 kg. The square content of Location3 was ø 2.56 kg. Location4 displayed the lowest dry weight content with kg and ø 1.41 kg.

4.2.4 Biomass production *H. opuntia*

Per location (1-4) the annual biomass production of an individual. *H. opuntia* per $1m^2$ was calculated. Location1 showed the highest annual biomass production per individual of ø 31.6 kg/year/$1m^2$. Location2 showed a *H. opuntia* biomass production of ø 9.04 kg/year/$1m^2$.The biomass production of Location3 was ø 6.2 kg/year/$1m^2$ and the lowest measured biomass production was ø 3.82 kg/year/$1m^2$ at Location4.

4.2.5 $CaCO_3$ content *H. opuntia*

The analysis of the calcareous sediment production of *H. opuntia*, showed, that treated *H. opuntia* individuals (N= 50) were ø 7.08 cm in height, ø 5.35 cm in width and ø 3.38 cm in diameter. The number of segments per individual was ø 344.60. *H. opuntia* individuals had a dry weight of ø 9.54 g before the HCL treatment and ø 0.99 g afterwards. The $CaCO_3$ content was ø 8.55 g per individual. This results in a $CaCO_3$ content of *H. opuntia* (N= 50) of ø 90%.

4.2.6 Calcareous sediment production of *H. opuntia*

4.2.6.1 Calcareous sediment production of *H. opuntia* per individual

The average $CaCO_3$ content of 90% was fitted to the new growth of each Location (1-4) and projected to an annual calcareous sediment production of (28.93 ± 29.44 g $CaCO_3$ yr^{-1}) at Location1, (26.28 ± 19.66 g $CaCO_3$ yr^{-1}) at Location4, (19.87 ± 16.40 g $CaCO_3$ yr^{-1}) at Location2 and (18.33 ± 13.51 g $CaCO_3$ yr^{-1}) at Location3 per *H. opuntia* individual. The Kruskal-Wallis test revealed however, that the calcareous sediment production per *H. opuntia* individual, between the locations (1-4) does not differ significantly (H (3) = 2.6901, p= 0.4419). The difference in $CaCO_3$ production is visualized in *Figure 20.*

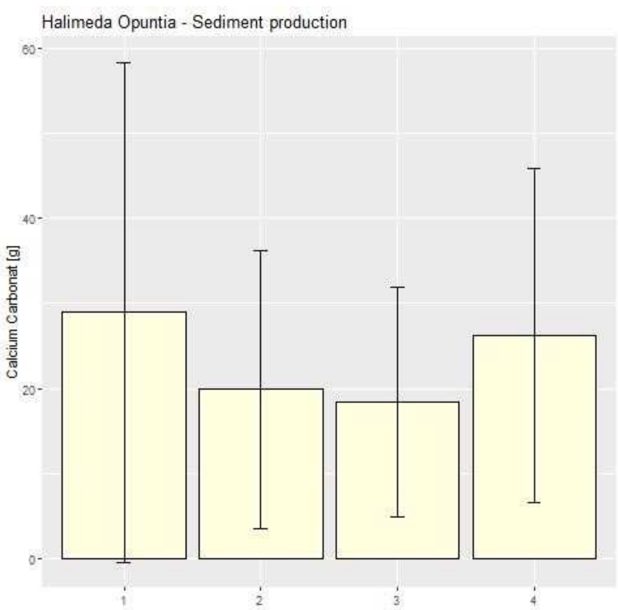


Figure 20: Mean calcium carbonate production of *H. opuntia* (g yr^{-1}) (± SE) per Location (1-4).

4.2.6.2 Calcareous sediment production of *H. opuntia* per 1m²

Location1 showed the highest calcareous sediment production of *H. opuntia* per 1m² with ø 28.44 kg. Location2 showed a *H. opuntia* calcareous sediment production of ø 8.14 kg. The calcareous sediment production of Location3 was ø 5.58 kg and the lowest measured calcareous sediment production was ø 3.43 kg at Location4.

4.2.7 Sediment displacement

The analysis of the sediment trap content collected at the research Location (1-4) showed, that the by far highest sediment displacement took place at Location2, where 205.09 g sediment were caught in 7d and 457.11 g sediment in 14d. The sediment at Location2 was white greyish and consistent of broken *Halimeda spp.* segments. At Location3 the second highest amount of sediment was caught with 32.05 g in 7d and 75.10 g in 14d. The sediment consisted of broken *Halimeda* spp. segments, fine sand and organic matter. In 7 days, 18.36 g of sediment were caught at Location1 and 37.87g in 14d. White coloured, broken pieces of *Halimeda* spp. dominated the sediment caught at Location1. The lowest amount of sediment with 1.53g, was caught in 7d at Location4, whereby the sediment collected in 14d with 38.75 was comparable with the amount caught at Location1, 14d. At Location4, the sediment was very fine and most particles were smaller than mesh size (<1mm). The caught sediment consisted mainly of larger organic material (like seagrass leaves).

4.3 Investigating *Halimeda* spp. in Lac Bay

4.3.1 Growth characteristics *H. opuntia*

The analysis of the pictures taken during the distribution and coverage measurements included the 18 grids covered with *H. opuntia* and 31 grids not covered with *H. opuntia*. Most of the covered grids showed growth characteristics comparable with Location3 and were therefore classified in K_3. Location1 however, had the lowest amount of associated grid points. The classification of each grid point, in one of the four key classes (K_1-K_4), can be seen in the following table.

Table 4: Grid points fitted to the growth key per location (1-4).

Key	**Grids**
K_1	B5
K_2	A3, B1, B2
K_3	A4, D2, F1, H1, I1, J2, J3, K3
K_4	C1, C4, E5, G1, H3, H6
0	A2, B3, B4, C2, C3, C5, D1, D3, D4, D5, E1, E2, E3, E4, F2, F3, F4, F5, G0, G2, G3, G4, G5, H2, H4, H5, I2, I3, I4, I5, I6

4.3.2 Biomass production of *H. opuntia* in Lac Bay

The annual biomass production was calculated per location (1-4) and projected to the grid area (300m x 400m) with a coverage of 100%. In the following table the results are displayed.

Table 5: Growth key per location, presenting the biomass production calculated for a 100% coverage.

Key	**Location**	**Biomass (C=100%)**
K_1	Location1	3.792.257 kg
K_2	Location2	1.084.851 kg
K_3	Location3	743.509 kg
K_4	Location4	457.899 kg

The results of the biomass production per location were fitted to the coverage per grid. Herby, grid B5 showed the highest biomass production with 3.438.440 kg/year and the lowest production was found at grid H3 and C1, with 708 kg/year. The biomass of all grids add together, revealed a biomass production in Lac Bay (580ha) of 5.010.181 kg/year. In the *Appendix D*, the results of the biomass production per grid can be found.

4.3.3 Calcareous sediment production of *H. opuntia* in Lac Bay

The calcareous sediment production was calculated per location (1-4) and projected to the grid area (300m x 400m) with a coverage of 100%. The results are displayed in the following table.

Table 6: Growth key per location, presenting the calcareous sediment production calculated for a 100% coverage.

