

# Eutrophication status of Lac, Bonaire, Dutch Caribbean Including proposals for measures

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## Summary

Lac is a semi-enclosed lagoon located on the south-eastern side of Bonaire, and contains a diversity of shallow water coral reef associated habitats in close proximity such as mangroves, seagrass beds, *Halimeda* algal beds, the back reef and sand flats. These habitats support a diversity of fish and invertebrates. The bay has numerous international and national legal protections. The Bonaire National Marine Park regulations and various Island Decrees facilitate from the local perspective. However, despite all regulations, the bay faces several changes, and management and protection of the bay is hampered by a lack of scientific information regarding current environmental status.

Nutrient poor waters are a requirement for healthy coral reefs. When these become enriched with nutrients, it results e.g. in increased algae and affected reef condition. One area of interest for management is the eutrophication status of Lac. Eutrophication is a pressure that might explain some of observed changes in the bay. However, no baseline on the eutrophication status of Lac exists. IMARES and Environics NV conducted a snapshot assessment of the eutrophication status for current understanding and as a basis for future management. Environics conducted the field measurements at Lac, and most of the data analysis. IMARES analysed geographical data and together with Environics co-wrote the report.

The purpose of this baseline study was to assess the trophic status of Lac by analyzing 4 potential indicators of eutrophication simultaneously:

- Nutrient levels
- Levels of fecal indicator bacteria (enterococci)
- Epiphyte loads of seagrasses,
- Benthic community composition of the back reef

The monitoring was performed at 32 sites within the bay and 1 control site outside the bay in December 2010.

In this study, three of the four observed indicators point towards an ecosystem that is under stress from eutrophication. The levels of nutrients in the bay exceeded thresholds for open coral reef systems due to lack of better. Overall, concentrations show that enrichment with nitrogen was widespread and levels commonly exceeded threshold values. No clear source or "hotspot" could therefore be identified in this study. Phosphate only exceeded threshold values at a few locations, but no clear source was identified. The diffuse enrichment of nutrients across the bay probably results from multiple factors such as water circulation, residence time, freshwater input, rainfall, groundwater contamination, tidal range, and geology. Besides the (semi-) natural conditions the nutrient status is likely to be affected by human impacts as greywater inputs and lacking of proper sewerage. All these factors should be considered regarding the future state and measures to tackle the eutrophication of the bay.

Enterococci bacteria were detected at levels above acceptable levels as determined by ISO for bathing waters. The mean levels of enterococci decreased as the distance from shore increases with the highest levels found at groundwater sites and zero enterococci found on the back reef sites. Based on this dispersion we assume that sources of enterococci in this study are most likely birds and cattle (donkey and goat manure). The identification of the true sources of enterococci in Bonaire is however compelling and further study on this aspect is necessary to protect public health.

The levels of epiphytes on seagrass blades, showed differences in biomass among studied stations. This could mean that seagrass beds in different regions of the bay are experiencing different levels of water

column nutrients but no clear relation between nutrient levels and epiphyte cover was observed in this study.

The benthic composition monitoring revealed high abundance of calcareous algae (*Ramicrusta* sp.). This abundance is likely to be a bloom (pers. observations over time). The bloom of *Ramicrusta* sp. might be indicative of nutrient enrichment and uptake occurring in Lac. The alga is currently taking over habitat where hard corals lived and changes the benthic composition of the back reef and potentially affecting the integrity of the reef crest. The degradation of the reef crest will diminish the protective role provided by the structure and increase exposure to wave and storm action from the adjacent sea.

Despite the current eutrophic state of Lac, studies elsewhere indicate that eutrophic bays may begin to recover within months after implementation of proper measures. To do so, natural sources of nutrients should be distinguished from anthropogenic sources.

Based on the results of this study and historical accounts of other bays in the Caribbean that have been degraded by eutrophication; the following recommendations for Lac are suggested:

- a. Reduction of nutrient and fecal bacteria inputs by removing donkeys and goats from the watershed, and ensuring adequate toilet facilities and sewerage at Cai and Sorobon, including greywater disposal.
- b. Continuation of nutrient monitoring nutrient in order to locate clear sources and fate of the eutrophic state of Lac. We recommend adding urea to the suite of nutrients monitored in this study.
- c. Implementation of a regular monitoring program to identify sources and fates of fecal bacteria in order to support public health. Effectiveness of above measures can then be assessed as well.
- d. In general, to understand the outcomes of the water quality management plan it would be of great value to have an understanding of groundwater flows, circulation patterns and residence time of water in Lac.

# Contents

Summary .....	3
1 Introduction .....	6
2 Methods .....	8
2.1 Sampling site and regime.....	8
2.2 Nutrient analysis.....	9
2.3 Enterococci bacteria .....	9
2.4 Epiphytes on seagrass blades .....	10
2.5 Benthic composition of the back reef .....	10
3 Results .....	13
3.1 Nutrients .....	13
3.2 Enterococci bacteria .....	17
3.3 Epiphytes on seagrass blades .....	19
3.4 Benthic composition of the back reef .....	19
4 Conclusion and discussion .....	23
4.1 Eutrophication assessment .....	23
4.2 Factors contributing to eutrophication in Lac .....	25
4.2.1 Sources of nutrients .....	25
4.2.2 Sources of bacteria .....	25
4.2.3 Erosion via overgrazing.....	25
5 Recommendation and outlook.....	26
6 References.....	27
7 Quality Assurance .....	29
8 Justification.....	30
Appendix 1. Water Quality Parameters .....	31
Appendix 2. Station Locations .....	33
Appendix 3 Geographical illustration of data.....	34

# 1 Introduction

Lac is a semi-enclosed lagoon located on the southern-Caribbean island of Bonaire (Figure 1), that experiences mostly diurnal, microtidal Caribbean sea influences with a mean tidal height of about 10 cm (Kjerfve 1981). Although the tidal range can be in excess of 50 cm during spring or neap tides, it rarely exceeds 30 cm and is much lower in the mangrove sub-basins of the bay (van Moorsel and Meijer 1993, Hummelinck en Roos 1969). The reef rim of the lagoon consists of a reef crest along the eastern, outer edge of the bay that limits seawater exchange with the sea. At the northern extent of the rim, the reef crest is scored by a shallow (5 m) channel where it is assumed that most of the water exchange between the Caribbean sea and Lac occurs. Strong tradewinds produce waves on the eastern side of the island of 2 to 3.5 m (van Duyl 1985). The energy of the nearly constantly breaking waves is absorbed by the reef crest and the waters of Lac are relatively calm. There is a wide sand area on the lagoonal side of the reef crest and a back reef that is created by shoreward movement of large carbonate sand grains from the reef crest area. The inner perimeter of the bay is lined with mangroves (*Rhizophora mangle*) and within the bay there are seagrass meadows and a shallow coral reef, the back reef, that lies just inside the reef crest. Lac is the only large lagoon on Bonaire and is exceptional in that it contains a diversity of shallow water coral reef associated habitats in close proximity (mangroves, seagrass beds, *Halimeda* algal beds, the back reef and sand flats) that support a diversity of fish and invertebrates.

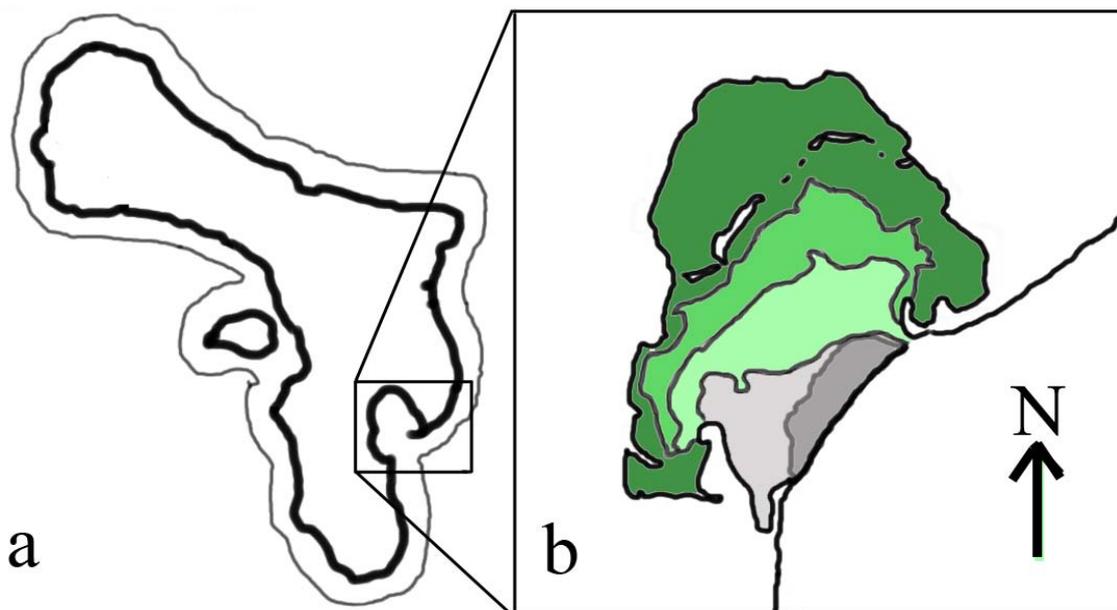


Figure 1. Line drawing of a) Bonaire and b) Lac bay, Dutch Caribbean, indicating major ecotypes. Dark green represents mangroves (past or present), medium green represents shallow seagrass habitat, light green represents deep seagrass habitat, dark grey represents the shallow back reef and coral dam, and light grey represents the sandy depositional area, *Awa blanku*.

Several legal frameworks, rules and regulations afford protection to Lac (Ramsar Convention on Wetlands, the Convention on Biological Diversity, the SPAW protocol of the Cartagena Convention, the Nature Ordinance of Bonaire including the Bonaire National Marine Park regulations and various Island Decrees, the Inter-American Convention for the Protection and Conservation of Sea Turtles, and the Convention of Migratory Species). However in light of all these protective frameworks and measures, mangroves and conch populations have been in decline in Lac for more than 30 years (Hummelinck and Roos 1969, Lott 2000) and it is possible that the overall condition of the bay is in decline as well due to pressures from land use practices, fishing and tourism. A new report highlights overgrowth of live corals

on the back reef and reef crest (Eckrich et al. 2011) that could have negative implications for the protective role provided by the physical structure of the reef crest and back reef. The reef crest protects the shallow bay from pounding waves and it appears that the structure is being weakened by boring organisms that are associated with the crustose alga that is overgrowing the live corals (R Peachey, personal observation). The back reef is also impacted by trampling by snorkelers and kayakers as snorkeling the back reef is promoted by the windsurf shops, which offer kayaks for rent. Other negative changes to Lac features include mangrove die-offs along the northern borders of Lac, ephemeral blooms of macroalgae on seagrasses in the southern reach of the bay and trampled and eroding seagrass beds and coral reefs due to day-users at Sorobon that are wading, swimming or windsurfing.

Numerous factors contribute to the changes in Lac (Lott 2001, Debrot et al. 2010), but until now, management and protection of the bay has been hampered by deficiencies in financial resources that impact implementation of management plans and enforcement of regulations. Furthermore, a lack of scientific information regarding current environmental status does not contribute to definition of accepted measures. One area of concern for management is potential eutrophication (enrichment by nutrients) of the bay. Eutrophication is a pressure that might explain some of the observed changes in the bay but no proper baseline on the status of eutrophication inside Lac exists. The Ministry of Infrastructure and Environment commissioned IMARES to conduct such a study and IMARES joined forces with Environics NV to provide a snapshot assessment of the eutrophication status for current understanding and as a basis for future planning.

The purpose of this baseline study was to assess the trophic status of Lac by analyzing 4 potential indicators of eutrophication simultaneously:

1. Nutrient levels of water samples in the bay were tested and are compared to thresholds levels that have been established for coral reefs. Results were also compared to other lagoons in the Caribbean to assess the relative level of eutrophication of Lac.
2. Levels of fecal indicator bacteria in water samples were tested and are compared to public health standards. These can be used to determine the need for monitoring in the bay to protect public health.
3. Epiphyte loads of seagrasses, which can negatively affect seagrass growth and reduce bed size by reducing light and interfering with nutrient uptake and respiration of seagrass blades. Epiphyte loads were determined from sites around the bay and compared to past studies of Lac and other bays in the Caribbean region. And lastly,
4. Benthic community composition of the back reef was assessed to detect the potential for a change from a coral dominated to algal dominated benthic assemblage, and which can be strong indicator of eutrophication in reef systems.

Environics conducted the field measurements at Lac, and most of the data analysis. IMARES analysed geographical data and together with Environics co-wrote the report.

