Water quality monitoring Bonaire

Results monitoring November 2011 and recommendations for future research

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Summary

On the island Bonaire, eutrophication is a point of serious concern, affecting the coral reefs in the marine park. Eutrophication can cause altered balance of the reef ecosystem because algae can outcompete corals, leading to a disturbed composition and deterioration of the biodiversity of the reef .
The reef of Bonaire faces nutrient input by various sources, of which enriched groundwater outflow from land to the reef is considered to be a substantial one. Groundwater is enriched with nutrients e.g. due to leaking septic tanks.
In order to reduce the input of nutrients on the reef via sewage water, a water treatment plant is being built on Bonaire. The treatment of sewage water will be extended in 2012 with a sewage system covering the so called sensitive zone, the urbanised area from Hato to Punt Vierkant. Based on the dimensions of the treatment plant and estimated connections to the plant, it can be assumed that a total of 17520-35040 kg of Nitrogen a year is removed from the sensitive zone, and will not leach out to the sea at the western coast of Bonaire. No estimates are known of the contribution of other sources to the total nitrogen load.

At the moment limited information is available about concentrations of nutrients in the marine environment. Therefore, Rijkswaterstaat Waterdienst asked IMARES to conduct a monitoring study.
The goal of this coastal monitoring study was to collect baseline water quality data to be able to study the effectiveness of the water treatment plant in coming years.

The study consisted of two phases and resulted in two reports:

1. recommendations for baseline monitoring in 2011,
2. monitoring, data evaluation, and recommendations

In this second report, monitoring data are presented and discussed, and recommendations for future monitoring are provided. Options for dissemination of data and data management are presented.

Monitoring:
In November 2011, field monitoring was performed at ten locations at the west coast, at two depths -6m and -20 m. Three of these locations lay with the “sensitive zone” and are suspect of enriched groundwater, being a diffuse source of nutrients. Other locations are regarded as relative reference locations, laying further offshore, north or south from the sensitive zone. The prevailing current is from south to north. The reference locations might be influenced indirectly by the (diffuse) source under study, or can be under pressure by other nutrient sources as e.g. the salt company in the south (see table).

Location	Outflow nutrient enriched groundwater	Other known influences	Treatment plant area	Reference
Playa Funchi	No	Indirect via wind/currents, salinas	No	Yes
Karpata	No	Indirect via wind/currents	No	Yes
Habitat	Yes, with sewage	Yes (fertilisers, brine)	Yes	No
Playa Lechi	Yes, with sewage	Yes (yachts)	Yes	No
18th Palm	Yes, with sewage	Yes (yachts, fertilisers)	Yes	No
Angel City	Yes, but not from sewage	Yes, via salt pans	No	relative
Cargill	Yes, but not from sewage	Yes, via salt pans (salpapanssnpannsplans)	No	relative
Red Slave	Yes, but not from sewage	No	No	relative
Ebo's Special (Klein Bonaire)	No	Indirect via wind/currents	No	Yes
South Bay (Klein Bonaire)	No	No	No	Yes

Samples were collected in triplo at -20 m and -6m water depth by SCUBA, and the following indicators were determined:

	indicative for		Analysis		environmental threshold
Indicator	**Treatment plant**	**other pressures**	**Method**	**laboratory/ institute**	
General (Temperature, pH, dissolved oxygen, salinity, turbidity)	indirect	yes (biotic, abiotic)	multimeter	In situ	3 NTU
Nutrients (NH4, NO2, NO3, PO4)	Yes	yes (biotic, abiotic)	continuous flow analyser	NIOO	DIN: 1 µmol/L, P: 0.1 µmol/L
Chlorophyll a	indirect	yes (biotic, abiotic)	aceton extraction	IMARES	0.5 µg/L
Stable isotope δ15N	Yes+	yes via foodweb	mass spectrometer	NIOZ	3 ‰
Bacteria (enterococci)	Yes	yes	Enterolert IDEXX	CIEE	>185 cfu/100ml >100 cfu/100ml >35 cfu/100ml
Benthic composition	Yes	yes	AGGRA	STINAPA, in prep	various

Monitoring data are compared to environmental threshold values for tropical ecosystems. In Figure I, a summary of this evaluation is presented. Data show that during this monitoring study, eutrophic conditions, based on DIN concentration, are observed at four out of ten locations: Habitat, Angel City, Cargill and Red Slave. No clear difference in eutrophic state between the sensitive zone and other locations is observed. Cargill, Red Slave and Angel city are influenced by percolation of enriched groundwater from the salt pans.

Nutrient concentrations in the "sensitive zone" do not clearly differ from reference observations at e.g. Playa Funchi, Karpata and Klein Bonaire, but bacteria counts do. Bacteria numbers at Habitat and Playa Lechi exceed EU, EPA and Caribbean Blue flag standards.

Stable nitrogen isotope ratios in macro algae show large variability and low average values near background levels, and are not specifically indicative for nitrogen related to sewage sources. Along developed coastlines with e.g. addition of inorganic fertilizer with low δ15N values will complicate the

study for a sewage signal. Analysing δ15N and organic N in groundwater should be considered in next monitoring in order to explain the low ratio found in this study.
Statistical similarity analysis between locations shows no similarity and relation to position of the location (within sensitive zone or reference). Location "Habitat" showed a clear dissimilarity compared to the other nine locations, and it is assumed brine effluent from WEB could be a steering factor in this observation.