Key	**Location**	**Calcareous sediment (C=100%)**
K_1	Location1	3.413.032 kg
K_2	Location2	976.366 kg
K_3	Location3	669.158 kg
K_4	Location4	412.109 kg

The results of the calcareous sediment production per location were fitted to the coverage per grid. Herby, grid B5 showed the highest calcareous sediment production with 3.094.596 kg/year and the lowest production was found at grid H3 and C1, with 701 kg/year. The calcareous sediment production of all grids add together, revealed a calcareous sediment production of 4.509.163 kg/year. In the *Appendix D,* the results of the calcareous sediment production per grid can be found. The calcareous sediment production in Lac Bay is presented in *Figure 21*. Hereby, the light blue areas, show no calcareous sediment production, whereby the light- brown to dark-brown areas have a low to high production of calcareous sediment.

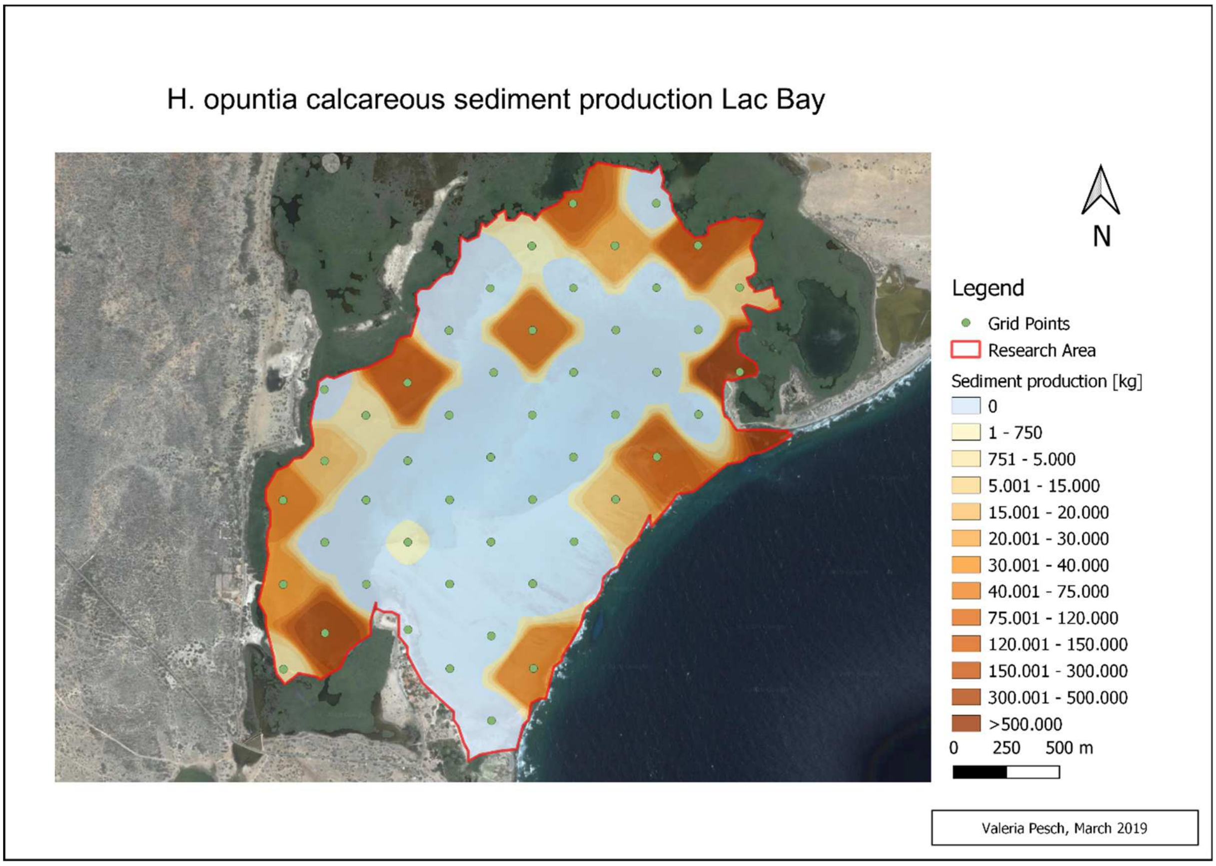


Figure 21: Calcareous sediment production of *H. opuntia* per grid

4.3.4 Extend of endogenous infilling by *H. opuntia* sediment

During the distribution and coverage analysis of *Halimeda* spp. measurements of the depth were taken at each grid point. The measured depth ranges from shallow (0.3m) to deep (5.0m) which can be seen in *Figure 22* and is indicated by the colour scheme (light blue-dark blue).

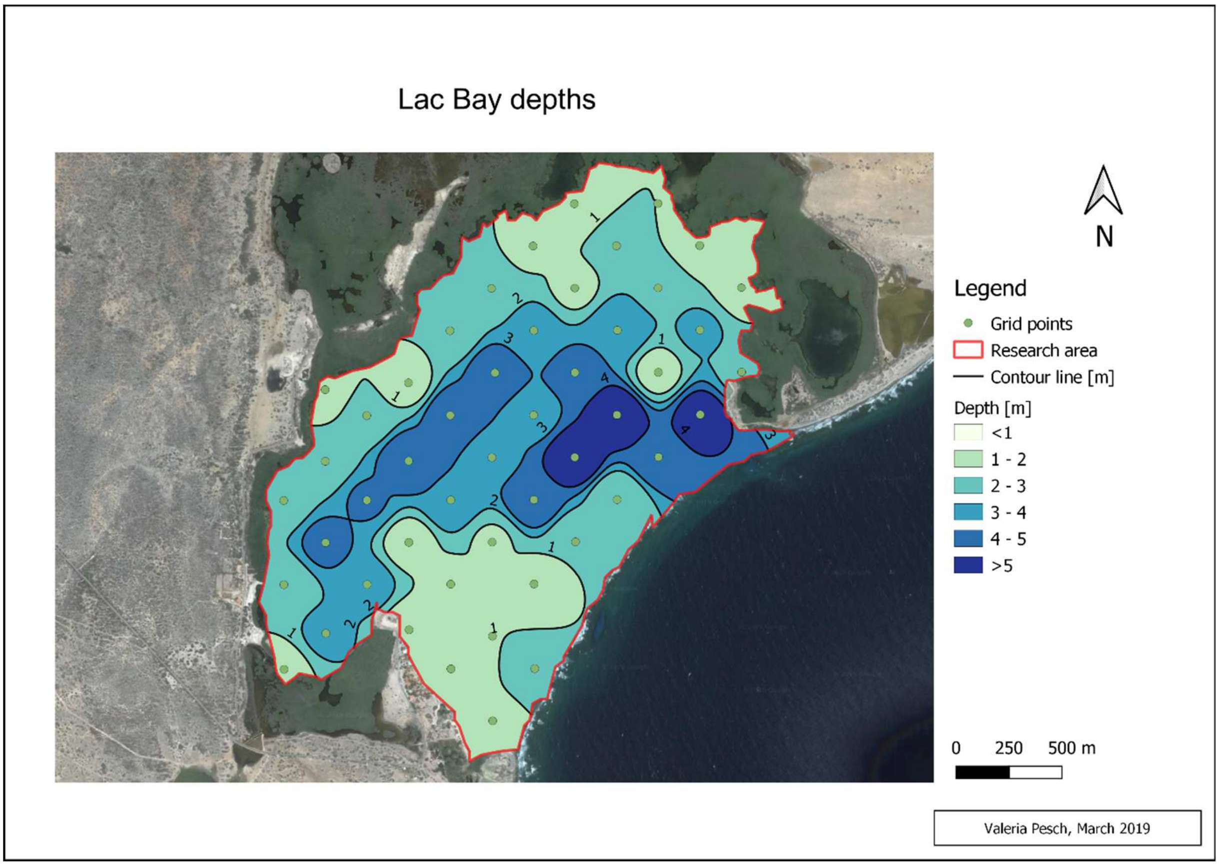


Figure 22: Calcareous sediment production of *H. opuntia* per grid

It is notable, that the shallowest areas, are the edges of Lac Bay in the north east and south west. From the edges, Lac Bay becomes continuously deeper until the deepest points at D4 and E4. At C5 in the north east, the inlet channel of Lac Bay is located. To determine, the depth change caused by the calcareous sediment production of *H. opuntia*, the sediment in kg/m^2 was translated into a production in g/m^3. The highest calcareous sediment infilling was found at grid B5, with a calcareous sediment increase of 8.801 mm /Year/m^2 and the lowest calcareous sediment increase was detected at grid H3, with 0.0019 mm /Year/m^2. In Lac Bay, the average calcareous sediment increase is 0.261 mm/Year/m^2. In the *Appendix* D, the infilling per grid can be seen. However, no maps of the depth change caused by the calcareous sediment production of *H. opuntia* were produced. This was due to the fact, that the calcareous sediment infilling was max. ≈ 9mm.