## 2 Methods

### 2.1 Sampling site and regime

To identify possible areas of eutrophication, 32 sites within the bay and 1 "control site" (CS) that was located 1 km south of the bay were selected for sampling. The sites selected included all major ecotypes and sections of the Lac ecosystem. Sample sites were located in three main areas of Lac: the 18 "back bay" sites were shallow, "shoreline" sites ( $\approx 0.5$  m water depth) that were predominately mangrove habitat with the exceptions of sites A, M and N, which were sandy shoreline sites; the 8 "mid-bay" sites were seagrass habitats (1 to 3 m water depth); and the 6 "outer bay" sites were sand or back reef sites (1 to 2.5 m water depth)(Figure 2). To account for variability in levels of nutrients or bacteria during tidal exchange, water samples were collected on both incoming and outgoing tide for nutrients and bacteria. Additionally, there were two groundwater sites, landward of the bay (W2 and W3), that provided a comparison between groundwater and bay sites.



Figure 2. Google Earth image of sampling sites in Lac bay, Bonaire mapped using GIS data collected at the sampling sites. The CD sites represent coral dam sites, SF sites are sand flat habitat, SG sites are seagrass habitat and the remaining sites are mostly mangrove sites. Back bay sites are indicated with pink circles, middle bay sites are represented by green circles and outer bay sites are represented by yellow circles or white balloons. A nearby well (W2) and groundwater sampling site (W3) are indicated by blue circles. CS is a Caribbean sea site used as a control for the bay sites (white circle).

## 2.2 Nutrient analysis

Eutrophication of coral reefs is a particularly well known cause of coral reef degradation and loss of reef corals in the Caribbean and tracks coastal development and population growth closely (Goreau et al. 1997). Improper land use and sewage treatment are major causes of nutrient enrichment throughout the Caribbean region (Burke and Maidens 2004). Since there are no published threshold values for semi-enclosed coral reef systems, levels of nutrients measured in Lac will be compared to thresholds developed for open coral reef systems where thresholds have been established since the early 1990s (Bell 1991, Lapointe et al. 1993). The thresholds are equivalent to the Standards Bonaire (Werkgroep Milieunormering Nederlandse Antillen, 2007) of  $0.014 \text{ mg l}^{-1} \text{ N}$  ( $1.0 \text{ }\mu\text{M NH}_4$  or  $\text{NO}_2 + \text{NO}_3$ ) or  $0.003 \text{ mg l}^{-1} \text{ P}$  ( $0.1 \text{ }\mu\text{M PO}_4$ ). Semi-enclosed areas are more susceptible to nutrient enrichment due to reduced mixing of water with the open sea, allowing nutrients levels to build up over time and damage the ecosystem as was first documented for a semi-enclosed coral reef ecosystem in Kaneohe bay, Hawaii (Maragos 1972). Groundwater is an important source of nutrients to coral reefs, especially where septic tanks are in use, and nutrient enriched groundwater was instrumental to the eutrophication of Discovery bay, Jamaica and other semi-enclosed coral reef coastal habitats in the Caribbean region (Lapointe 1997). Recovery of corals is possible when good water quality is restored and by improving water quality, the harmful effects of climate change on coral reef communities were nutrient levels have become elevated are ameliorated (Wooldridge and Done 2009).

Nutrient sampling at 33 marine sites (including the site outside the bay CS) occurred on an incoming and outgoing tide from 22 - 24 December 2010. One nutrient sample was collected at each site during each tide (incoming and outgoing). Water samples were collected by hand just below the water surface ( $\approx 25 \text{ cm}$ ) in acid-washed 250 ml polyethylene bottles that were placed on ice until returned to the laboratory for processing. Immediately following the collection of each nutrient sample, the following water quality parameters were measured at the station with a calibrated YSI 556 multimeter: water temperature, conductivity (salinity), and pH (Appendix 1). Water samples for nutrient analysis were not taken at groundwater sites.

In the lab, 60 ml Luer-Lok plastic syringes were filled with 10 ml of sample water, shaken and the rinse water discarded, 3 times. Then the syringe was filled with sample water and fitted with a Sterivex  $0.22 \text{ }\mu\text{m}$  filter cartridge. A 60 ml sample was forced through the filter and the syringe refilled. Three rinses of 5 ml each of sample water were introduced into 20 ml scintillation vials with foam PE lined screw caps. A 15 ml sample was dispensed and frozen and subsequently air-freighted to the Woods Hole Oceanographic Institute Department of Marine Chemistry and Geochemistry for analysis of  $\text{NH}_4^+$ ,  $\text{NO}_2^- + \text{NO}_3^-$ ,  $\text{SiO}_4^-$ , and  $\text{PO}_4^{3-}$ . The facility operates a Lachat Instruments QuickChem 8000 four-channel continuous flow injection system to determine dissolved nutrient concentrations including: ammonium, nitrate, nitrite, orthophosphate, silicate, and total dissolved nitrogen. The methods used for analysis are specified and approved by the United States Environmental Protection Agency (1985).

## 2.3 Enterococci bacteria

Enterococci are useful for monitoring fecal bacteria in marine waters because they are persistent in saltwater whereas, coliforms such as *Escherichia coli* experience high rates of decay in seawater (Anderson et al. 1979). The occurrence of gastroenteritis among users of marine bathing waters has been directly related to levels of enterococci, which are the only indicator bacteria used to monitor water quality in the US (EPA 1986). Enterococci are quantitatively linked to illnesses in swimmers (Kay et al. 1994, Wade et al. 2003) and levels are monitored in countries around the world to issue warnings when densities exceed threshold values established by the various counties.

Water samples were collected at the same sites as the nutrient samples and tested for enterococci bacteria using the Enterolert system (IDEXX, Philadelphia PA). In addition to the marine sites, 2 groundwater sites (W2 and W3) were sampled and tested for enterococci bacteria. Samples were collected in sterile 125 ml polyethylene bottles and stored on contained ice pending transport to the laboratory. In the lab, Enterolert reagent was added to a 10 ml sample of the seawater, filled to the 100 ml line with sterile freshwater and gently shaken until dissolved. Groundwater samples did not require dilution and the reagent was added directly to the 100 ml water sample. Samples were poured into a Quanti-tray, sealed and stored in an incubator at 41°C for 24 h. Quanti-tray cells were examined under a 365 nm UV light for fluorescence and interpreted according to the mean probable number table supplied by IDEXX.

The number of colony forming units (cfu) 100 ml<sup>-1</sup> were compared to the International Organization for Standardization (ISO) Guideline number of 100 cfu 100 ml<sup>-1</sup> (ISO 1996) for coastal waters and transitional waters. Enterococci levels below 100 cfu 100 ml<sup>-1</sup> are indicative of excellent water quality whereas, levels above 100 cfu 100 ml<sup>-1</sup> indicate water quality as average or non-compliant (ISO 1996). Based on a study of 11,000 EU bathers, Kay et al. derived a 95<sup>th</sup> percentile value for enterococci level of 185 cfu 100 ml<sup>-1</sup> that has a risk of illness factor of 5% (1 in 20 bathers) for bathing waters, which is used as an indication of unacceptable water quality for the World Health Organization (WHO) that rounded the number up to 200 cfu 100 ml<sup>-1</sup> and uses that as an important bathing water standard for insuring public health (Kay et al 2004).

## 2.4 Epiphytes on seagrass blades

Epiphyte load of the seagrass, *Thalassia testudinum*, was determined at 8 sites in the open, middle region of the bay. Three 25 cm x 25 cm quadrats were haphazardly placed within the seagrass bed at each station. Blades were clipped from 5 sub quadrats and returned to the lab where the width and height of each blade was measured. Blades were scrapped gently with a single edge razor to remove epiphytes, which were placed into pre-weighed aluminum boats and dried at 60°C for 24 h. Dry weight of epiphytes per cm<sup>2</sup> seagrass blade surface was determined and compared among the 8 sites. Epiphytes were not separated into plant or animal and, although some animals that live epiphytically on seagrass blades build calcareous tubes, tubes were not extricated from the material because an abundance of filter feeding tube-building organisms is also an indicator of eutrophication. Additionally, calcareous algae also respond to over-nutrition and will add to the dry weight of the epiphytic material. Dry weight was inclusive of everything attached to the surface of the seagrass blades to be compared among sites selected at a range of sites within the bay.

## 2.5 Benthic composition of the back reef

On the back reef of Lac, 50 – 10 m transects were examined to determine the percent cover of live coral, macroalgae, *Ramicrusta* sp. (an encrusting calcareous algae that is overgrowing corals), dead coral (category includes turf algae, carbonate pavement/coral rubble) or sand along the back reef habitat (Figure 3). The shallow (< 2 m) back reef habitat hosts an isolated coral reef that is just over 1 km long and 0.25 km wide and is oriented at about 45° east of north in an area previously reported to have good coral development including thickets of staghorn and elkhorn corals (*Acropora palmata* and *A. cervicornis*) and live coral cover on the back reef ranged from 0 – 25% cover (Lott 2001). Other coral species that have been identified living on the back reef are *Montipora annularis*, *Agaricia agaricities*, *Diploria strigosa*, *Favia fragum*, *Dichocoenia stokesii*, *Portites porites*, *P. astroides* and *Millepora* spp. (Engel 2008, Lott 2001, Roos 1971).

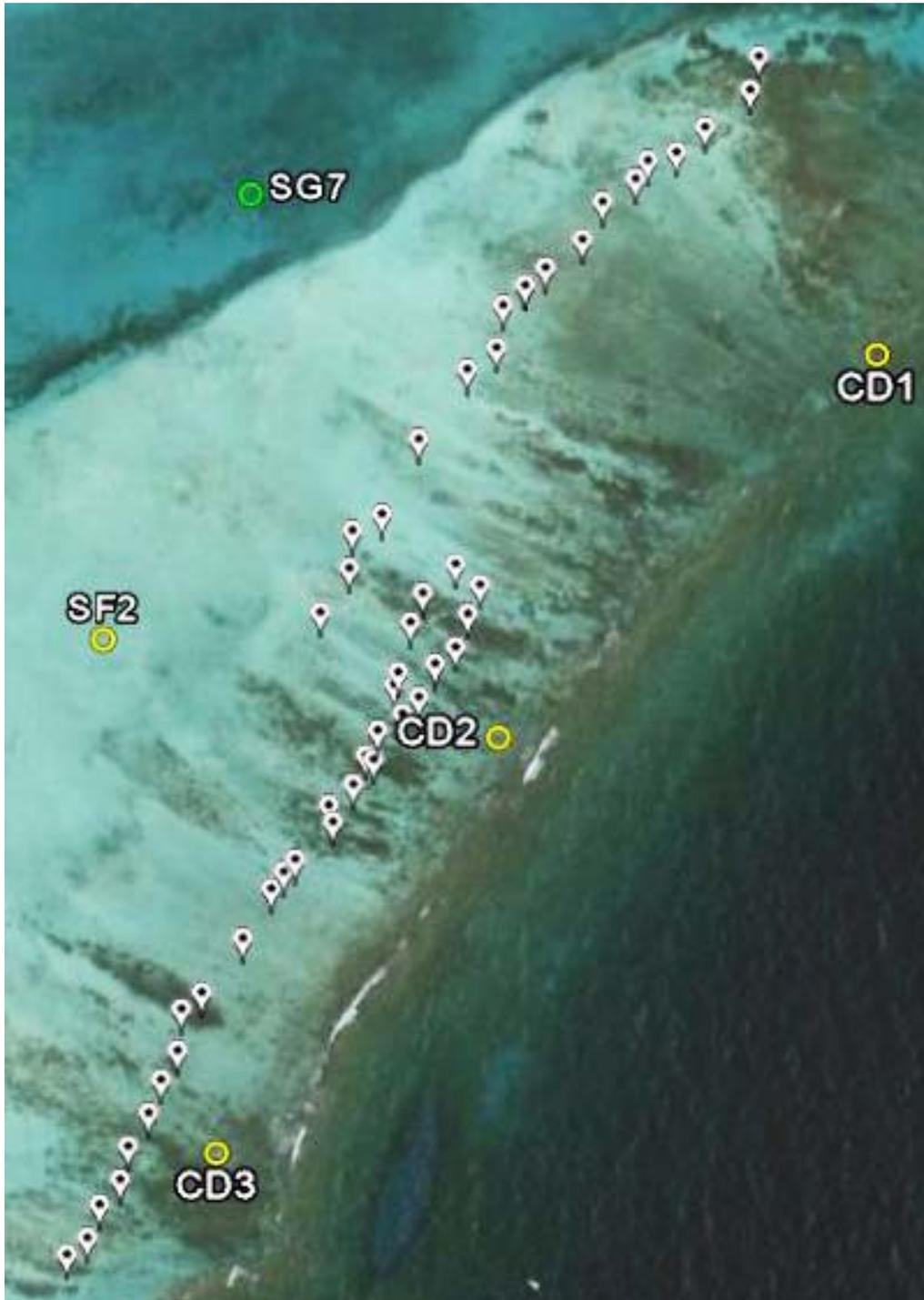


Figure 3. Enlargement of back reef area from Figure 2 indicating the location of fifty 10 m benthic cover line transects (white balloons). Other coral dam (CD), seagrass (SG) and sand flat (SF) sites are included for geographic reference.