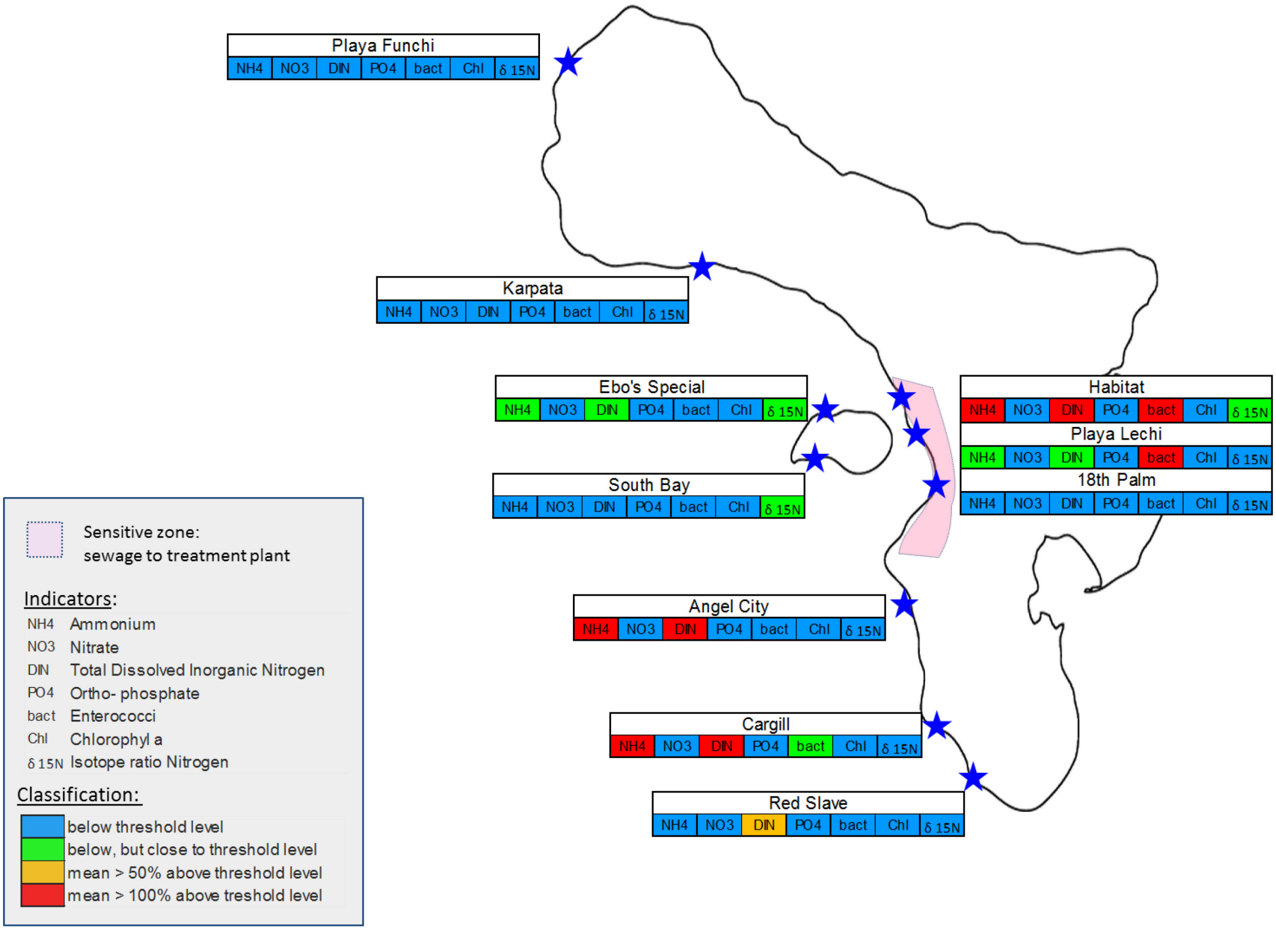


Figure I Summary of results, based on mean values.

The study of November 2011 leads to the following conclusions:

- Benthic surveys were not included in this study, and add largely to a whole ecosystem assessment on eutrophication. In upcoming research this should be included.
- Based on nutrient levels, in the south and in one location in the sensitive zone a eutrophic status was observed. The other locations did not have nutrient levels harming the development of a healthy coral reef, based on nutrient concentrations alone. Nutrients levels are however in a constant flux, and data should be considered in an ecosystem context.
- Enriched groundwater with nutrients from sewage is not the only source of nutrients. Other sources as nutrients from the salt pans in the south and from brine near Habitat probably add to the eutrophic status at these locations. Furthermore percolation and surface run off from Salinas and stormwater via roois are probably a source of nutrients as the isotope values at the other locations are low too.
- Monitoring in the coastal zone alone, will not provide adequate indication of the effectiveness of the treatment plant. Monitoring in the coastal zone is effective to detect areas at risk, and to detect long term changes in overall water quality (= so called "surveillance monitoring").

- Monitoring in the coastal zone should be supported by additional so called "investigative monitoring" at the sources to quantify the relative contribution of each of these sources in order to be able to discuss additional measures.

Above mentioned preliminary conclusions need to be considered using additional monitoring. Based on a one time monitoring activity no definite conclusions are possible related to the treatment plant.