4.4 Management of *Halimeda* spp. in Lac Bay

4.4.1 Current management of *Halimeda* spp. in Lac Bay

In the following, a reflection on the current management policy of STINAPA on *Halimeda* spp. in Lac Bay will be given.

Management of Lac Bay

Lac Bay is part of the *Bonaire National Marine Park* (BNMP) and therefore managed by STINAPA (STINAPA, 2018a).

The Lac Bay area is protected by multiple legal frameworks, rules and regulations which protect species or habitat types in Lac Bay (Slijkerman et al., 2011). In the following the different conventions are listed.

- **Bonaire national marine park**
  (STINAPA, 2018b)
- **Ramsar Convention on Wetlands**
  (Debrot, Meesters & Slijkerman, 2010; Slijkerman et al., 2011)
- **Convention on Biological Diversity**
  (Slijkerman et al., 2011)
- **SPAW protocol of the Cartagena Convention**
  (Slijkerman et al., 2011)
- **Nature Ordinance of Bonaire**
  (Slijkerman et al., 2011)
- **Inter-American Convention for the Protection and Conservation of Sea Turtles**
  (Slijkerman et al., 2011)
- **Convention of Migratory Species**
  (Slijkerman et al., 2011)
- **IUCN IBA (Important Bird Area)**
  (Debrot, Meesters & Slijkerman, 2010; STINAPA, 2018c)
- **Marine park including Lac Bay: International Coral Reef Action Network (ICRAN) demonstration site**
  (STINAPA, 2018b)

Halimeda spp. in Lac Bay

Halimeda spp. in Lac Bay, are originally invasive (Verbruggen et al., 2009) and main producer of endogenous sediment (Debrot et al., 2012). Furthermore, did some researchers observed an increase of *Halimeda* spp. over time. This could be due to the Lac Bay´s eutrophicated waters, which increase *Halimeda* spp. abundance (Debrot et al., 2010).

Research and management of *Halimeda* spp. in Lac Bay

In the past, *Halimeda* spp. were poorly investigated in Lac Bay. Information on *Halimeda* spp. in Lac Bay are therefore very limited. *Halimeda* spp. have been described as part of other studies in Lac Bay. Debrot et al. (2012) for example mentions the role of *Halimeda* spp. in Lac Bay. Hylkema et al. (2014) delivers valuable information on the occurrence of *Halimeda* in "*Thalassia*/*Halimeda* habitat types". No literature could be found on the management strategy of STINAPA regarding *Halimeda* species. Recently, the interest of STINAPA on *Halimeda* spp. in Lac Bay has increased. Presumably due to the above-mentioned characteristics of *Halimeda* spp. and the ecological development of Lac Bay. Students are now investigation *Halimeda* spp. in the frame of the project "Ecological restoration Lac Bay and south coast, Bonaire" (Engel, 2017). A baseline study on *H. incrassata* was conducted in 2017 by Timmermans (2018).

Halimeda spp. as a threat

Halimeda spp. are invasive to the whole Caribbean (Verbruggen et al., 2009). In the eutrophic Spanish water of Curacao, *H. incrassata and H. opuntia* have caused an infilling of isolated parts (Debrot et al., 2012). *Halimeda* spp. are scarcely investigated, in contrast to other invasive species, like the seagrass *H. stipulacea* which is intensively investigated in Lac Bay (Christianen et al., 2018). Due to the fact, that *Halimeda* spp. are also invasive and increasing in number like *H. stipulacea, Halimeda* spp. should as well move into the focus of STINAPA. Similar areas to Lac Bay, like the Spanish water in Curacao, have experienced a *Halimeda* spp. induced infilling (Debrot et al., 2012). Considering the current ecological status of Lac Bay, this scenario could become a threat to Lac Bay too.

4.4.2 Adapted management of *Halimeda* spp. in Lac Bay

In the following, possible approaches for the management of *Halimeda* spp. in Lac Bay will be given.

Direct management approaches *Halimeda* spp.

A direct management approach to tackle spreading of *Halimeda* spp. in Lac Bay, is the manual removal. Due to the fact, that especially *H. opuntia* has filamentous holdfasts, which are often very loose or not even attached to the sediment, a removal can be done without heavy equipment. Furthermore, is the water depth considerably shallow at most spots. *H. opuntia* mounds could therefore be collected manually, while snorkelling (standing in the water). This would allow a low-cost, simple intervention approach. In the past, fisherman kept the channels with an extensive *Halimeda spp.* growth open, by removing their biomass (Debrot et al., 2012). It is conceivable, that this practice could be resumed. Furthermore, it is imaginable, to organize the removal with volunteer groups. There are already volunteer groups operating in Lac Bay, like the "Mangrove maniacs", who are working in the mangroves and could occasionally organize *Halimeda* spp. removals.

Indirect management approaches *Halimeda* spp.

Lac Bay is experiencing signs of eutrophication (Slijkerman et al., 2011). *Halimeda* spp. are known, to show an increased abundance in eutrophicated waters (Debrot et al., 2010). Furthermore, do the growth rate and the pigment concentration in *H. opuntia* increases, when the water is enriched with nutrients. This enables *H. opuntia* to populate deeper areas with less light penetration (Fricke et al.,2018). If the eutrophication in Lac Bay continuous the areas suitable for *H. opuntia* growth could extend and areas with a considerable low coverage could increase in biomass.
Therefore, the control of parameters like the nutrient influx should be managed. This is not only necessary to limit *H. opuntia* growth, but also to maintain the health of ecosystems like the mangroves. To improve the hydrology in Lac Bay, STINAPA is already clearing channels through the mangroves and is installing sediment traps on the road to Lac Bay (Engel, 2017). Furthermore, the water surface needs to be increased for the seagrass restoration, by the removal of accumulated sediment (Engel, 2017). This approach could also be applied in areas with a high amount of *Halimeda* spp. sediment. If *Halimeda* spp. become a threat to the Lac Bay ecosystem, several legal frameworks could be considered for funding of research projects and intervening measurements. To ensure a sustainable ecosystem development, a holistic ecosystem-based management approach (Kay, 2005) should be implied to manage *Halimeda* spp*.* in Lac Bay.