There are no previous reports of *Ramicrusta* sp. in the back reef habitat, or at any other location in Bonaire, even though numerous algal taxonomists have studied the area (van Moorsel and Meijer 1993, Lott 2001, S Engel and C Eckrich personal communication). In the van Moorsel and Meijer (1993) baseline survey a species called "cf *Peyssonnelia* sp" was documented. This species is from the same family as *Ramicrusta* sp. (Peyssonneliaceae) but no previous study documents extensive benthic areas of Lac covered by an encrusting red algae. If the *Peyssonnelia* sp. reported by van Moorsel and Meijer

(1993) is the same as *Ramicrusta* sp. that occurred in the past, it was integrated into the macroalgal community, which is not the condition it is found in currently in Lac. There are benthic areas where *Ramicrusta* sp. covers 100% of the solid substrate over distances greater than 1m x 1m.

The 50 transect stations were approximately 25 m apart and at each station a transect tape was extended in a direction perpendicular to the reef crest (approximately 135° east of north) for a distance of 10 m to measure and categorize the benthic habitat. Benthic composition was measured to the nearest 5 cm along each 10 m transect. Percent cover of the major benthic groupings was plotted to detect areas with high percent cover of *Ramicrusta* sp. or macroalgae, which were not commonly reported in the back reef in the past.

### 3 Results

#### 3.1 Nutrients

In appendix 3 nutrient concentrations are plotted in a geographical illustration of Lac. Figures presented in this appendix cover both incoming as outgoing tide illustrations.

TIN ( $\text{NH}_4 + \text{NO}_3 + \text{NO}_2$ ) concentrations ranged from undetectable ( $<0.05 \mu\text{M}$ ) to  $88.02 \mu\text{M}$ . The mean concentration of all the sites within the bay on the incoming tide ( $n = 32$ ) was  $1.51 \pm 1.36 \mu\text{M}$  and on the outgoing tide the mean was  $5.45 \pm 16.42 \mu\text{M}$  ( $n = 32$ ). The Caribbean sea station (CS) sample values are not included in the calculations of the means for the sites within the bay as that station was selected to provide a comparison with the sea outside the bay. Of 64 samples, 36 (56%) exceeded the acceptable threshold of  $1.0 \mu\text{M}$  for total inorganic nitrogen (TIN) for open coral reef systems (Bell 1992) (Figure 4). When the results are categorized into the 3 major zones (shoreline sites: A,B, C, D, E, F, G, H, I, J, K, M, N, P; middle bay sites: L, O, Q, R, SG 1-8; "outer bay" sites: SF 1-2, CD1-4) the mean TIN levels of incoming and outgoing tides for the shoreline sites, "mid bay" sites and "outer bay" sites all exceed the threshold and the TIN levels during the outgoing tide in the middle bay and outer bay are higher than the incoming tide (Figure 5). The lower levels on the incoming tide may be due to dilution as water from the Caribbean sea moves into the bay. The level of TIN at the CS site was  $0.06$  on the incoming tide and  $2.68 \mu\text{M}$  on the outgoing tide. The higher levels of TIN on the outgoing tide may indicate that the nutrients from the bay were transported to the south of the bay on the outgoing tide.

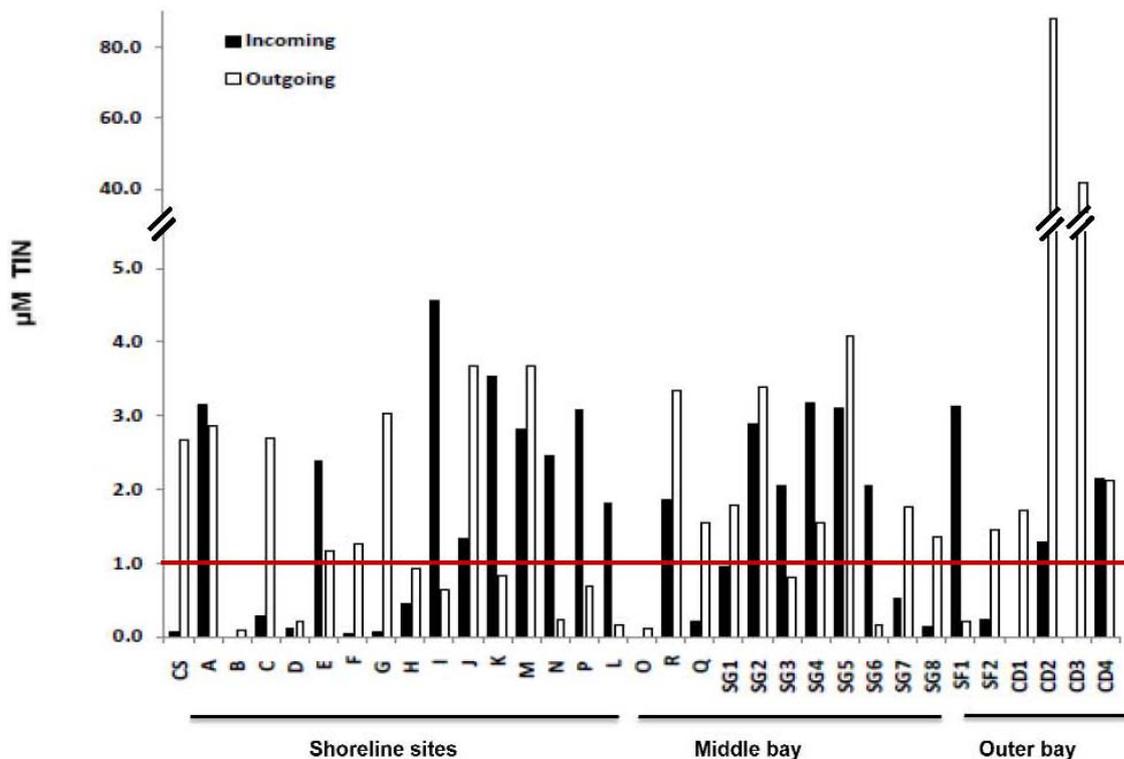


Figure 4. Concentration of TIN ( $\text{NH}_4 + \text{NO}_3 + \text{NO}_2$ ) detected in water samples in Lac bay, Bonaire on incoming and outgoing tides in December, 2010. Red line indicates a threshold for total inorganic nitrogen (TIN) levels on open coral reefs above which reefs are at risk to eutrophication (Bell 1992).

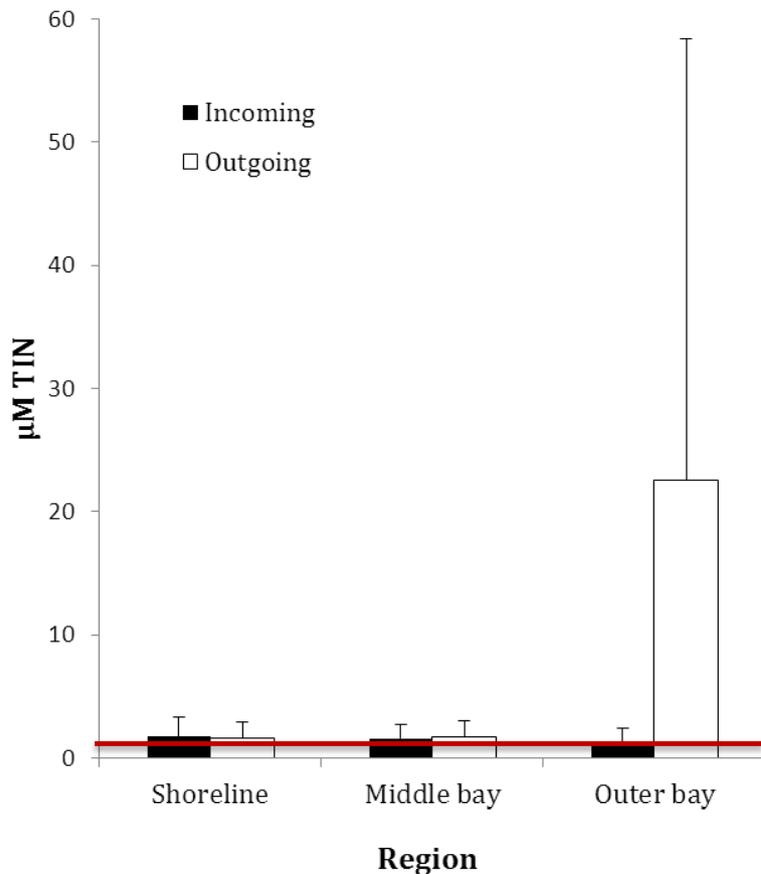


Figure 5. Mean concentration of TIN ( $\pm$  SD) in water samples from Lac bay, Bonaire on incoming and outgoing tides between 22 – 24 December 2010 in 3 regions of the bay; shoreline ( $n=14$ ), middle bay ( $n=12$ ) and outer bay sites ( $n=6$ ). Red line indicates threshold value for TIN for open coastal marine waters.

Levels of ammonia ( $\text{NH}_4$ ) at the 32 sites, on incoming and outgoing tides, ranged from undetectable ( $<0.05 \mu\text{M}$ ) to  $4.56 \mu\text{M}$ , with a mean of  $1.48 \pm 1.41 \mu\text{M}$  on the incoming tide and a mean of  $1.41 \pm 1.37 \mu\text{M}$  on the outgoing tide (Figure 6). Ammonia was the main TIN component in most of the water samples and independently exceeded the limit for TIN in more than 50% of the water samples analyzed from within the bay. The level of  $\text{NH}_4$  at the CS site was non-detectable on the incoming tide and  $2.48 \mu\text{M}$  on the outgoing tide.

Concentrations of  $\text{NO}_3 + \text{NO}_2$  ranged from undetectable ( $<0.05 \mu\text{M}$ ) to over  $88.02 \mu\text{M}$ . At three outer bay sites CD2, CD3 and CD4, (during the outgoing tide, levels of  $\text{NO}_3 + \text{NO}_2$  far exceeded the threshold for TIN whereas, levels of  $\text{NO}_3 + \text{NO}_2$  at the other sites in the bay were less than the threshold level on incoming and outgoing tides (Figure 7). The level of  $\text{NO}_3 + \text{NO}_2$  at the CS site was  $0.06 \mu\text{M}$  on the incoming tide and  $0.20 \mu\text{M}$  on the outgoing tide.

Levels of phosphate ( $\text{PO}_4$ ) at 32 sites, on incoming and outgoing tides ranged from undetectable ( $<0.05 \mu\text{M}$ ) to  $0.16 \mu\text{M}$ , with a mean of  $0.05 \pm 0.04 \mu\text{M}$  on the incoming tide ( $n = 32$ ) and a mean of  $0.03 \pm 0.04 \mu\text{M}$  on the outgoing tide ( $n = 32$ ). Of 64 samples, 4 were above the acceptable threshold of  $0.1 \mu\text{M}$  for phosphate for open coral reef systems (Bell 1992) (Figure 8). Two of the 4 samples were taken at the same sites that exceeded the threshold for  $\text{PO}_4$  on the outgoing tide (CD2 and CD3, Figure 8). The other sites that were above the threshold for  $\text{PO}_4$  were shoreline sites, F and P during incoming tide. The level of  $\text{PO}_4$  at the CS site was  $0.06 \mu\text{M}$  on the incoming tide and  $0.05 \mu\text{M}$  on the outgoing tide.