"*Surveillance" monitoring* in the coastal zone will identify areas at risk, determine long-term changes in water quality, and can be used to evaluate environmental risk assessment.
Indicators to include are: nutrients (NH4, NO2, NO3, DIN, PO4, Total P, organic (kjeldahl) nitrogen) bacteria, benthic composition. The added value of N15 is questioned because of the average low response and high variability. A reference locations further offshore has to be added.
A clear advise on minimum frequency cannot yet be given as seasonal and diurnal variance is evident, but the extent not yet identified. Seasonal and diurnal dynamics (and thus variance) in nutrient availability is common at reef systems. Factors steering this seasonal variance are e.g wet and dry season, dynamics in regional upwellings, atmospheric pressure, biannual tidal regime, and irregular discharge in both quality and quantity. Suggestions for getting grip on this variance is provided in the report. A minimum frequency of monitoring in dry (May/June) and wet season (October/November) is suggested by parties involved. This frequency is a starting point, but could however be too low to detect significant trends. Future data have to be evaluated and monitoring has to be adapted according to the new results. Integration of these data with benthic survey data is considered to be a priority.

"Investigative monitoring" should be directed to measurements and evaluation of the quantity and quality of the sources and can be used to establish causal relations. In relation to the effectiveness of the treatment plant, it is advised to direct "investigative" monitoring to:

- quantity and quality of the influent and effluent of the Water Treatment Plant
- quantity and quality of other sources of nutrients via e.g. groundwater monitoring
    - Industrial sources (salt company, WEB brine effluent)
    - Salinas and roois

Indicators to include are: BOD, COD, bacteria, nutrients (NH4, NO2, NO3, DIN, kjeldahl N, PO4, total P), and 15N. Scenarios for field work are presented and cost estimates provided in the report.

Synchronization and support of STINAPA research
Options to integrate and support ongoing research by STINAPA are discussed in the report. The processing of obtained data by the benthic surveys is time consuming and therefore not yet available. Second subject is the dissemination of results from project "light and motion" by the university of California. These data could very well fit into an exploration of remote sensing as a cost effective monitoring technique for water quality. Both subjects could contribute largely to the assessment of water quality in the coastal zone of Bonaire and aid management decisions. Data analysis via e.g. student projects should be considered as an option.

Data management and dissemination of results:
Regarding data management and dissemination of results it is advised to further explore and to contribute to the development of the WUR portal on BES data and use the ISO standard by SeaDataNet to describe metadata. The WUR portal provides the opportunity of storing all BES data in a format of choice. Excel tables and figures, including the reports can be uploaded, and could for the time being be suitable enough to disseminate the data. The portal is under development and options for dissemination will be gradually extended and improved. If chosen to describe the monitoring and data with a metadata format prescribed by international standards, in time, the (meta) data could be synchronised with any other system. The location of the portal is http://scomp0703.wur.nl/bioplanbes/.

Contents

1 Introduction

On the island Bonaire, eutrophication is a point of serious concern, affecting the coral reefs in the marine park. Eutrophication can cause altered balance of the reef system because algae shall outcompete corals, eventually leading to a disturbed composition of the reef.

The reef of Bonaire faces nutrient input by various sources:

- Enriched groundwater outflow to the reef. Enrichment of groundwater is caused by:
    - Discharge of untreated sewage water collected from resorts, households and companies.
    - Sewage leaking from septic tanks. Estimated is that a total of 118.275 m^3/year[1] flows into the reef ecosystem (Anonymous, 2008).
    - Fertilizers in resort gardens
- Run off via salina's and storm water
- Illegal discharge and overflows of septic tanks
- Discharge of yachts+ cruiseships
- Industrial discharge (e.g. salt company and WEB)

In order to reduce the input of nutrients via sewage water, a program was established to build a water treatment plant on Bonaire. Recently a preliminary treatment plant was built treating 200 m^3 a day (73000 m^3 a year). The treatment of sewage water will be extended in 2012 with a sewage system covering the so called <u>sensitive zone</u>, from Hato to Punt Vierkant (Figure 1). This treatment plant, located at LVV near Lagun, is capable of treating 1200 m^3 a day (438000 m^3 a year), and Van Kekem et al. 2006 estimated that the total nitrogen balance shows a total reduction of nitrogen input due to the foreseen connections of septic tanks to the treatment plant (with 2006 specifications) about 70% (6.5 tonnes per year) in the sensitive zone (by the year 2017 compared to 2005) ..
Based on MIC, 2011 average influent conditions in practice are however assumed to be different (Table 1). Based on the details in table 1, it can be assumed that a total of 17520-35040 kg of Nitrogen is removed from the sensitive zone, and will not leach out to the sea at the western coast of Bonaire. The effluent will be discharged at the LVV area or used as irrigation water for agriculture. Part of the effluent might discharge to the sea at the eastern coastline, or infiltrates into the groundwater. The groundwater flows are unknown.

Table 1 Assumed influent and effluent conditions (MIC, 2011)

Aspect	**Specification**	**Equels to**
Average flow rate	480 m3/day	175200 m3/year
Influent Total Nitrogen	100-200 mg/l	17520-35040 kg/year
Influent total Phosphorus	75-200 mg/l	13140-35040 kg/year
Effluent Total Nitrogen	46 mg/l	8059 kg/year
Effluent total Phosphorus	65 mg/l	11388 kg/year

[1] This equals roughly to 21 m3/hour (in case of constant flow, which is not the case due to variable outflow).