5 Discussion

Distribution and coverage analysis *Halimeda* spp. in Lac Bay

The *H. opuntia* map shows, that the species is mainly growing around the mangrove edges in the north, especially north-east and in the south-western part of Lac Bay. *H. incrassata* is present in the north, especially north- west and south-western part, along the mangroves. These findings confirm observations made by Hylkema et al. (2014), who describes a predominant occurrence of *Halimeda* spp. in the shallow water of the mangrove peripheral regions of the central bay. Moreover, do *Halimeda* spp. growth in the mangrove channels (Debrot et al., 2012). The greater *Halimeda* spp. coverage close to the mangroves can be explained by the higher nutrient availability in these areas. Mangrove forests act as nutrient traps (Debrot et al., 2010), which increases the nutrient concentration (Hogarth, 2015). *H. opuntia* is known to increase in numbers, when the water is eutrophicated (Debrot et al., 2010). Furthermore, does *H. opuntia* grow on the coral reef at the shoreline, which fits the sediment preference as a lithophyte species, that mainly grows on, or around hard substrate (Hillis-Colinvaux, 1980). *H. opuntia* populated the coral rubble, whereby *H. incrassata* was absent on this hard substrate. It was however striking, that *H. opuntia* also heavily populates areas with sandy or silty sediment. Most of the areas populated by *H. opuntia* showed a sandy or silty sediment composition. Though, no actual sediment analysis took place. The findings of this study do query the species preference and only occasional presence on sandy substrate described by Hillis-Colinvaux (1980). Observation showed, that *H. opuntia* often grew on degraded *Halimeda* spp. which could provide a stable ground for new growth. *H. incrassata* presents the highest coverage in the sandy, silty areas in the north. The sediment in the area consists of calcareous segments of *Halimeda* species. This confirms the species preference for soft substrate types (Hillis-Colinvaux, 1980, Perry et al., 2016).

With 90-100% coverage, *H. incrassata* also presents the highest coverage at the grid points A3, B1 and C1 and in the north, especially north- west and south-western part, along the mangroves. These results comply with the findings of Timmermans (2018), were with 200-530 *H. incrassata* individuals per m^2, the most comprehensive and highest coverage was detected at the grid points A1-A3 in the north. The findings by Timmermans (2018) could present a more detailed picture in the north, due to a different sampling technique used close to the mangrove edges.

When the distribution of *Halimeda* spp. is linked to the depth of Lac Bay, it can be seen, that there is a higher presence of both species in the shallow areas of Lac Bay. Especially the northern part which is less than 1m in depth, shows a high coverage of both species. This could also be explained by the availability of light.

Exact information on the distribution and coverage of *Halimeda* spp. in Lac Bay are scarce. It can be noted that maps from the past, including the distribution and coverage of *Halimeda* spp. in Lac Bay are rare. Though, there is a species distribution map including *Halimeda* spp., produced in 1969 by Wagenaar-Hummelink & Roos, which can be found in the *Appendix E.* This map shows the occurrence of *Halimeda* spp. in the north-east, in close distance to the mangroves and at the shallow reef at the edge of the lagoon. Furthermore, does the species distribution map show a sporadic *Halimeda* spp. presence throughout the whole bay. These observations do show a similar picture, like the occurrence analysis of Timmermans (2018) and distribution observations made during this research. In contrast to the distribution of *Halimeda* spp. in 1969, there is nearly no occurrence of *Halimeda spp.* throughout the bay in 2018 (Timmermans, 2018). During this research the same results were found. However, no differentiation between *Halimeda* spp., nor the coverage of *Halimeda* spp. was considered in the research by Wagenaar-Hummelink & Roos (1969).

For the distribution sampling of *H. opuntia* and *H. incrassata*, predetermined grids (300 x 400 meters) were sampled 6 times with an $1m^2$ PVC frame. At some locations this method seemed applicable, due to a relatively uniform picture of the sea bottom coverage. However, several grids displaced a different picture, with changing sedimentation and vegetation every few meters. During sampling, these factors were considered, though due to the enormous variety at some spots might be misrepresented. For a more efficient, less time intense sampling, the 6 samples divided between the two researchers. Though the researchers experience were equal, personal assessment of the samples could have been subjective. Sampling was done by visual inspection of the morphological features of *H. opuntia* and *H. incrassata*. This method revealed some issues concerning the accuracy, especially at locations with environmental influences like a strong serge, or ingrowing vegetation. However, at locations with a better visibility, pictures of the measurements can be taken for later investigation with a coverage analysis software like CPCe (Coral Point Count with Excel extensions) (Kohler, 2006). This would decrease the time needed at a site and would allow a more accurate way of gathering the data. The maps produced for the distribution and coverage visualization have been produced with the IDW function of QGIS. This interpolation estimates the coverage in between the grid points. The visual presentation of the coverage in between the grid points, could therefore be misleading.

Regrowth experiment *H. opuntia*

During the regrowth measurements, Location 1-4 did not show signs of new growth of *H. opuntia* at any monitoring point. However, the cleared space offers opportunities for other species of algae and seagrass to take over. The ingrowth pressure of the surrounding *H. opuntia* can be explained by the propagation of this species, which is marked by fusion of the segments. New growth does not occur by spreading through the sediment. This can be explained by the fact, that *H. opuntia* as an algae does not spread through the holdfast, which is mostly not even anchored in the sediment (Hillis-Colinvaux, 1980).

Weight increase of *H. opuntia*

Originally the water displacement method should have been used, to evaluate the volume change of *H. opuntia* over time. This method however showed some flaws according the accuracy in volume change. In some cases, new grown fragments are very light, like experienced during this research (smaller 10mg) where multiple segments showed nearly no weight and no change in water displacement could be seen. Results showed, that most samples decreased in weight, which was probably due to a loss of biomass, caused by physical forces. To measure a weight increase, or the volume of *H. opuntia* species over time, the method applied during this research needs to be adapted. Due to the quite inflexible nature of this algae, caused by the calcified segments, shape and size can change quite frequently. Segments naturally shed or break off due to physical forces (Hillis-Colinvaux, 1980). New grown segments can weight <0,001g, which can most scales cannot detect. Furthermore, there is still the natural or forced loss of biomass. To cope with this, it would be imaginable to put the specimen individually in bags with a small mesh size, were shed segments will be collected. This could however influence their ability to photosynthesize and might restrict water flow. Though not suitable for *H. opuntia*, the growth characteristics of *H. incrassata* seem to show better preconditions. They do have an actual holdfast, can therefore be embedded again and their structure is more stable.

Discussion individual growth

H. opuntia growth characteristics differ, depending on the location where they occur. Different to *H. incrassata*, which has a strong holdfast and distinctive thallus, *H. opuntia* lacks a thallus and anchors itself with filamentous holdfasts, which makes it hard to determine an individual. During this research *H. opuntia* specimen were considered as an individual, when they did not show multiple filamentous holdfasts. However, the holdfast can hardly be identified as one and most *H. opuntia* specimen have multiple holdfasts. Even if it was tried, to choose specimen with a single holdfast, it was not always workable. Literature which defines an individual *H. opuntia* specimen was not found. Furthermore, is it questionable, if excessive *H. opuntia* patches like at Location1 are even identifiable as individuals. *H. opuntia* individuals collected during this research, were all in the dame size rang. This is due to the Alizarin set up, which was restricted by the size of the PVC pipe. Furthermore, the 50 individuals collected for the carbonate content analysis were in the same size range as the *H. opuntia* samples, to have a similar growth characteristic. This focus on a certain size range could lead to a misrepresentation of the actual biomass and calcareous sediment production, which could be different in the thick *H. opuntia* mounds. Besides, the age of the specimen was not considered. Differences in age could lead to different growth rates/calcification rate. Though, there was no literature found discussing this topic on *H. opuntia. H. opuntia* growth characteristics differ depending on the location where they occur. Different to *H. incrassata*, which has a strong holdfast and distinctive thallus, *H. opuntia* lacks a thallus and anchors itself with filamentous holdfasts, which makes it hard to determine an individual. If sediment produced at the mangrove edges gets transported to the open bay, with no *Halimeda spp.* occurrence the might not even cause a threat to an infilling and could even be a useful in terms of providing sediment for other species to growth in. During this research it was observed, that locations that expertise a greater surge growth smaller and thicker (Location4). *H. opuntia* growing on the coral reef also showed a more horizontal growth. Furthermore, it was noticeable that *H. opuntia* segments showed different shapes and sizes, which is typical for their species. However, there were similarities between locations with calm water and more sunlight (1&2) where segments sometimes were crown shaped and very small. Locations with a greater surge showed the typical *H. opuntia* form and segments were bigger. They were also thicker and more calcified.