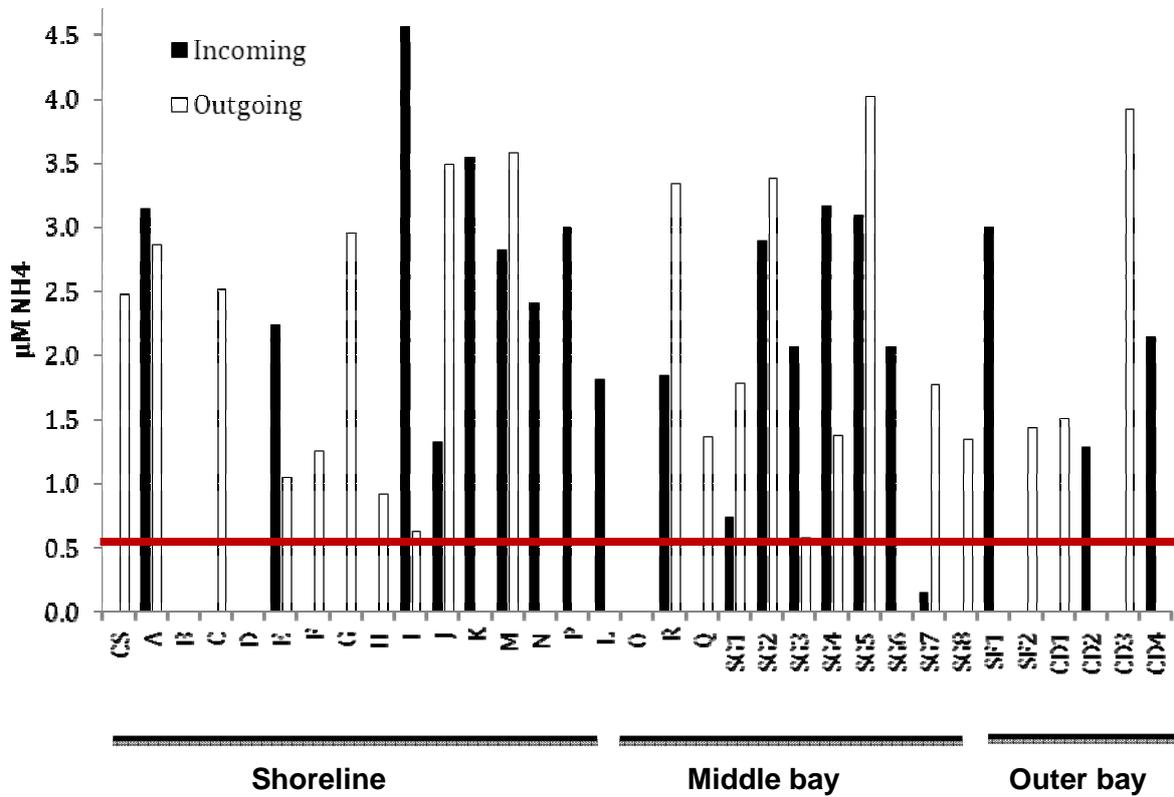


Figure 6. Concentration of  $\text{NH}_4$  detected in water samples in Lac bay, Bonaire on incoming and outgoing tides between 22 - 24 December, 2010. Red line indicates a threshold for total inorganic nitrogen (TIN) levels on open coral reefs above which reefs are at risk to eutrophication (Bell 1992).  $\text{NH}_4$  is a component of TIN.

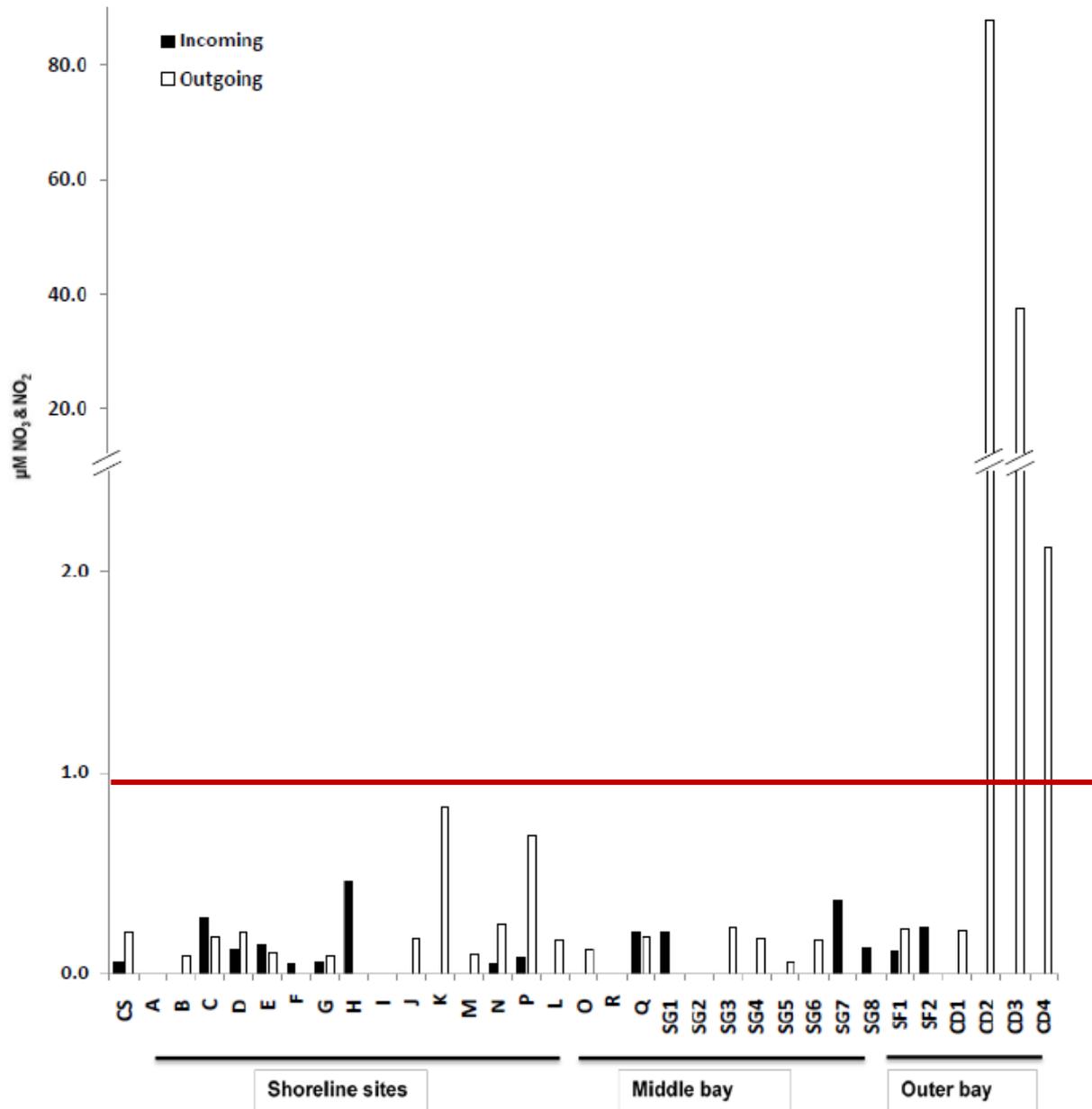


Figure 7. Concentration of  $\text{NO}_3 + \text{NO}_2$  detected in water samples in Lac bay, Bonaire on incoming and outgoing tides in December, 2010. Red line indicates a threshold for total inorganic nitrogen (TIN) levels on open coral reefs above which reefs are at risk to eutrophication (Bell 1992).  $\text{NO}_3 + \text{NO}_2$  are components of TIN.

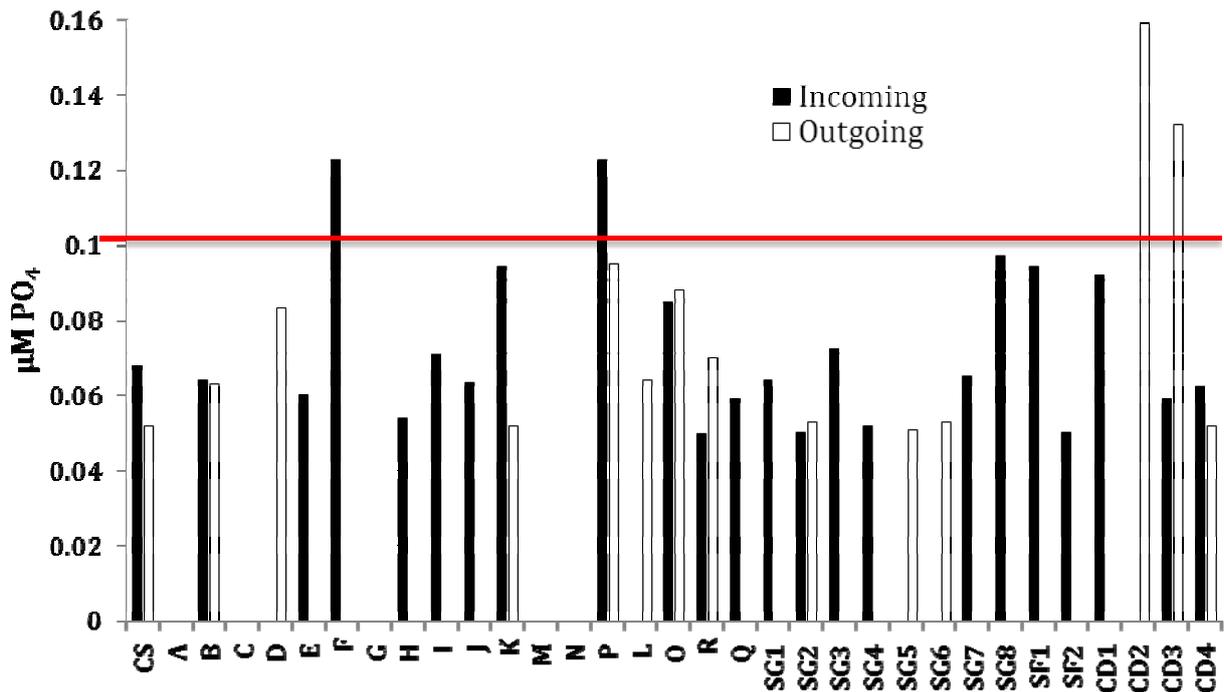


Figure 8. Levels of PO<sub>4</sub> in water samples from Lac bay, Bonaire on incoming and outgoing tides, December 2010. Red line indicates a threshold for levels of PO<sub>4</sub> on open coral reefs above which reefs are at risk to eutrophication (Bell 1992).

### 3.2 Enterococci bacteria

In appendix 3 enterococci concentrations are plotted in a geographical illustration of Lac. Figures presented in this appendix cover both incoming as outgoing tide illustrations.

Eleven of the 64 water samples collected within the bay exceeded ISO Guideline standard for enterococci for marine waters of 100 cfu 100 ml<sup>-1</sup>. Levels of enterococci at marine sites, on incoming and outgoing tides, ranged from undetectable to 399 cfu 100 ml<sup>-1</sup>, with a mean for bay stations of 39.5 ± 0.04 cfu 100 ml<sup>-1</sup> on the incoming tide (n = 32) and a mean of 45.1 ± 90.1 cfu 100 ml<sup>-1</sup> on the outgoing tide (n = 32). For the 2 well sites that are just inland of the bay (15 – 600 m), enterococci ranged from 3.1 to 658.6 cfu 100 ml<sup>-1</sup>, which is well within the range of enterococci detected in the well water of Bonaire, which in recent testing exceeded 2,400 cfu 100 ml<sup>-1</sup> (R Peachey, unpublished data). In this study, the mean levels of enterococci decrease as the distance from shore increases with the highest levels found at groundwater sites and zero enterococci found on the back reef sites (Figure 9). The highest levels of enterococci are found on the shoreline locations along the northern mangrove area, as can be seen in Figure 10, in which enterococci concentrations during outgoing tide are modeled. In appendix 3 an overview of concentration

Obviously, more samples of groundwater surrounding the bay are needed to determine the nature of the relationship as the 2 locations included in this study may not be representative of the groundwater surrounding the bay and may instead be a result of a localized source.

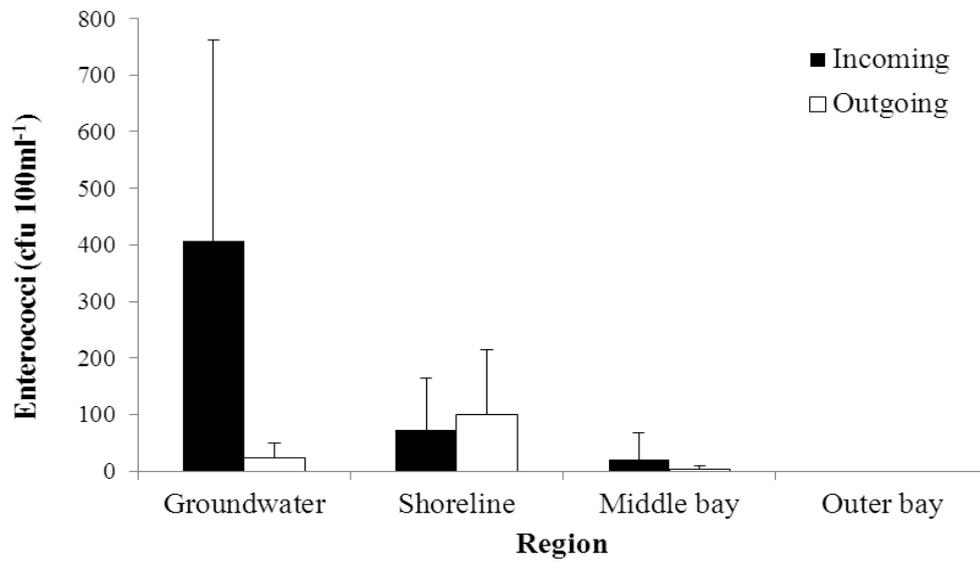


Figure 9. Mean enterococci levels ( $\pm$  SD) in water samples from Lac bay, Bonaire on incoming and outgoing tides, 22-24 December 2010 in groundwater (n=2), shoreline (n=15), middle bay (n=13) and outer bay (n=6) sites.