Figure 1 Map of Bonaire. Stars indicate the boundaries of the sensitive zone between Hato (north) and Punt Vierkant (south)

At the moment limited information is available about the total amount of nutrients in the marine environment, and the contribution per source.

Rijkswaterstaat Waterdienst asked IMARES to conduct a study, consisting of three subtasks :

1. suggest a monitoring program to monitor eutrophication in the marine environment of Bonaire in which the relation to the treatment plant can be made clear;
2. conduct a baseline study based on this program;
3. based on the results, advise on a monitoring program for upcoming years

The subtask are reported in two separate reports. This is report 2 of this series. The first report describes the results from subtask 1. This report (2) describes in brief the planned approach (see report 1 for details), and deviations based on field and laboratory possibilities and experiences. The data are described and discussed. Recommendations for future monitoring are presented. Options for data management and dissemination of results is included as well.

2 Planned approach and deviations

2.1 Locations

Planned was to collect samples from the following locations at 20 m and 6m water depth:

1. Playa Funchi
2. Karpata
3. Habitat
4. Playa Lechi
5. 18th Palm
6. Angel City
7. Red Slave
8. Cargill/ salt company
9. Ebo's Special
10. South Bay
11. Lagun (only surface water due to risk of diving)

In Table 2 the specifications of the locations in terms of relevance to enriched groundwater with sewage from septic tanks are given.

Table 2 Overview of locations and their specifications.

Location	Outflow enriched groundwater	Other influence	Sensitive zone	Treatment plant area	Reference
Playa Funchi	No	Indirect via wind/currents, salinas	No	No	Yes
Karpata	No	Indirect via wind/currents	No	No	Yes
Habitat	Yes, with sewage	Yes (fertilisers, brine)	Yes	Yes	No
Playa Lechi	Yes, with sewage	Yes (yachts)	Yes	Yes	No
18th Palm	Yes, with sewage	Yes (yachts, fertilisers)	Yes	Yes	No
Angel City	Yes, but not from sewage	Yes, via salt pans	No	No	relative
Cargill	Yes, but not from sewage	Yes, via salt pans	No	No	relative
Red Slave	Yes, but not from sewage	No, via salt pans	No	No	relative
Ebo's Special (Klein Bonaire)	No	Indirect via wind/currents	No	No	Yes
South Bay (Klein Bonaire)	No	No	No	No	Yes
Lagun	Yes	No	No	Yes, via LVV	No

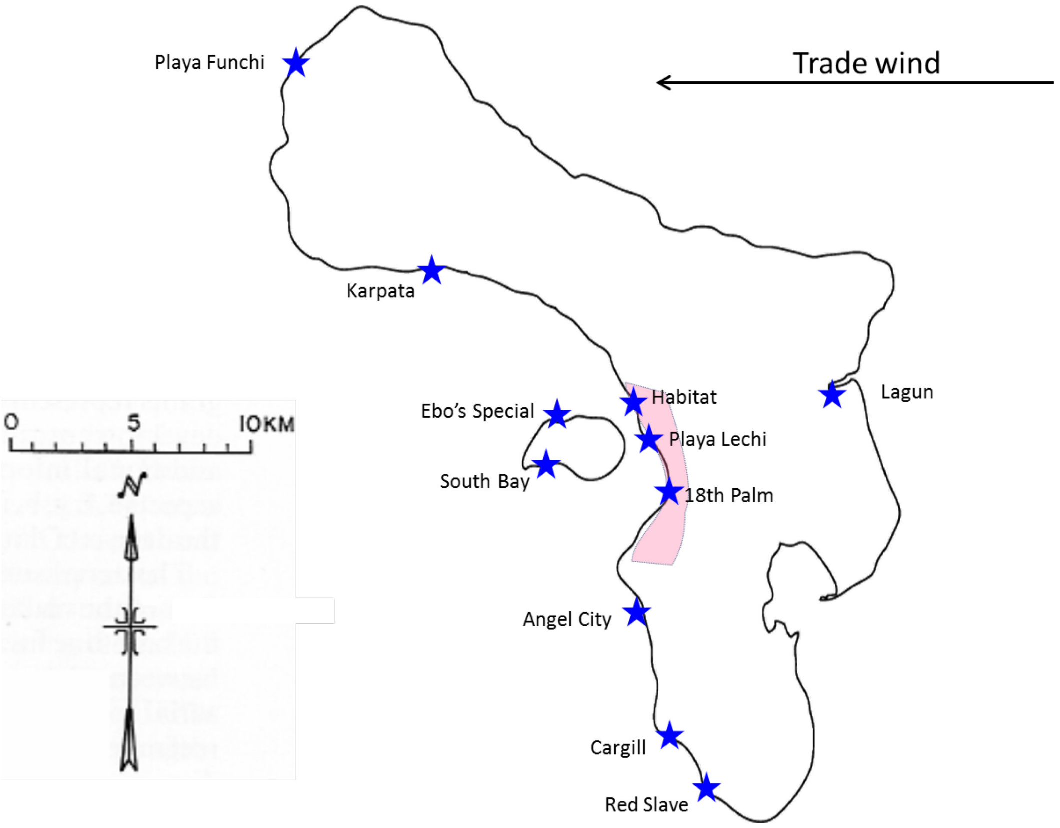


Figure 2 Geographical overview of locations.

Sampling was conducted according to plan, except that Lagun could not be visited due to time constraints. For Cargill/ Salt company the location is the same as dive spot Tori's reef. The channel of Cargill towards the reef was closed at time of sampling. GPS coordinates on shore were plotted during field sampling.