Sediment displacement

During this research the sediment caught in the traps, was solely weighted. Neither the sediment composition, particle size, nor the sediment origin was analyzed. This limited the analysis and did not allow a valid statement about the sediment transport in Lac Bay. If sediment produced at the mangrove edges gets transported to the open bay, with no *Halimeda* spp. Occurrence, they might not even cause a threat to an infilling and could even be a useful in terms of providing sediment for other species to growth in.

Total sediment production by H. opuntia in Lac Bay and contribution to the overall infilling

Research by Timmermans (2018) also pointed out, that there has been an overall decrease of 40cm in depth of Lac Bay during the last fifty years. *H. opuntia* contributes ø 0.261 mm/Year/m^2 to that decrease, which seems to be an insignificant amount. The depth of Lac Bay is not significantly influenced by the sedimentation of *Halimeda* species.

Management

The eutrophication of Lac Bay (Slijkerman et al., 2011) might lead to an ongoing increase of *Halimeda* spp. (Debrot et al., 2010). The manual removal would be appropriate at locations with an extensive *H. opuntia* growth and important position particularly interesting. Location1 for e.g. is in a channel that leads to the back mangroves and were an infilling could hinder the water flow to the back mangroves. Here a removal could be vital, to allow an appropriate water flow and mixing of the water. At other locations, were *H. opuntia* just shows a very limited growth and is not hindering the water flow, a removal is probably not necessary. The manual removal seems to be a cost effective, minimally invasive, long-term approach, to tackle the continuous infilling with *H. opuntia* sediment at sensitive locations.

6 Conclusion

1. a) What is the spatial distribution of *H. opuntia* and *H. incrassata* in Lac Bay?

At more than half of the visited grids *Halimeda* spp. were present. However, *H. incrassata* was growing at more grids than *H. opuntia.* Both species were primarily growing around the mangrove edges in the north and south and at the reef crest. This was not unexpected, due to the information of other studies, that described a presence of *Halimeda* spp. around the mangrove edges in Lac Bay.

1. b) What is the coverage of *H. opuntia* and *H. incrassata* throughout Lac Bay?

Data on the coverage of *H. opuntia* in Lac Bay was unavailable. Though, descriptions of thick *H. opuntia* mounds were found in literature. The exact location of these mounds and the coverage throughout Lac Bay were unknown. It was found, that the highest coverage of *H. opuntia* was in the north east and in the south. Due to the knowledge of another research on *H. incrassata* is was expected, that the species shows the highest coverage around the mangroves in the north-west. This expectation was found to be true and could be due to the greater availability of nutrients near the mangroves and the relatively shallow water. *H. incrassata* showed a higher average coverage within the grids, than *H. opuntia*.

1. c) What are the growth characteristics of *H. opuntia* in Lac Bay and do they differ between locations?

No information on the different growth characteristics of *H. opuntia* in Lac Bay were given. Investigations of the research location (1-4) revealed however, that *H. opuntia* shows different growth characteristics between the research locations. The fact, that *Halimeda* spp. show various morphological features was described in literature and therefore expected. Though, the range in height and width of *H. opuntia* was unexpected wide.

After the manual removal of *H. opuntia* species, no new growth within a 2-month period could be detected at any of the research locations (1-4). However, the installed squares experienced an ingrowth pressure from surrounding *H. opuntia* specimen. This seems logical, due to the fact, that *H. opuntia* is a sprawling species and as an alga does not have roots for propagation. *H. opuntia* individuals analysed within the Alizarin Red-S set up were in the same size range and had therefore a predictable average size and dry weight range. During the alizarin Red-S staining experiment, the new growth of *H. opuntia* segments in seven days was measured. The average weight of new grown segments did not differ significantly between the four research locations. However, the difference in numbers of new grown segments did differ significantly between locations with the highest number of new grown segments at Location1 and lowest at Location4. However, the segment counting method is not very accurate and therefore the differences in weight should be considered. To compensate for the limitations of the Alizarin Red- S setup, the grammage per research location was also taken into account. This guaranteed a more realistic picture of the actual growth at each location. The grammage between the research locations (1-4) varied a lot and was highest at Location1 and lowest at Location4. Furthermore, was the annual biomass production per individual also highest at Location1 and lowest at Location4. *H. opuntia* consisted nearly entirely of calcium carbonate. This high percentage was also found in other studies on the species. Literature suggested, that the sediment displacement varies greatly in Lac Bay. The results of the sediment displacements analysis showed the same picture, whereby Location2 experienced a way greater sediment displacement as the other locations. The lowest amount of sediment was caught at location4. This is perfectly logical due to the differences in particle size and composition of sediment at each location

(1-4). Furthermore, does the hydrology between the locations vary greatly. However, no actual sediment analysis, nor current analysis took place.

1. d) How much biomass is produced by *H. opuntia*?

The biomass produced by *H. opuntia* various greatly between the research locations. The minimum amount of biomass was produced at Location4 and the maximum at Location1. When the appropriate growth key was fitted to the coverage of the grid, Grid B5 showed the highest biomass production in kg and grid H3 and C1 the lowest. Add together, the grids had a biomass production of 5.010.181 kg/year.

1. e) How much calcareous sediment is produced by *H. opuntia*?

Halimeda spp. store $CaCO_3$ in form of aragonite. Like described by literature, treated *H. opuntia* individuals mainly consisted of $CaCO_3$. The $CaCO_3$ sediment production of an *H. opuntia* individuals between the four research locations was investigated and no significant differences were found. However, the calcareous sediment production between the research locations differs massively, whereby the greatest amount of $CaCO_3$ sediment was produced at Location1 and the lowest at Location4. When the appropriate growth key was fitted to the coverage of the grid, the calcareous sediment production per grid could be determined. Grid B5 showed herby the highest annual $CaCO_3$ sediment production in kg and grid H3 and C1 the lowest. Add together, the grids had a $CaCO_3$ sediment production of 4.509.163 kg/year.

1. f) How is the depth of Lac Bay influenced by the sediment production of *H. opuntia?*

Due to the fact, that Lac Bay is a shallow lagoon, assumptions were made, that the calcareous sediment production of *H. opuntia* would decrease the depth of Lac Bay greatly. Though, the annual depth increase with *H. opuntia* sediment in Lac Bay, is with less than 0.5mm considerably insignificant. At some grids however, the infilling with calcareous sediment was much higher with more than 8mm per year and m^2.

1. What is the total sediment production by *H. opuntia* in Lac Bay and what contribution to the overall infilling of endogenous sediment does it represent?