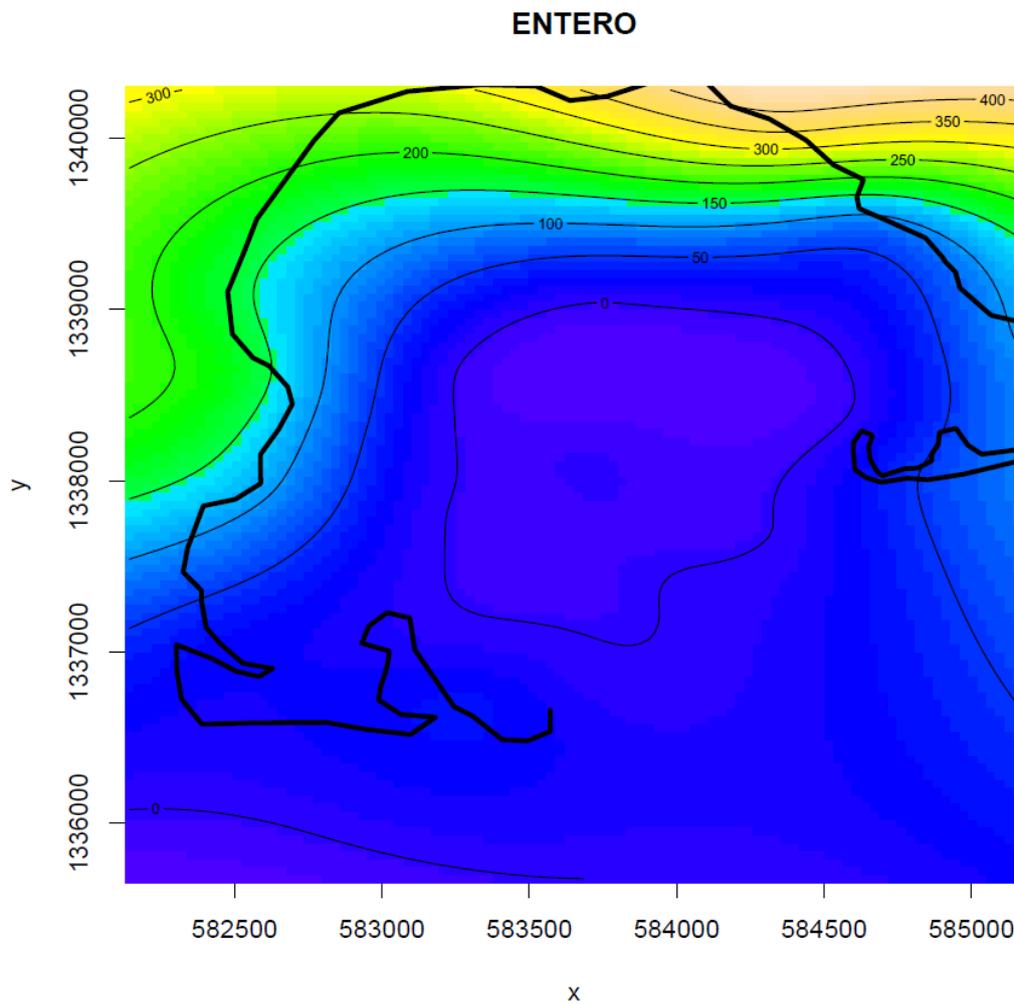


Figure 10 Enterococci data modelled. Based on data obtained during outgoing tide in December 2010.

### 3.3 Epiphytes on seagrass blades

Epiphyte loads ranged from 0.1 mg cm<sup>-2</sup> to 20.9 mg cm<sup>-2</sup>. The seagrass station with the highest epiphyte load was SG5 and SG6 had the second highest level (Figure 11). There is no discernable pattern of epiphyte load in the bay with sampling at the scale chosen for this study with sites ranging from 0.5 to 1.0 km apart. Although numerous studies show that algal epiphytes respond strongly to water column nutrient enrichment and that the increases in epiphytes on seagrasses are localized (see review in Hughes et al. 2004); no clear relationship between nutrients and epiphyte load was observed in this study. Since increases in epiphytes are localized around nutrient sources, perhaps SG5 is a hotspot that is worth further investigation. An interesting note is that the water depth at SG5 is over 3 m and it is located in the central deeper region of the bay, in fairly close proximity to the channel. It may be possible that there is some sort of hydrodynamic effect on epiphyte load in this location possibly due to high flow rates in the area that would decrease the boundary layer around the epiphytes, delivering more nutrients, or it could be possible that there is some stagnation (or pooling) in the deepest part of the bay where nutrients could build up due to reduced dilution effects from the sea.

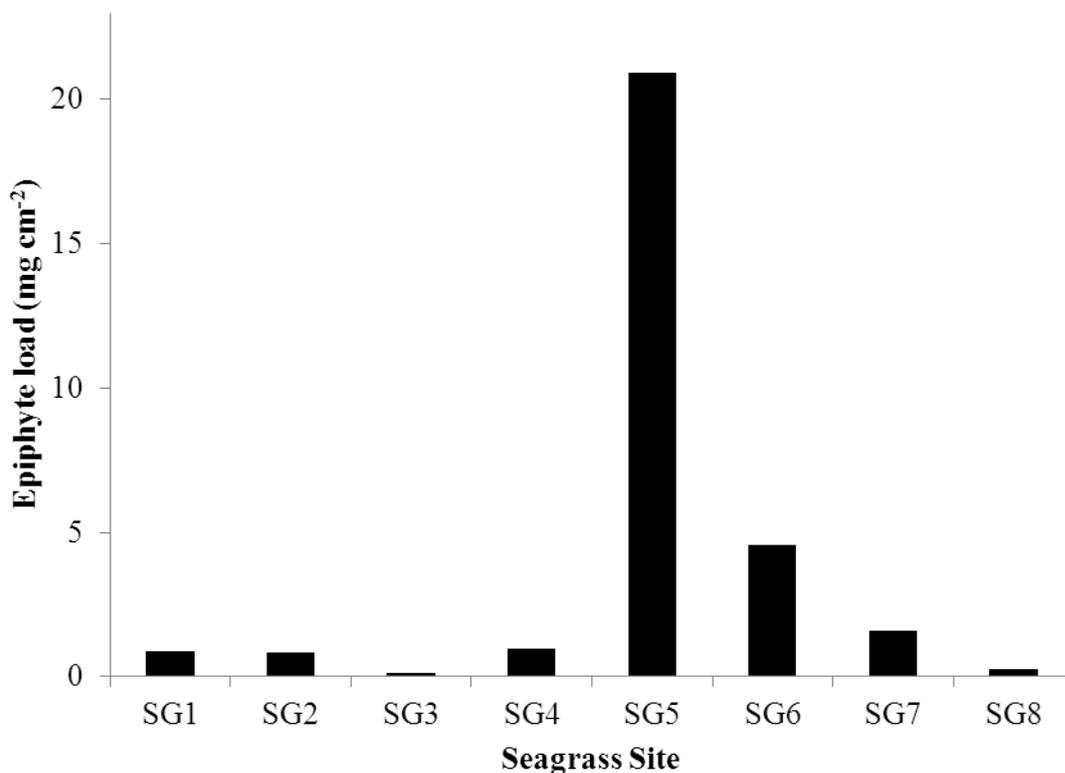


Figure 11. Epiphyte load in dry weight of epiphytes cm<sup>-2</sup> seagrass blade surface area from 8 sites in the middle bay region of Lac bay, Bonaire in December 2010.

### 3.4 Benthic composition of the back reef

The analysis of the benthic composition on the back reef habitat (n = 50) indicated that *Ramicrusta* sp. (31%), Dead Coral/Turf (29%) and Sand (27%) were the dominant benthic cover types, followed by Macroalgae (10%) and Live coral (3%)(Figure 12). When the categories of Macroalgae and the encrusting alga, *Ramicrusta* sp. are combined the mean percent cover of algae is more than 40% of the substrate on the back reef. The Dead Coral/Turf category is also covered in a thin layer of algae, called

turf algae and when included with the other algal categories, 70% of the back reef benthic community is covered in some type of algae.

The percent cover of *Ramicrusta* sp. was plotted for each transect in geographical order from north to south. *Ramicrusta* sp. was present at all sites with a range of 1.9 to 82.7 percent cover and there is a distinct increase in percent cover of *Ramicrusta* sp. in the central portion of the back reef (Figure 13). Additionally, the mean of 5 adjacent transects are plotted as a moving average, the north end of the back reef environment near the channel has the lowest moving average of the habitat (4%); the moving average of the northern reach peaked at about 25% whereas, in the central region, the moving average approaches 75%, and drops off in the southern reach of the bay. However, rather than dropping off like it does in the north, the moving average drops to nearly 10% then increases to 30% in the southernmost reach of the bay where circulation is limited.

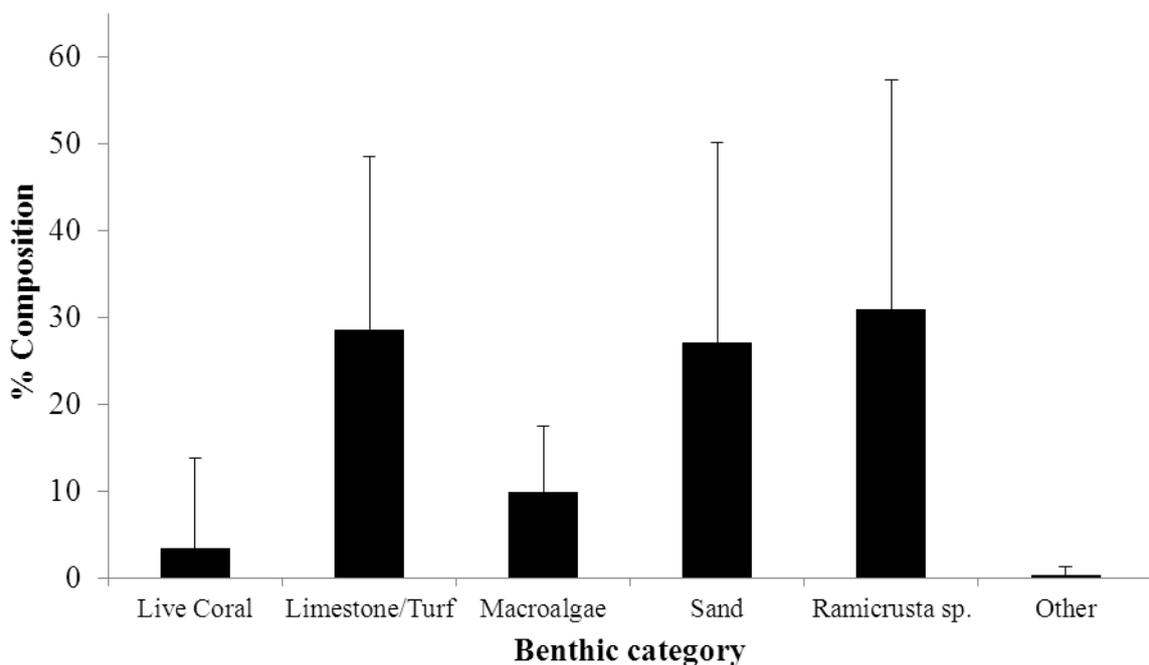


Figure 12. Mean percent composition ( $\pm$  SD) of benthic cover in the back reef habitat of Lac bay, Bonaire in January 2011. Means are the result of analyzing fifty 10 m line transects.