2.2 Overview of field and lab work

Fieldwork for the baseline monitoring took place in the period November 11- November 17, 2011 under coordination by IMARES.
Ramon de Leon (STINAPA) conducted the field sampling by means of scuba. Diana Slijkerman (IMARES) assisted in the field. The preparation of field samples and analysis of entero-bacteria was conducted in the laboratory of CIEE by Diana Slijkerman. General water quality parameters were analyzed in the field if possible, otherwise in the lab of CIEE immediately after returning there.

Each day, 2 field locations were planned to be visited in the morning. At each location, water sampling was done at two depths, 20 m, and 6 m. At each point, 3 sample bottles of 500 ml were filled for nutrient analysis, two dark bottles of 1 L for chlorophyll a and bacteria analysis. Macro algae were collected in a zip lock bag.
After sampling, the samples were prepared in the CIEE laboratory according to the protocols (report 1 for details, Slijkerman et al., 2012). Entero-bacteria analysis was done immediately after returning to the CIEE lab as these samples needed to incubate for 24 hours. After the bacteria processing the nutrient samples were prepared and filtered. The chlorophyll a samples were processed afterwards. Bottles and jars were cleaned according to protocol for the next day. Macro algae were stored in the refrigerator until a time window became available for processing. Analyses of nutrients was performed by NIOO laboratory

in Yerseke (Netherlands), isotope analysis at NIOZ, Texel (Netherlands) and Chlorophyll a at IMARES Den Helder (Netherlands).

2.3 General water quality parameters

Planned was to assess water quality parameters by means of a multimeter, including temperature, dissolved oxygen, pH, turbidity and salinity. These meters were expected to be available at CIEE, STINAPA, PROES or DROB. However, probes at all meters for dissolved oxygen and pH were not working properly and could not be calibrated. These parameters were thus not included in this field monitoring. Turbidity probe was not present.
In the field, one sample bottle of each depth was measured (temperature and salinity) as no deviations between sampling bottles were observed during the first day. The probe was cleaned after each measurement with acetone and Milli-Q water to avoid contamination.

2.4 Nutrients

Planned was to collect three water samples of one liter of each depth per location. The first sampling day we experienced that the caps were not suitable for collecting water by means of scuba. Other bottles of 500 ml were prepared for upcoming sampling. A total of 10 bottles of 500 ml were available, and additional two samples bottles of 250 ml were used. A small inconvenience was introduced by replacing these bottles. The replaced bottles have a narrow neck and regarding the processing of the samples in the lab, the syringe could not be put into the bottle. An intermediate step had to be introduced. The water of the sample bottle was put into a wider jar in which the syringe could take up the sample. This jar was cleaned according to protocol, and cleaned between each sample.

One 500 ml sample bottle was lost during sampling (Playa Lechi S3). This sample bottle was replaced by another 250 ml bottle for the next day.

Samples were directly put in coolers with icepacks and transported to the lab. Temperature decreased with approximately 4 degrees. Upon arrival in the lab, the samples bottles were stored in the refrigerator prior to further processing.

Of each sample bottle, a sample was prepared for nutrient analysis by means of filtering 20 ml over a 22 µm filter. The filtered sample was stored in the freezer. Additionally, of 2 bottles per depth extra samples were prepared to have an spare set of samples in case samples would defreeze during transport. This spare set was kept in the freezer on Bonaire during the transport of the first set.

The first 2 sampling days we experienced that the freezer had not enough capacity to freeze all samples within 24 hours. These samples might have been influenced by this delay in freezing. Data should be evaluated accordingly. Adjustments were made after this observation and the following samples were frozen within 24 hours.