The results of the calcareous sediment production of *H. opuntia* in kg seem relatively high. However, due to the low density of the stored aragonite in the segments, the sediment depth increase per year is minimal. Furthermore, are great parts of Lac Bay not covered with *H. opuntia* species, which explains the moderate production of sediment. However, at production rates vary within Lac Bay. Certain locations with mounds of *H. opuntia* may experience an increased infilling with calcareous *H. opuntia* sediment.

2. a) How are *Halimeda* spp. currently managed in Lac Bay?

Currently, STINAPA does not have a management approach regarding *Halimeda* spp. in Lac Bay. *Halimeda spp.* are partly described in other studies. Research on the species is however very limited. In the frame of the “Ecological restoration Lac Bay and south coast, Bonaire” students started investigating *Halimeda* spp*.* in Lac Bay. The first research was conducted on *H. incrassata.*

2. b) How could the *Halimeda* spp. management be adapted?

Halimeda spp. could either be managed using a direct management approach like the manual removal, or indirect approach by managing the environmental factors, that lead to a *Halimeda* spp. increase. *Halimeda* spp. should be seen as part of the ecosystem and therefore managed using a holistic approach.

2. How could *Halimeda* spp. be managed regarding the endogenous infilling of Lac Bay?

Though, the infilling with calcareous *H. opuntia* sediment throughout is not a threat yet, local sedimentation could be an issue already and might become an issue for the Lac Bay ecosystem in the future. Especially if eutrophication continuous and *Halimeda* spp. spread. Direct management approaches like the manual removal could provide long term solution. Indirect management approaches would tackle abiotic factors, like eutrophication, which are also important for other species. *Halimeda* spp. become part of other investigations and projects in Lac Bay. However, more research on *Halimeda* spp*.* is needed. This would also enable STINAPA to set up an appropriate management approach for *Halimeda* species.

7 Recommendations

Replication study

To overcome the limitations experienced during this study suggestions for improvement for a replication study are given in the following. For a more accurate reflection of the coverage and distribution of *Halimeda* spp. in Lac Bay, it is recommended to choose a smaller grid size, or scale up the number of measurements taken within each grid. Moreover, it is advised to analyse the measurements by taking pictures of the PVC frames, for later analysis with a software like CPCe. The research material was limited during the field research and analysis part of this study. For a replicate study it would be desirable, to improve the research material availability and ensure, that *Halimeda spp.* can be analysed under laboratory conditions.

During this study the terminology for an individual species of *H. opuntia* was based on easy identifiable morphological features, which were important for this research. How to scientifically identify *H. opuntia* individuals based on their morphology is unknow. Therefore, a further investigation into the topic is necessary.

Due to the limitations of the alizarin Red-S research set up, individuals were all in the same size range and did not reflect the actual growth characteristics at a site. Therefore, it is recommended to adapt the alizarin Red-S research set up, by for e.g. developing a larger construction, to include different growth characteristics.

If the alizarin Red-S staining method is applied in further research on calcifying species, it is recommended, to experiment with the amount of Alizarin Red-S being used. Due to the high costs of the alizarin Red-S stain, it would be desirable to determine the minimum quantity needed, to successfully conduct the new growth measurements. During this research, *H. incrassata* was investigated in a very limited extend. Though, did the alizarin Red-S stain results show, that the method is appropriate for the species new growth measurements. It is recommended to apply the method in future research on *H. incrassata.*

During the *H. opuntia* investigation, segments showed a range of different shapes, sizes and weight. Due to lack of time and manpower it was impossible to investigate *H. opuntia* segments in depth. It is therefore recommended to analyse the growth characteristics of the segments.

The sediment displacements method was very basic and neither the sediment samples particle size, nor composition was analysed. For future research it is recommended to install more sophisticated sediment traps and analyse the sediment and its composition. Furthermore, were the origin of the sediment and abiotic factors at sight unknow. It would be necessary to identify the dynamics of sediment transport to understand, if the *Halimeda* spp. sediment causes a local infilling.

Management

So far, there are no management approaches in place, that concern *Halimeda* spp. in Lac Bay. However, the results of this research show, that *Halimeda* spp. should be included in a holistic, ecosystem, based management approach of Lac Bay. Therefore, *Halimeda* spp. research should be implemented in ongoing projects. Furthermore, it is necessary to frequently monitor *Halimeda* spp. in Lac Bay, to assess changes in their coverage and distribution. If interventions concerning the distribution and coverage of *H. opuntia* are necessary, it is recommended to consider short-term and long-term approaches. A direct, straight forward approach is the manual removal of *H. opuntia* mounds, which implementation should be further investigated. The eutrophication of Lac Bay, which possibly causes an increase of *Halimeda* spp. should be tackled using a long-term holistic approach.

Follow-up research

Due to the nearly complete lack of data concerning *Halimeda* spp. in Lac Bay, follow-up research topics are numerous. First, it is recommended to replicate this study with adapted methods and to gather more quantitative data on the distribution of *H. opuntia* and *H. incrassata* in Lac Bay. Besides, the growth characteristics of both species should be further investigated. Furthermore, it is advised to in assess the growth dynamics between *H. opuntia* and *H. incrassata*. During this research no abiotic parameters were analysed. Therefore, it would be interesting to investigate abiotic parameters at *Halimeda* spp. locations and link them for e.g. to the growth characteristic of *H. opuntia.* So far, no data on the competition of *Halimeda* spp. with other species has been gathered. Especially information about the competition with threatened seagrass and coral species could be valuable. A research concerning this topic is therefore advised. To fully understand *Halimeda* as a species, it is advised, to link research on *Halimeda* spp. with other topics, like eutrophication, terrigenous sedimentation and the overall endogenous sediment production in Lac Bay. Furthermore, should the role of *Halimeda* spp. be investigated within the different ecosystems present in Lac Bay, like the coral reefs, seagrass fields and mangrove forests.

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Appendices

Appendix A: Further stakeholder

In the following table an overview of the stakeholders with a primarily economical interest are presented.

Local businesses

Stakeholder	**Interest**
Bonaire Windsurf place	Locally owned windsurf school operating at Sorobon, Lac Bay (Bonaire Windsurf Place, 2018).
Local Fisherman	The family Solina has a little bar at Cai beach, were the sell beverages.
Jibe City	Operate at Sorobon, Lac Bay. This includes: windsurfing, a shop and a bar. (Jibe city, 2017)
Horse Ranch Bonaire	Offer Horseback riding and swimming in Lac Bay (Horse Ranch Bonaire, 2013).
Kayak	Offer guided kayak and solar boat tours (inclusive snorkeling) though the mangrove forest of Lac Bay. Follow zoning plan and “Code of Conduct for Kayakers” provided by STINAPA (The Mangrove Info Center, 2017).

Private sector

Stakeholder	**Interest**
Short term tourism and Recreational tourism	Especially the short-term cruise ship tourism and their interest in Lac Bay has increased in the last years (Debrot et al., 2010). Sorobon in the Lac Bay area is a touristic hotspot (Slijkerman et al., 2011).
Locals	Locals use the Lac Bay for recreation.

Appendix B: GPS data

The table below shows the original labelling of the 49 grid points(Site ID old), the adapted Site ID, GPS coordinates and depth(m) measured at each grid point.