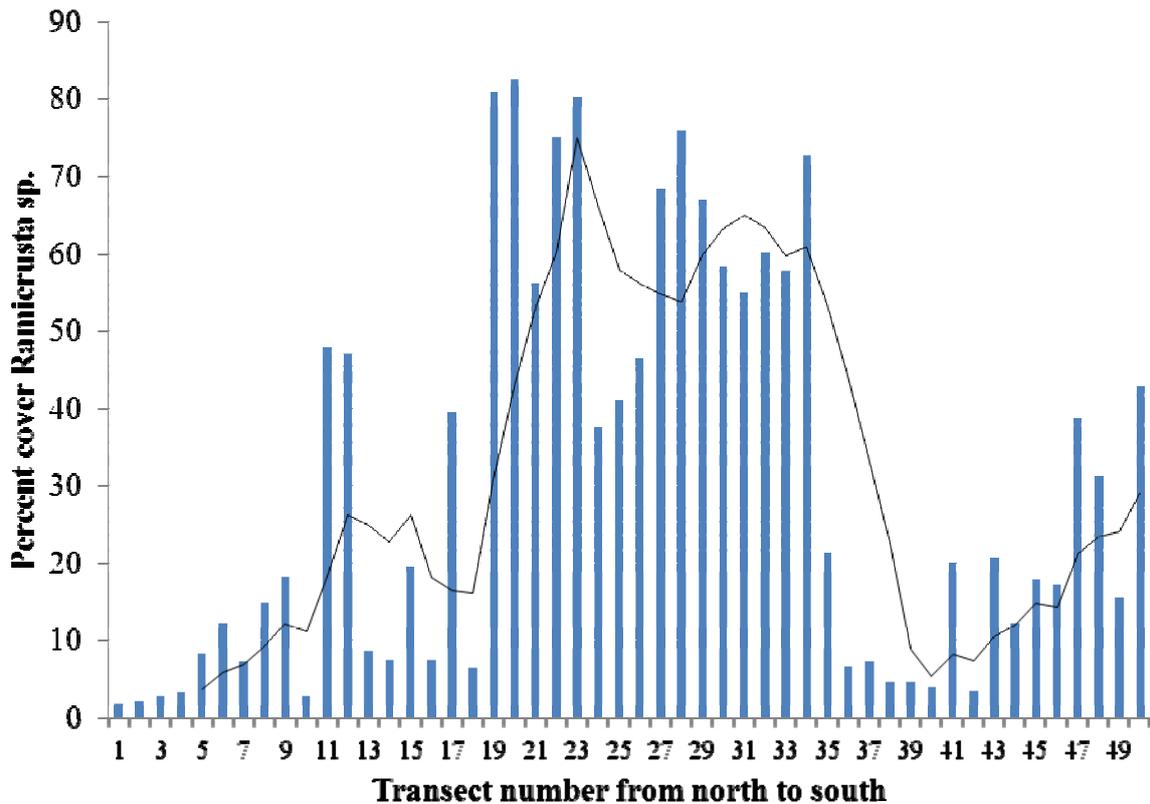


Figure 13. Percent cover of *Ramicrusta* sp. for each transect that was surveyed in order from the north end of the back reef habitat to the southern extent. Surveys were conducted in Lac bay, Bonaire in January 2011.

Since there was a distinct pattern within the back reef habitat from north to south, the percent cover by the different benthic groups was determined for the 3 regions. The central region had the highest percent cover of *Ramicrusta* sp. and macroalgae and the lowest percent cover of live coral (Figure 14). The percent cover of Sand was 3 times higher in the northern and southern reaches of the back reef than the central region. An analysis of benthic cover, excluding Sand, where algae cannot attach and grow, shows that the percent cover of *Ramicrusta* sp. of each transect ranged from 5 to 89% along the back reef, specifically on substratum where algae can grow (data not shown). The mean percent cover of *Ramicrusta* sp. is similar in the northern and southern regions of the back reef and is 2.8 times higher in the Central region and Live Coral cover is negligible (Figure 15). The percent cover of the Other category was less than 1% and is not shown in the figure. The pattern of mean percent cover of Dead Coral/Turf Algae is the inverse of the pattern for *Ramicrusta* sp. It is important to note that there is a lot of substratum available for *Ramicrusta* sp. or coral recruitment in the northern and southern regions of the back reef. The only information regarding the benthic composition are lists of coral species said to be common there but no data, either qualitative or quantitative, to compare with the results of this study.

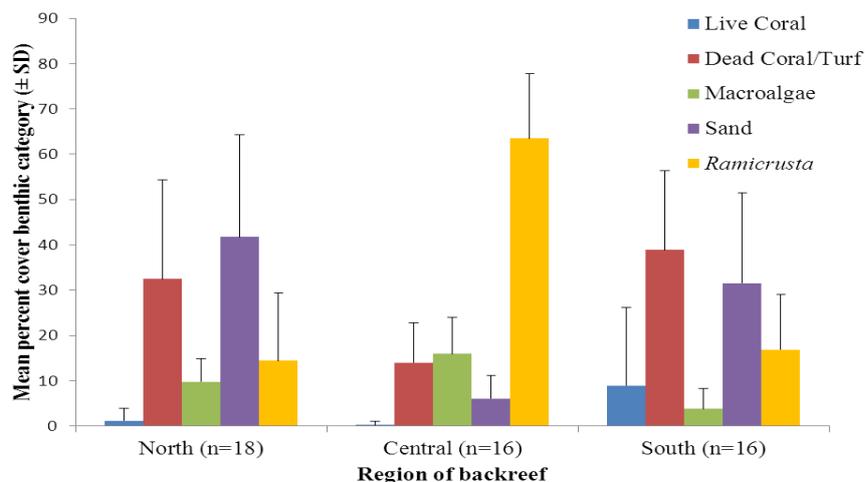


Figure 14. Spatial analysis of mean percent cover ( $\pm$  SD) of benthic groups on the back reef of Bonaire in January 2011 by region of the back reef habitat. The category Other is not shown because the mean is  $< 1\%$  in all regions.

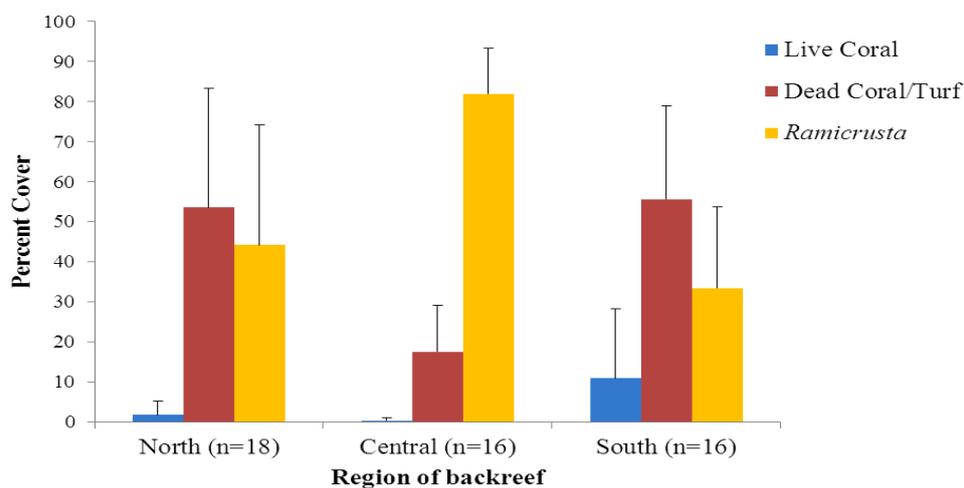


Figure 15. Spatial analysis of mean percent cover of benthic categories ( $\pm$  SD) in line transects conducted in the back reef habitat of Lac bay, Bonaire in December 2010. The back reef is divided into 3 regions north, central and south for this analysis and only substrata that are available for colonization by coral or algae are included (the Sand category was removed).

## 4 Conclusion and discussion

Many Caribbean lagoons have been degraded and corals and seagrass beds have been lost due to eutrophication (Goreau 1992, Littler and Littler 2000, McManus et al. 2000), which also negatively affects coastal fisheries through loss of critical habitat for fish (Wynne and Cote 2007). Lac is a semi-enclosed lagoon and based on the results of this study show notable indications of eutrophication.

### 4.1 Eutrophication assessment

In this study, four potential indicators for eutrophication were assessed to provide a snapshot of the eutrophication status of Lac. Three of the four observed indicators in this study point towards an ecosystem that is under stress from eutrophication.

Firstly, the levels of nutrients in the bay exceed thresholds for open coral reef systems. The application of thresholds values for nutrients on coral reefs for open systems are not completely appropriate (Goreau 2003) but are applied in this study for the lack of better.

Overall, concentrations show that enrichment of the bay waters with nitrogen was widespread and levels commonly exceeded threshold values. Phosphate was detected in samples from all regions of the bay but only exceeded the threshold values in 6% of the samples. Nitrogen is usually the growth-limiting nutrient for algae in marine systems (D'Elia and Wiebe 1990, Nixon et al. 1996) whereas, phosphate is the limiting nutrient in freshwater systems and some estuaries (Jickels et al. 1989). This makes the results of this study, wherein nitrogen commonly exceeded threshold levels, particularly noteworthy.

Secondly, fecal indicator bacteria are at levels above acceptable levels as determined by the ISO for bathing waters. The results of this study show a pattern of higher levels in groundwater and shoreline sites with decreasing levels as distance from the shore increases. The pattern will need further investigation due to the low number of samples from the wells. However, recent groundwater research at various locations around Bonaire showed that out of 17 samples of groundwater that were analyzed from wells, caves and surface water; all were tested positive for enterococci (Table 1). The levels of enterococci in groundwater were very high, exceeding 1000 cfu 100 ml<sup>-1</sup> in some places. The identification of the sources of enterococci in Bonaire is compelling and monitoring and further study is necessary to protect public health.

Thirdly, there is an expansive bloom of a calcareous alga that is capable of overgrowing other living organisms, including corals. It has become the dominant benthic cover in the central portion of the back reef environment. The bloom of *Ramicrusta* sp. is indicative of nutrient enrichment and uptake occurring in Lac. The alga is taking over habitat where hard corals lived and it has changed the benthic composition of the back reef. Additionally, the algal bloom is affecting the structural integrity of the reef crest, which is currently being degraded by boring organisms, associated with the algae that are eroding the reef structure. The limestone back reef (dead coral) that is covered by *Ramicrusta* sp. has become very weak and fractures easily with little application of force (R Peachey, personal observation). Degradation of the reef crest can diminish the protective role provided by the reef structure and increase exposure to wave and storm action from the adjacent sea. The reef crest has been a source of sand replenishment for the bay (Lott 2001) and the collapse of this important geologic feature of Lac would result in changes in the circulation pattern and energy regime within the bay with unknown consequences for the sand area (landward of the back reef), beaches, seagrass beds and mangroves.

Table 1. Levels of enterococci in water samples from wells, caves and surface water at various sites around Bonaire in 2009 (Peachey unpublished data).

Name	Location	Source	cfu 100 ml <sup>-1</sup>
Well LVV	Central	well	48.0
Well LVV	Central	well	48.5
Well LVV	Central	well	75.4
Well north of Lac	Central	well	571.7
Well north of Lac	Central	well	755.6
Well north of Lac	Central	well	791.5
Groto Lourdes	North	well	58.3
Dos Pos	North	well	1.0
Molina Bentura	North	well	1.0
Joubert Kunuku	North	well	57.2
Cai holes	Central	surface	1011.2
Bonaco ditch	Central	surface	164.4
Belnam shrimp	South	surface	1011.2
cave 1 hilltop	North	cave	16.8
cave 2 hilltop	North	cave	25.4
cave 3 Sabadeco	North	cave	98.0
cave 4 airport	Central	cave	41.0

The fourth indicator, levels of epiphytes on seagrass blades, showed differences among stations. This could mean that seagrass beds in different regions of the bay are experiencing different levels of water column nutrients or grazing. No clear relation between nutrient levels and epiphyte cover was observed in this study. Direct comparisons of epiphyte load on seagrasses between this study and previous studies in Lac bay were not possible because the previous studies did not quantify epiphytes but rather categorized seagrass epiphytes (Lott 2001, Engel 2008). The results of the Lott (2001) and Engel (2008) reports indicated a higher epiphyte load at stations in the northern reaches of the bay (near SG6 and SG8) and Lott reported fairly high epiphyte loads at sites just inshore from the back reef in 2001. Station SG5 had the highest epiphyte load in this study, but former studies indicated that epiphytes were medium to light in the same area of the bay and the category decreased slightly from 2001 to 2009 (Lott 2001 (Map 13, p 69), Engel 2008 (Chart 24, p 18)).

Direct comparisons between epiphytes in Lac and other areas in the Caribbean region are also difficult because investigators use a variety of measurements that are not directly comparable (mg epiphytes g blade<sup>-1</sup>, mg chl A of epiphytes g blade<sup>-1</sup>, g dry weight shoot<sup>-1</sup>, and categorical data). To summarize, epiphyte observations varied among this study and the previous studies in Lac and can be explained by natural variation in time and space, differences in sampling technique or hydrodynamics in the bay. However, nutrient dynamics do affect epiphyte levels on seagrasses as well and can be important indicators when temporal and spatial relationships are considered carefully in experimental design. Epiphytes are good indicators of localized nutrient inputs but leaf tissue nutrient content of the seagrass blades may be a better indicator of larger scale eutrophication in the bay (Frankovich and Fourqurean 1997) and should be considered as a potential indicator in future studies to try to detect potential nutrient hotspots.

## 4.2 Factors contributing to eutrophication in Lac

There are many additional factors that affect the potential for eutrophication of Lac including: water circulation (Lac is a lagoon with restricted flow), residence time, freshwater input, rainfall, groundwater contamination, tidal range, geology of the area and human impacts on the watershed. These aspects are known to be important steering factors within the bay, but their dimensions and thus implications are not quantified yet.

Although many factors are involved and should be considered regarding the current state of eutrophication of the bay it is clear that nutrient poor waters are a requirement for healthy coral reefs. When these become enriched with nutrients, it results in increases in algae because algal growth is a direct response to nutrient uptake (Smayda 1990).