Picture 1. Materials used during processing of the nutrient samples

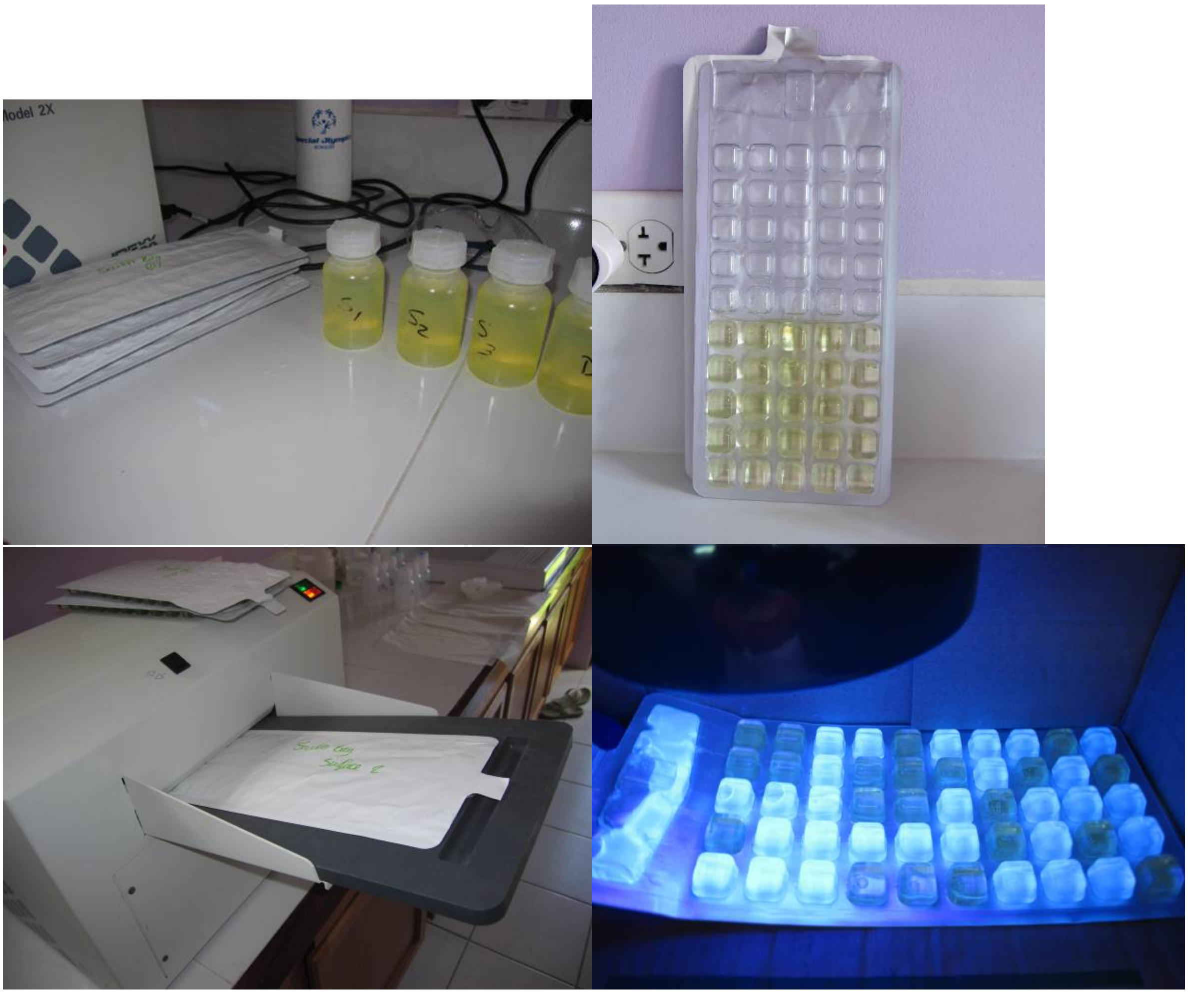
2.5 Faecal bacteria (enterococci)

Samples for faecal bacteria (enterococci) were collected in sterile 1000 ml dark bottles, and stored in the cooling box on ice prior to processing. Sterile syringes were used to sub-sample 10 ml and to transfer this to 100 ml sterile jars to which 90 ml of sterile water (locally obtained via Botika) was added. After day 1, additional surface water samples were collected in duplo[2].

Further analysis were performed according to protocol (see Slijkerman et al., 2012, report 1). Positive control media were not included in the ordered test kit, and could not be performed in this baseline study.

The first three days a large stove was used to incubate the samples at 41 °C. The other three days, the smaller “bacteria” incubator was used. This had two reasons: the first days the smaller stove was used for another project, and the larger stove was not in use for the macro algae samples yet. After the other project was finished, the macro algae were placed into the larger stove, and the smaller incubator was used for the Enterolert test. However, the capacity of this smaller stove showed to be limited: once samples were placed into the incubator, the heater switched on automatically to account for the loss of temperature. The heat temporarily raised to 45 °C (checked with inside hand-thermometer, instead of reading the outside thermometer logger), not only after opening the door, but also at times the heater needed to be on. This strong fluctuation in temperature was detected during the second series in this incubator. Samples near the heater (low level) could be influenced by this temperature elevation. Samples in the upper level of the incubator were more stable, and the thermometer did not show elevated temperatures there.

[2] Reagentia showed to have limited storage time. Additional samples from the surface could put additional data into the evaluation, and cost only limited extra time and money as spare reagentia were taken into account.



Picture 2. Overview of enterolert screening. Upper left: samples jars with dissolved reagent. Upper right: sample in tray. Lower right: sealer for trays . Lower right: screening for results under blacklight. Blue cells are positive for bacteria darks cells negative.

2.6 Chlorophyll a

Chlorophyll samples were taken from the same 1000 ml dark bottles that were used for the bacteria sampling . Of each bottle 500 ml was filtered using syringes and 0.22 µm glassfibre filters.
Filters were folded and stored in alu-foil and frozen immediately after processing. Filters were transported to the IMARES lab for analysis. Data indicated that 500 ml was enough to detect chlorophyll a.

Picture 3. *Overview of chlorophyll a processing. Water of 10 syringes with 50 ml were poured through a 0.22 µm filter. The filter is stored in a alufoil for freezing and transport.*

2.7 Stable isotopes in macro algae

Planned was to collect two species of macro algae at each depth per location, assuming that one species would be present across all locations. However, collecting two species per depth was not always possible as species were not easily to be found, and/or biomass could be very limited.
In cases were more than two species were found, these species were all included in the analysis in order to account for variance in species presence in upcoming sampling campaigns.

After collection, the samples were stored in the refrigerator in the lab prior to further processing. Time was too limited to process the samples the same day. At Tuesday, Wednesday and Thursday all samples were processed and stored in the 60°C stove.
Samples could be a mixture of species. In that case a choice was made for the prevailing species across all locations. Sand and debris, and epiphytes were washed-off with filtered water, obtained from the chlorophyll a analysis (waste). Forceps were used to handle the algae and filter paper was used to drain most of the water before putting the sampling in the stove. Pictures of all samples were taken in order to be able to recheck samples and species names.