Site ID (old)	**Site ID**	**East**	**North**	**Depth in m**
Ra	A2	12,09250E	-68,24167N	1
Ua	A3	12,09611E	-68,24167N	0,4
Wa	A4	12,09972E	-68,24167N	0,5
Ja	B1	12,09403E	-68,23986N	0,5
Na	B2	12,09792E	-68,23986N	1,2
Ta	B4	12,10139E	-68,23986N	2,1
Va	B5	12,10444E	-68,23986N	1,7
Ea	C1	12,09611E	-68,23806N	0,9
Ia	C2	12,09972E	-68,23806N	0,8
Ma	C3	12,10333E	-68,23806N	2,9
Oa	C4	12,09417E	-68,23625N	0,6
Sa	C5	12,09792E	-68,23625N	4,5
Y	D1	12,10139E	-68,23625N	1,1
Da	D2	12,10472E	-68,23625N	2,4
Ha	D3	12,09250E	-68,23444N	3,3
Qa	D3	12,09611E	-68,23444N	1,8
La	D4	12,09972E	-68,23443N	4,9
Pa	D5	12,10333E	-68,23444N	3,8
S	E1	12,10694E	-68,23444N	1
X	E2	12,09028E	-68,23264N	3,6
Ca	E3	12,09389E	-68,23264N	2,6
Ga	E4	12,09792E	-68,23264N	4,9
Ka	E5	12,10153E	-68,23264N	--
N	F1	12,10514E	-68,23250N	0,6
R	F2	12,10875E	-68,23264N	3,8
W	F3	12,09250E	-68,23081N	2,7
Ba	F4	12,09611E	-68,23083N	3,8
Fa	F5	12,09972E	-68,23083N	--
G	G0	12,10333E	-68,23083N	0,8
J	G1	12,10694E	-68,23081N	1,1
M	G2	12,11056E	-68,23083N	3,9
Q	G3	12,09792E	-68,22903N	2,9
V	G4	12,10153E	-68,22905N	--
Aa	G5	12,10514E	-68,22903N	0,6
F	H1	12,10875E	-68,22903N	1
I	H2	12,11236E	-68,22904N	3,1
L	H3	12,09972E	-68,22722N	0,5
P	H4	12,10333E	-68,22722N	--
U	H5	12,10694E	-68,22719N	--
Z	H6	12,11056E	-68,22722N	--
C	I2	12,10514E	-68,22542N	1,1
E	I3	12,10153E	-68,22542N	3,3
H	I3	12,10875E	-68,22542N	2,5
K	I4	12,11236E	-68,22542N	0,3
O	I5	12,10333E	-68,22361N	--
T	I6	12,10694E	-68,22361N	0,9
B	J2	12,11056E	-68,22361N	1,1
D	J3	12,10514E	-68,22181N	2,2
A	K2	12,10875E	-68,22181N	0,6

Appendix C: *H. opuntia* grammage

Location 1:			
Sample	**Date**	**Dry weight *H. opuntia* in kg**	**Notes**
Sample 1.1	05-11-2018	0.530 kg	
Sample 1.2	05-11-2018	0.695 kg	
Sample 1.3	05-11-2018	0.570 kg	
Sample 1.4	05-11-2018	0.455 kg	
Sample 1.5	05-11-2018	0.655 kg	

Location 2: Fogon?			
Sample	**Date**	**Dry weight *H. opuntia* in kg**	**Notes**
Sample 2.1	07-11-2018	0.475 kg	
Sample 2.2	07-11-2018	0.020 kg	*H. incrassata* present in frame: 0.345 kg *H.incrassata*
Sample 2.3	07-11-2018	0.255 kg	
Sample 2.4	07-11-2018	0.575 kg	
Sample 2.5	07-11-2018	0.195 kg	

Location 3			
Sample	**Date**	**Dry weight *H. opuntia* in kg**	**Notes**
Sample 3.1	07-11-2018	0.115 kg	
Sample 3.2	07-11-2018	0.255 kg	
Sample 3.3	07-11-2018	0.175 kg	
Sample 3.4	07-11-2018	0.160 kg	
Sample 3.5	07-11-2018	0.095 kg	

Location 4			
Sample	**Date**	**Dry weight *H. opuntia* in kg**	**Notes**
Sample 4.1	08-11-2018	0.200 kg	
Sample 4.2	08-11-2018	0.100 kg	
Sample 4.3	08-11-2018	0.020 kg	*H. incrassata* present in frame: 0.030 kg *H.incrassata*
Sample 4.4	08-11-2018	0.055 kg	
Sample 4.5	08-11-2018	0.065 kg	