### 4.2.1 Sources of nutrients

Levels of nitrogen throughout the bay are exceeding quality standards. No single hotspot was found as almost all locations show concentrations higher than the quality standard. It is obvious that with outgoing tide TIN levels are higher than with incoming tide. Sources of nitrogen are inside the bay, and might be multiple and scattered around the bay. Nutrients and bacteria may have a number of sources including leaching of untreated sewage and grey-water from docks, beaches, restaurants and resorts; birds (nesting/roosting/feeding); groundwater flows from the surrounding watershed and runoff from the watershed, which might include nutrients from donkey and goat manure<sup>1</sup>. During sampling of the middle and outer bay locations by boat, two cruise ships were in town, and many passengers visited Lac bay these days to recreate. This might have influenced this baseline observation. Current data do not show a direct relation with touristic hotspot location a Sorobon, but cannot be discarded as well. Increased numbers of tourist in future ask for proper measures to rule out any relationship.

### 4.2.2 Sources of bacteria

Enterococci levels are higher at shore locations near mangrove area. These bacteria levels most probably originate from animal feaces such as from water birds or cattle in the watershed. One shoreline location with bacteria level exceeding bathing water standards is found near the tourist area of Lac (location C). These bacteria might originate from human sources. Additional monitoring including the use of smart techniques to differentiate between sources could help to pinpoint the (different) sources.

### 4.2.3 Erosion via overgrazing

Overgrazing has depleted the areas bordering Lac of crucial ground cover vegetation. Wind, vehicle traffic and rainwater move sediments into Lac. The sediments are unable to wash out of the system and further accumulate in sub-basins and tidal creeks. In addition, the land loses the relatively thin layer of valuable sediment for plants. It is therefore, the first step in restoring Lac (especially mangrove area) to stabilize shorelines to reduce sediment transport into the system. This can be accomplished by enforcement of existing laws requiring farmers to keep livestock within fenced boundaries of their properties and decreasing vehicular traffic in off road areas. Communication and awareness will be key steps in achieving these goals.

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<sup>1</sup> Many donkeys and goats live within the Lac watershed, , potentially causing manifold problems (Debrot et al 2010)

## 5 Recommendation and outlook

Lac is an important conservation area that is critical to Bonaire's wildlife, above and below the water. The area provides many ecosystem services. It is an important recreational area that is essential to the tourism industry and therefore is in need of immediate protective action to stop further decline. Poor bathing water quality might hamper its touristic value. Furthermore the bay provides a critical nursery area for fish (Nagelkerken et al. 2000, 2002; Nagelkerken and Van der Velde, 2004) and a critical feeding area for juvenile green sea turtles. Loss of critical fish habitat will potentially impact artisanal fisheries and piscivorous fish abundance on the reefs. Measures to address the watershed issues in Lac are important as they will contribute to sustainable economic benefits for Bonaire.

The short time frame for this study limited the data analysis to a snapshot of processes that change constantly. Further study over time is needed for a better understanding of the sources and fates of the chemicals and bacteria. Nevertheless, the results clearly indicate that there are harmful levels of nutrients and bacteria present in the bay.

In order to take proper measures, natural sources of nutrients should be distinguished from anthropogenic sources. If the nutrients and bacteria that are entering the bay from anthropogenic sources are stopped immediately, studies elsewhere indicate that the bay may begin to recover within months. For instance, recovery has happened in other nutrient-impacted coral reefs such as Dragon Bay, Jamaica. When sewage stopped being released to the bay in 1996, weedy algae began to die back in a very brief time (2 months) and have not returned since the inputs stopped and corals are still recovering in the bay (Goreau 2003).

Based on the results of this study and historical accounts of the many lagoons in the Caribbean that have been degraded by eutrophication; we make the following recommendations for Lac:

1. Some potential sources of nutrient and fecal bacteria are known in the Lac area. Based on the presence of fecal indicator bacteria and high levels of nitrogen in Lac, we recommend that a program should be implemented to reduce nutrient and fecal bacteria inputs immediately by:
  - a. removing donkeys and goats from the watershed
  - b. ensuring that there are adequate toilet facilities for visitors at Cai, Sorobon and the restaurants and resorts
  - c. ensuring that sewage is removed from the area and not leached into the groundwater or bay
  - d. ensuring that restaurants are properly disposing of all greywater.
2. In order to manage water quality in Lac to ensure public health: a regular monitoring program should be implemented to assess public health risk and to identify sources and fates of fecal bacteria. When hot spots are identified, their sources may be obvious and the potential for reducing or eliminating the source can be assessed. If the source of the hot spot is not obvious, implement microbial source tracking technology, such as enterococci antibody resistance patterns and PCR, to identify the various host species so that management efforts can be directed at sources.
3. In order to manage water quality in Lac to ensure ecosystem health: initiate a monitoring study of nutrient types and levels and locate hot spots that might facilitate management action. If hot spots are not located using the broad scale spatial monitoring of Lac; implement nutrient source tracking using technology such as isotope analysis to pinpoint the origin of nutrients so that management can direct resources at specific sources. We recommend adding urea to the suite of nutrients monitored in this study.
4. In order to understand the outcomes of the water quality management plan it would be of great value to have an understanding of groundwater flows, circulation patterns and residence time of water in Lac. Our recommendation is that high-quality aerial or satellite mapping of the watershed will be essential for understanding sources and fates and an assessment of groundwater flow and quality is also essential to understanding the system.

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## 7 Quality Assurance

Environics conducted the field research.

Nutrient sampling and analysis was performed according to US EPA (1985) protocols. Woods Hole Oceanographic Institute Department of Marine Chemistry and Geochemistry conducted the nutrient analysis.

Enterococci bacteria were monitored according to US EPA (1986) protocols.

For seagrass and benthic cover monitoring regional accepted protocols were applied.

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 57846-2009-AQ-NLD-RvA). This certificate is valid until 15 December 2012. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

## 8 Justification

Report C093/11  
Project Number: 4305201101

The scientific quality of this report has been peer reviewed by a colleague scientist and the head of the department of IMARES.

Approved: Dr. A.O. Debrot  
Research scientist

Signature:

Date: 25 July, 2011



Approved: J.H.M. Schobben, MSc.  
Head of Department

Signature:

Date: 25 July, 2011



## Appendix 1. Water Quality Parameters

Measured at Time of Nutrient Sampling, Lac Bay, December 22 -24, 2011

22 AND 23 DECEMBER

Station	Temperature (C)	Temperature (C)	Conductivity (ppt)	Conductivity (ppt)	pH	pH
	Incoming Tide	Outgoing Tide	Incoming Tide	Outgoing Tide	Incoming Tide	Outgoing tide
CS	28.59	29.4	35.65	35.65	7.91	7.76
A	28.24	30.99	35.80	35.83	7.96	8.07
C	27.91	30.44	35.72	36.01	7.93	8.05
D	28.06	29.56	34.75	34.41	7.55	7.64
E	27.83	28.72	34.10	33.50	7.47	7.50
F	28.12	28.24	24.07	29.80	7.50	7.34
G	27.79	30.53	24.62	24.66	7.43	7.48
H	31.04	31.69	27.18	31.27	7.90	7.91
I	28.75	30.76	33.97	34.87	7.41	7.62
J	30.76	29.34	31.32	34.96	7.68	7.55
K	28.68	29.23	35.91	38.84	7.40	7.44
M	28.92	28.77	35.97	35.90	7.97	7.93
N	29.19	28.86	36.05	35.92	7.77	7.81
P	29.47	28.76	36.21	36.12	7.57	7.52
W2	33.47	-----	1.95	-----	7.95	-----

24 DECEMBER

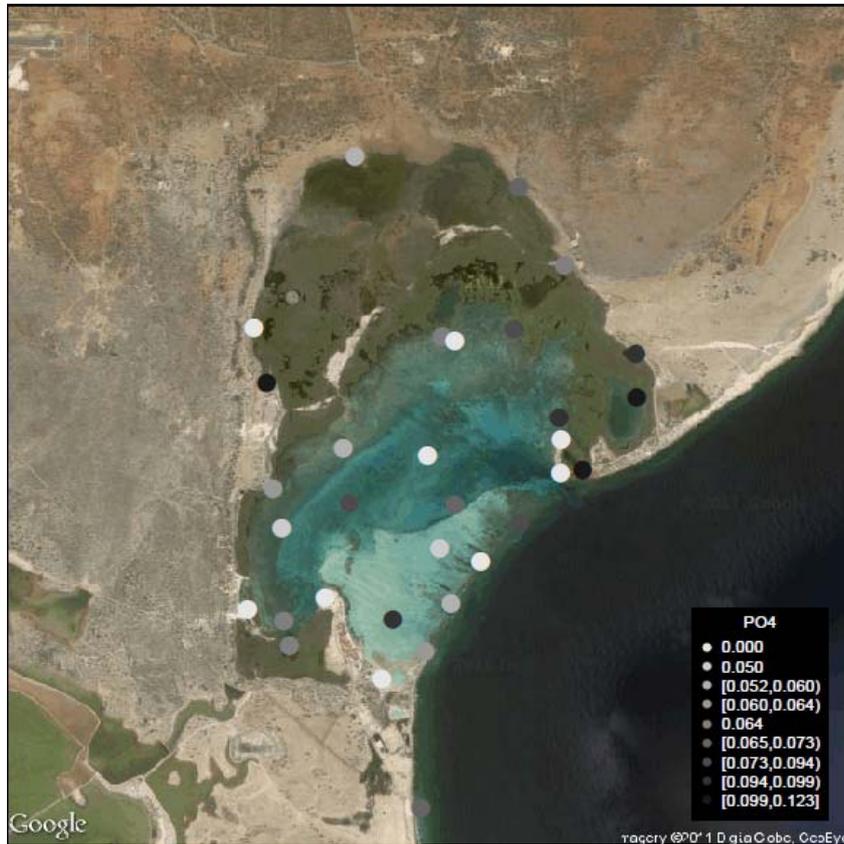
Station	Temperature (C)		Conductivity (ppt)		pH	
	Incoming Tide	Outgoing Tide	Incoming Tide	Outgoing Tide	Incoming Tide	Outgoing tide
B	29.50	29.39	35.55	35.55	7.74	7.75
SG1	28.76	29.31	35.33	34.97	7.75	7.77
SG2	28.70	29.37	34.20	34.71	7.60	7.84
SG3	28.37	28.97	35.80	35.74	7.96	7.98
SG4	29.02	29.45	35.89	35.96	7.94	8.01
SG5	28.70	28.61	35.75	35.77	7.97	7.97
SG6	29.20	29.56	35.99	35.97	7.83	7.96
SG7	29.05	29.03	35.75	35.74	8.02	8.04
SG8	29.19	29.41	35.92	35.90	7.97	8.01
CD1	28.70	28.50	35.74	35.73	7.95	7.99
CD2	28.71	28.76	36.67	35.74	8.01	8.03
CD3	28.61	28.69	35.60	35.76	8.01	8.03
CD4	28.92	28.90	35.71	35.81	8.02	8.06
SF1	28.69	28.54	35.74	35.78	7.92	7.99
SF2	28.61	28.44	35.70	35.70	7.99	8.00
Q	29.38	29.62	35.70	35.73	7.73	7.85
P	29.08	29.53	35.63	35.80	7.72	7.83
O	29.30	29.57	35.02	35.80	7.74	7.85
L	28.76	29.01	36.13	36.03	7.63	7.58
S	28.93	-----	36.06	-----	7.51	-----
T	28.90	-----	36.13	-----	7.50	-----
W2	-----	30.25	-----	2.02	-----	8.11