At Saturday November 19th, samples were collected from the stove and put into ziplock bags. Due to the limited time that was available, not all samples were completely dried until stable weight by then.
Back in the laboratory of IMARES the samples were dried further and processed according to protocol.

Picture 4 *Overview of macroalgae processing*

Picture 5 Overview of macro algae after processing in the stove.

2.8 Transport

Frozen nutrient and chlorophyll samples were transported in a cooling box with cooling devices. A test was done in the days before transport to assess whether the coolpacks would still be frozen after 8 hours in 28 °C. The coolpacks were solid frozen after this test, and since airplane ambient temperature is much lower it was assumed that the frozen samples would be able to stay frozen during transport to the Netherlands.

The samples were almost all solid frozen when inspected after transport (19th of November/20th November). Only 1 sample which came in contact with the outer part of the coolbox was slightly defrosted (only limited, core was still solid).

In the Netherlands, the samples were stored in the freezer of IMARES laboratories at -20 °C and transported in the freezer to the NIOO laboratory on November 30th.

The macro algae samples were dried and packed in zip-lock bags and transported in hand luggage. No export permit was needed (accompanied letter by STINAPA to confirm).

2.9 Statistical analysis

All statistical analyses have been implemented and executed in R version 2.12.2 (The R Foundation for Statistical Computing, Vienna).

2.9.1 ANOVA analyses

For each of the measured parameters, an ANOVA (ANalysis Of VAriance) is performed. An ANOVA tests whether means of all groups are equal ($p < 0.05$). Hence, when the test rejects the null hypothesis, not all means of the groups are equal. Such ANOVA analyses have been performed individually for each nutrient, bacteria, chlorophyll-a, and δ15N isotopes as response variable. For the latter, it was tested to what extend the factor ‘location’, ‘depth’ and ‘macroalgae in which the isotope was analysed’ contributed to the variation of δ15N. For the other response variables, only the contribution of the factors ‘Location’ and ‘Depth’ to the variance was tested.
One of the assumptions in the ANOVA analyses is that the data is normally distributed. In order to get more normal like distributions, all data, except δ15N data, are fourth root transformed before analysis. Log transformation is not possible as our data contains a lot of zero values.

ANOVA analyses are followed by a post hoc Tukey's 'Honestly Significant Difference' test, in order to determine which groups differ significantly (remember that the ANOVA only tests whether or not all means are equal and does not compare individual groups). In the results section summaries of the significance testing are included. In Annex 3 more details are provided.

2.9.2 Box Plots

Box plots are used to visualise data per factor (either depth or location). Each box has a bold line somewhere in the middle, indicating the median value for that specific factor. The boxes indicate the first and the last quartile of the data. In other words, 50% of all observations (for the specific factor) lies within the box. Whiskers indicate the minimum and maximum values, excluding outliers. Outliers are shown as markers (◦). In the box plots, data are considered to be outliers if they deviate with more than 1.5 times the interquartile range from the first or third quartile. Box plots give a simple overview of the range of the observations.

2.9.3 Cluster analysis

In order to determine which locations share characteristics and which are dissimilar, a cluster analysis is performed. The nice thing about the cluster analysis is that it can include multiple characteristics at once. In the present study, the following parameters were included in the analysis: bacteria count, ammonia concentration, nitrogen dioxide concentration, nitrogen oxide (NOx) concentration, phosphate concentration and chlorophyll-a concentration. Nitrate concentration was not included as it highly correlates with NOx, which is included in the analysis. δ15N isotopes were not included because they were determined in different matrices (macro algea) for each location.
Data was first fourth root transformed, after which it was scaled such that the average value of each parameter equals zero, and has a standard deviation of one. Then , for each combination of locations (also distinguishing between deep and shallow samples), the Euclidean distance was calculated. The greater this Euclidean distance, the more dissimilar the locations are. Based on these distances a cluster dendrogram was generated, in which locations are clustered. Locations in the same cluster (on the same "branch" of the "tree") share characteristics, whereas locations in separate clusters (on the different "branches" of the "tree") are more dissimilar.

3 Results

In the figures, locations are plotted on the x-axes, and geographical ordered from North to South. Locations at Klein Bonaire (reference) cannot be ordered properly by geographical order, and are placed last. Locations laying within the sensitive zone (habitat, Playa Lechi and 18th Palm), and assumed to receive nutrient enriched groundwater are marked with an asterisk.
Data are compared to available studies on nutrient monitoring.

3.1 General water quality parameters

On average, water temperature at all sampled locations was 28.9 C ± 0.31. Average salinity was 36.7 ± 0.17. Temperature and salinity are not different between deep and shallow sampling depths. Oxygen and pH were not included as the probes were not working.

In annex 1 an overview is presented of the results of water quality aspects, as well as information on the tidal regime. Additional remarks as water depth, coordinates and weather specifications are included in the table.

3.2 Nutrient concentrations

If ANOVA analysis reveals depth to be a significant factor, data per location and depth are presented separately (n=3). If depth is not a significant factor, samples of both depths were not separated (n=6).

The data are discussed in line with the assumed influence of enriched groundwater in the sensitive zone versus reference area. The data of Wieggers (2007) are the only available data to compare with, and is done for the nutrient data and chlorophyll a.

Summaries on statistical results are provided. Only significant differences are mentioned. Meaning of significance asterisk in summaries: *< 0.05, ** < 0.01, *** < 0.001. Other differences are not significantly different.