Appendix D: Gathered data per grid

Site-ID	X	Y	Depth	Coverage HO	Coverage HI	Key	new growth	sediment production	Sediment Hight [cm]	100% Coverage	Sediment Volume [cm³]	Site-ID old	Coverage HO [%]	Coverage HI [%]
A2	12.11236	-68.22542	1	0	0,0283	0	0	0	0,00E+00	0	0	Ra	0	2,83
A3	12.11056	-68.22361	0,4	0,3033	0,6233	2	329.035	296.132	2,47E-06	1.084.851	101.068.857	Ua	30,33	62,33
A4	12.10875	-68.22181	0,4	0,02	0,29	3	14.870	13.383	1,12E-07	743.509	4.567.632	Wa	2	29
B1	12.11236	-68.22904	0,5	0,1233	0,9133	2	133.762	120.386	1,00E-06	1.084.851	41.087.340	Ja	12,33	91,33
B2	12.11056	-68.22722	1,2	0,03	0,2833	2	32.546	29.291	2,44E-07	1.084.851	9.996.920	Na	3	28,33
B3	12.10875	-68.22542	1,8	0	0,0583	0	0	0	0,00E+00	0	0	Qa	0	5,83
B4	12.10694	-68.22361	2,1	0	0,02	0	0	0	0,00E+00	0	0	Ta	0	2
B5	12.10514	-68.22181	1,7	0,9067	0	1	3.438.440	3.094.596	2,58E-05	3.792.257	1.056.176.032	Va	90,67	0
C1	12.11056	-68.23083	0,9	0,0017	0,8083	4	778	701	5,84E-09	457.899	239.108	Ea	0,17	80,83
C2	12.10875	-68.22903	0,8	0	0,0333	0	0	0	0,00E+00	0	0	Ia	0	3,33
C3	12.10694	-68.22719	2,9	0	0	0	0	0	0,00E+00	0	0	Ma	0	0
C4	12.10153	-68.22542	3,8	0,3117	0	4	142.727	128.454	1,07E-06	457.899	43.841.087	Oa	31,17	0
C5	12.10333	-68.22361	4,5	0	0	0	0	0	0,00E+00	0	0	Sa	0	0
D1	12.10875	-68.23264	1,1	0	0	0	0	0	0,00E+00	0	0	Y	0	0
D2	12.10694	-68.23081	2,4	0,1517	0	3	112.790	101.511	8,46E-07	743.509	34.645.486	Da	15,17	0
D3	12.10514	-68.22903	3,3	0	0	0	0	0	0,00E+00	0	0	Ha	0	0
D4	12.10333	-68.22722	4,9	0	0	0	0	0	0,00E+00	0	0	La	0	0
D5	12.10514	-68.22542	0,6	0	0	0	0	0	0,00E+00	0	0	Pa	0	0
E1	12.10694	-68.23444	1	0	0,015	0	0	0	0,00E+00	0	0	S	0	1,5
E2	12.10514	-68.23250	3,6	0	0	0	0	0	0,00E+00	0	0	X	0	0
E3	12.10333	-68.23083	2,6	0	0,005	0	0	0	0,00E+00	0	0	Ca	0	0,5
E4	12.10153	-68.22905	4,9	0	0	0	0	0	0,00E+00	0	0	Ga	0	0
E5	12.09972	-68.22722	1	0,045	0,1167	4	20.605	18.545	1,55E-07	457.899	6.329.320	Ka	4,5	11,67
F1	12.10472	-68.23625	0,6	0,3	0,0567	3	223.053	200.747	1,67E-06	743.509	68.514.474	N	30	5,67
F2	12.10333	-68.23444	3,8	0	0	0	0	0	0,00E+00	0	0	R	0	0
F3	12.10153	-68.23264	2,7	0	0,1083	0	0	0	0,00E+00	0	0	W	0	10,83
F4	12.09972	-68.23083	3,8	0	0	0	0	0	0,00E+00	0	0	Ba	0	0
F5	12.09792	-68.22903	1	0	0	0	0	0	0,00E+00	0	0	Fa	0	0
G0	12.10444	-68.23986	0,8	0	0,06	0	0	0	0,00E+00	0	0	G	0	6
G1	12.10333	-68.23806	1,1	0,01	0,002	4	4.579	4.121	3,43E-08	457.899	1.406.515	J	1	0,2
G2	12.10139	-68.23625	3,9	0	0	0	0	0	0,00E+00	0	0	M	0	0
G3	12.09972	-68.23443	2,9	0	0	0	0	0	0,00E+00	0	0	Q	0	0
G4	12.09792	-68.23264	0,9	0	0	0	0	0	0,00E+00	0	0	V	0	0
G5	12.09611	-68.23083	0,6	0	0	0	0	0	0,00E+00	0	0	Aa	0	0
H1	12.10139	-68.23986	1	0,0267	0,0133	3	19.852	17.867	1,49E-07	743.509	6.097.788	F	2,67	1,33
H2	12.09972	-68.23806	3,1	0	0	0	0	0	0,00E+00	0	0	I	0	0
H3	12.09792	-68.23625	0,5	0,0017	0,4067	4	778	701	5,84E-09	457.899	239.108	L	0,17	40,67
H4	12.09611	-68.23444	0,8	0	0	0	0	0	0,00E+00	0	0	P	0	0
H5	12.09389	-68.23264	1	0	0	0	0	0	0,00E+00	0	0	U	0	0
H6	12.09250	-68.23081	1,2	0,17	0	4	77.843	70.059	5,84E-07	457.899	23.910.763	Z	17	0
I1	12.09972	-68.24167	1,1	0,0767	0,235	3	57.027	51.324	4,28E-07	743.509	17.516.867	C	7,67	23,5
I2	12.09792	-68.23986	3,3	0	0	0	0	0	0,00E+00	0	0	E	0	0
I3	12.09611	-68.23806	2,5	0	0	0	0	0	0,00E+00	0	0	H	0	0
I4	12.09417	-68.23625	0,3	0	0	0	0	0	0,00E+00	0	0	K	0	0
I5	12.09250	-68.23444	0,4	0	0	0	0	0	0,00E+00	0	0	O		
I6	12.09028	-68.23264	0,9	0	0	0	0	0	0,00E+00	0	0	T	0	0
J2	12.09611	-68.24167	1,1	0,0483	0,155	3	35.911	32.320	2,69E-07	743.509	11.030.830	B	4,83	15,5
J3	12.09403	-68.23986	2,2	0,485	0,0017	3	360.602	324.542	2,70E-06	743.509	110.765.067	D	48,5	0,17
K2	12.09250	-68.24167	0,6	0,0067	0,0033	3	4.982	4.483	3,74E-08	743.509	1.530.157	A	0,67	0,33
							5.010.181	4.509.163	0,000001		1651707918			

Appendix E: Lac Bay species distribution

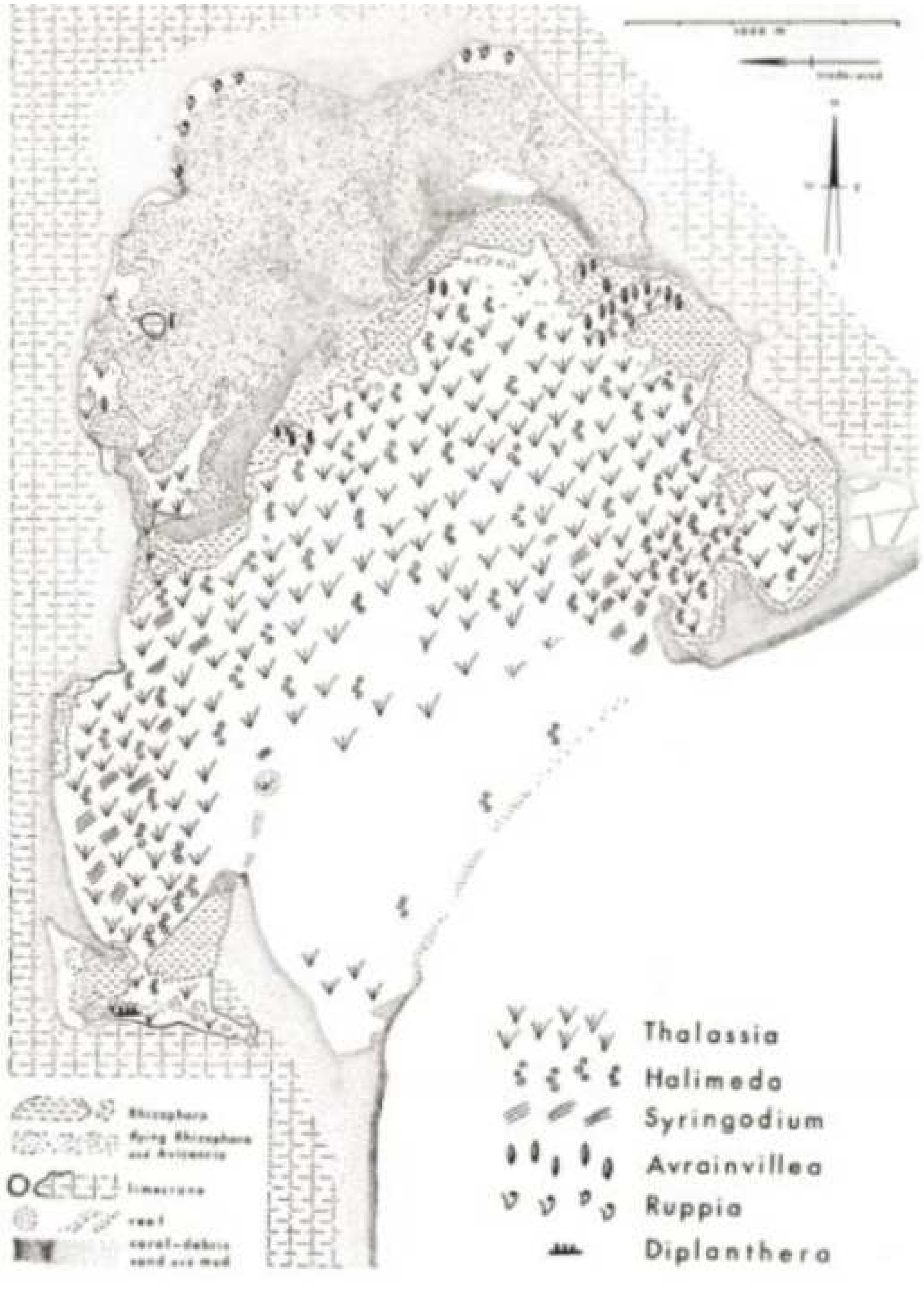

Figure 3 Species distribution map (Wagenaar-Hummelink & Roos, 1969).