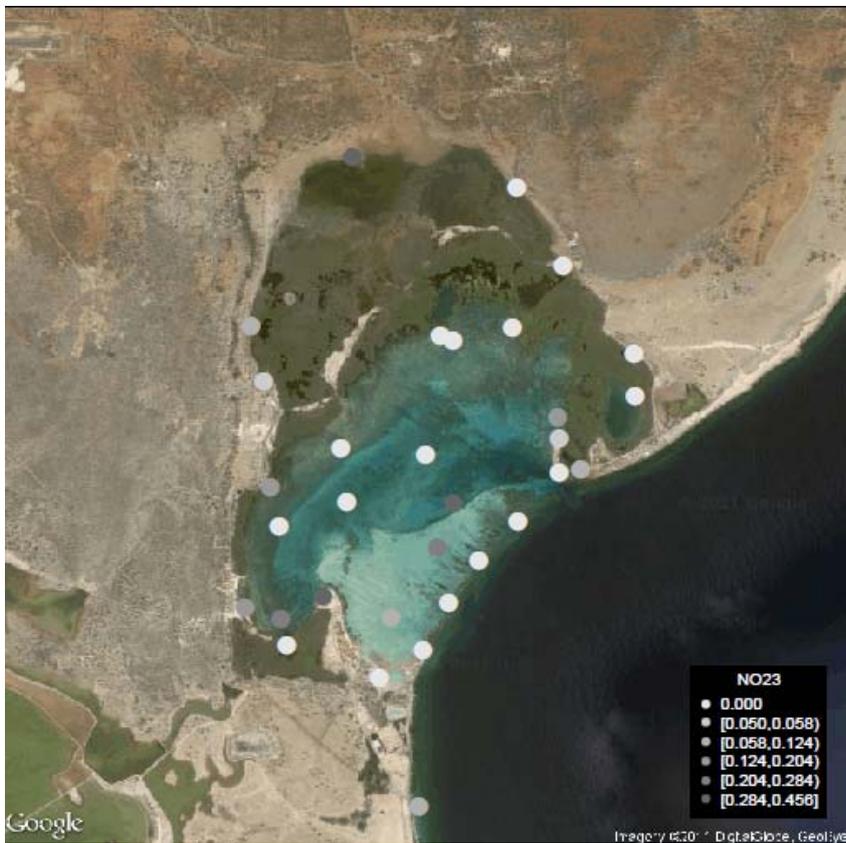
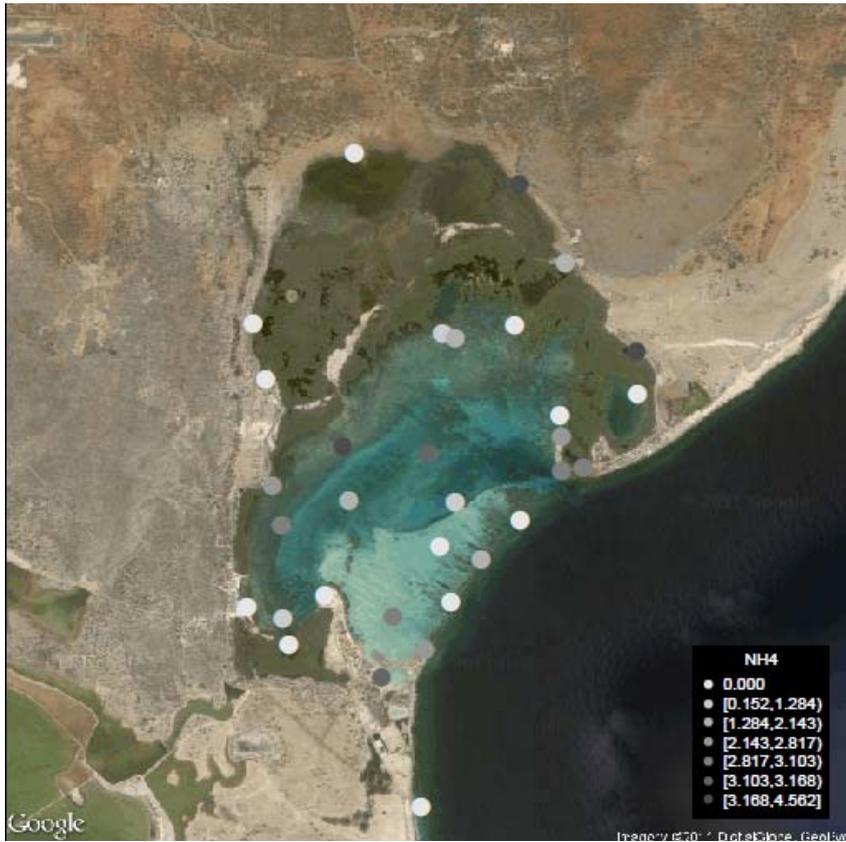
## Appendix 2. Station Locations

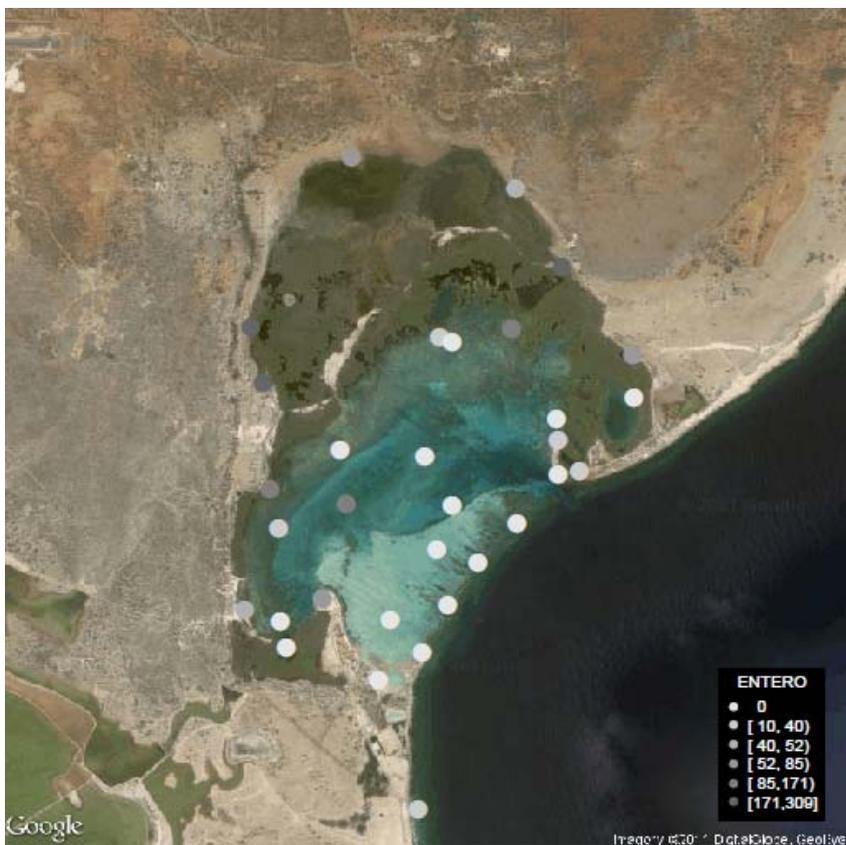
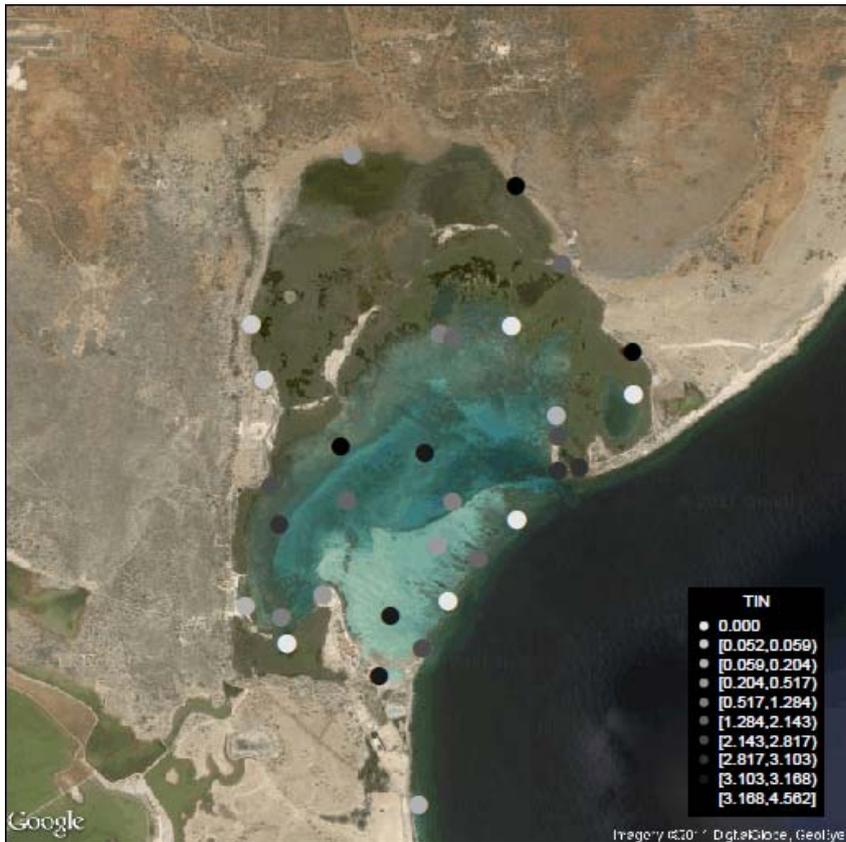
Station	Latitude	Longitude
A	12°5'28.8" N	68°14'03.1" W
B	12° 5'30.34"N	68°14'23.64"W
C	12°5'41.5" N	68°14'15.3" W
CD1	12°5'58.9" N	68°13'29.7" W
CD2	12°5'49.6" N	68°13'38.9" W
CD3	12°5'39.9" N	68°13'45.9" W
CD4	12°5'29.2" N	68°13'51.9" W
CS	12°4'53.2" N	68°13'55.1" W
D	12° 5'38.76"N	68°14'33.37"W
E	12°6'7'7" N	68°14'27.9" W
F	12°6'29.8" N	68°14'28.9" W
G	12°6'43.7" N	68°14'31.9" W
H	12°7'23.1" N	68°14'08.3" W
I	12°7'16.8" N	68°13'29.8" W
J	12°6'58.3" N	68°13'19.2" W
K	12°6'37.6" N	68°13'02.7" W
M	12°6'10.9" N	68°13'19.8" W
N	12°6'17.4" N	68°13'19.8" W
O	12°6'44.7" N	68°13'29.9" W
P	12°6'10.7" N	68°13'15.1" W
Q	12°6'30.1" N	69.14'08.1" W
R	12° 6'41.70"N	68°13'47.90"W
S	12° 6'27.60"N	68°13'2.50"W
SF1	12°5'37.7" N	68°14'006" W
SF2	12°5'52.8" N	68°13'49.6" W
W2	12° 7'32.35"N	68°14'32.40"W
W3	12°6'47.9" N	68°13'7'9" W
SG1	12°5'6'3" N	68°14'42.7" W
SG2	12°5'03.6" N	68°14'25.0" W
SG3	12°6'03.2" N	68°14'09.6" W
SG4	12°6'15.8" N	68°14'11.1" W
SG5	12°6'14'2" N	68°13'51.3" W
SG6	12°6'40.6" N	68°13'44.9" W
SG7	12°6'02.9" N	68°13'44.9" W
SG8	12°6'22.8" N	68°13'20.5" W

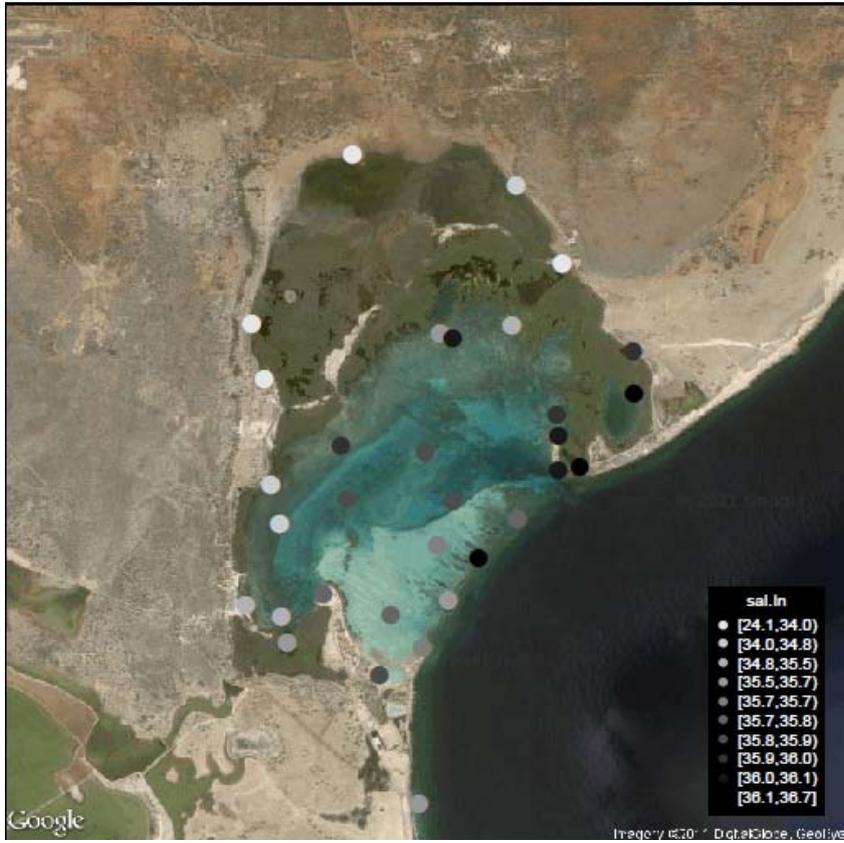
## Appendix 3 Geographical illustration of data

Overview of different parameters and concentrations at the visited locations in Lac during incoming tide









Overview of parameters and concentrations at the visited locations in Lac during outgoing tide.