3.2.1 Ammonium: N-NH4

In figure 1 the results for NH_4 are presented as boxplots. Average concentrations are presented in Figure 9. N-NH4 does not vary between deep and shallow sampling depths (see annex 3 for statistics) and therefore no distinction for depth is made in figure 1 and further data analysis. The replicates showed some variance, and the dataset is corrected for outliers (based on assumptions as described in section 2.8). This correction resulted in the discard of one datapoint: Red Slave S1.

No clear deviation was observed between reference locations (Ebo's Special, South Bay, Red Slave, Angel City, Karpata, Playa Funchi) and locations in the sensitive zone (Playa Lechi, habitat, 18th Palm). Lowest concentrations were observed at South Bay. Habitat, Angel City and Cargill show elevated concentrations of N-NH4, and are significantly higher than 18th Palm and South Bay. The environmental standard for nitrogen is 1 µmol/l. Habitat, Angel City and Cargill exceed this standard.

Cargill can be affected by enriched groundwater of the salt company. Habitat can be affected by WEB and resorts nearby this location. No clear explanation can be given for the high concentration at Angel City.

Wieggers (2007) found average NH_4 concentrations of 0,92 µmol/l, with lowest concentrations at Playa Lechi, Playa Funchi and Karpata and highest at Red Slave, Angel City and 18th Palm. In this study average NH4 concentration is 0,82 µmol/l. Playa Lechi being location with lowest NH_4 concentration is a similarity between these studies (see PO4 for similar observation). A clear deviation is that 18th Palm in this studies shows low NH_4 values, whereas Wieggers observed high NH4 values at this location.
As these are independent measurements, no conclusions can be drawn on the significance of the lower values of NH4 observed in this study.

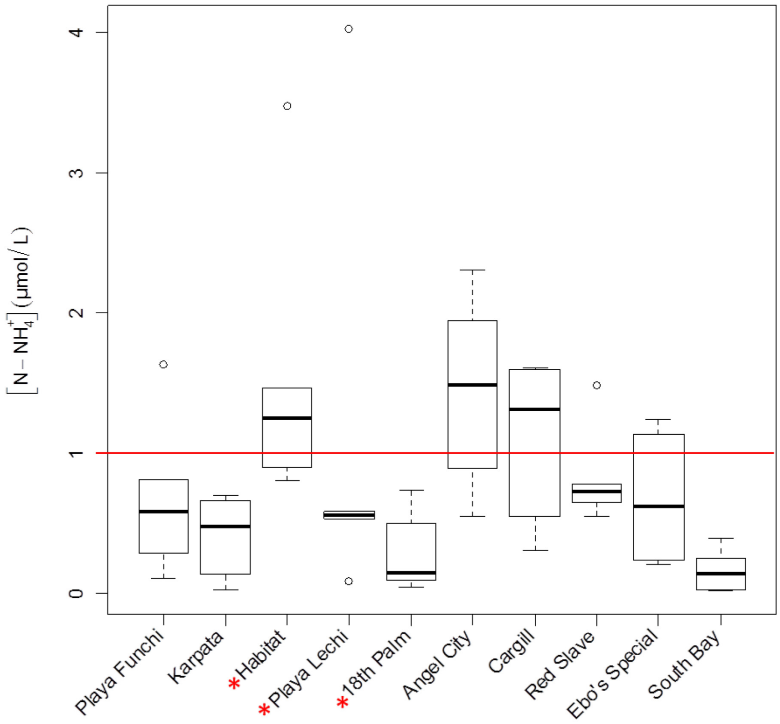


Figure 3 *Boxplot for N-NH4 concentrations (µmol/l) per location. No distinction between deep and shallow. Samples are pooled, n= 6. Red line at 1 µmol/l represents environmental standard. * indicate location within sensitive zone = assumed enriched area.*

Summary of statistical significant difference between locations. Locations in sensitive zone (enriched area) are written in bold:
Habitat>**18th Palm**** and South Bay***
Angel City> **18th Palm*** and South Bay***
Cargill> South Bay**
In annex 3 details are provided in the two way anova test.

3.2.2 Nitrate: N-NO_3

In Figure 4 and Figure 5 the results for NO_3 are presented as boxplots. Average concentrations are presented in Figure 9. N- NO_3 varies between deep and shallow sampling depths (see annex 3 for statistics) and therefore a distinction for depth is made in Figure 4 and figure 4 and further data analysis. The replicates showed some variance, but the dataset on NO3 did not contain significant outliers.

NO3- nitrogen does not exceed the environmental standard of 1 µmol/l N. Based on NO_3 nitrogen, no clear deviation was found between reference locations and locations within the sensitive zone. Playa

Lechi, a location assumed to be in the middle of the sensitive zone, and suspect to receive enriched groundwater, has a significant lower NO_3 concentration then most other locations (see summary of statistics for details). Karpata, Ebo's Special and South Bay, all locations assumed to serve as a reference for enriched groundwater, show higher NO_3 concentrations then some of the locations within the sensitive zone (e.g. Habitat and Playa Lechi).

Within locations, significantly higher N- NO_3 concentrations at -20 meter depth are observed for Habitat, Playa Funchi, and Red Slave compared to the shallow depth of -6 m. No other difference between depths were observed. The lower concentration at the -6 meter compared to concentrations at -20m is explained by consumption of NO_3 by corals, sponges, and macro algae between -20m and -6m.

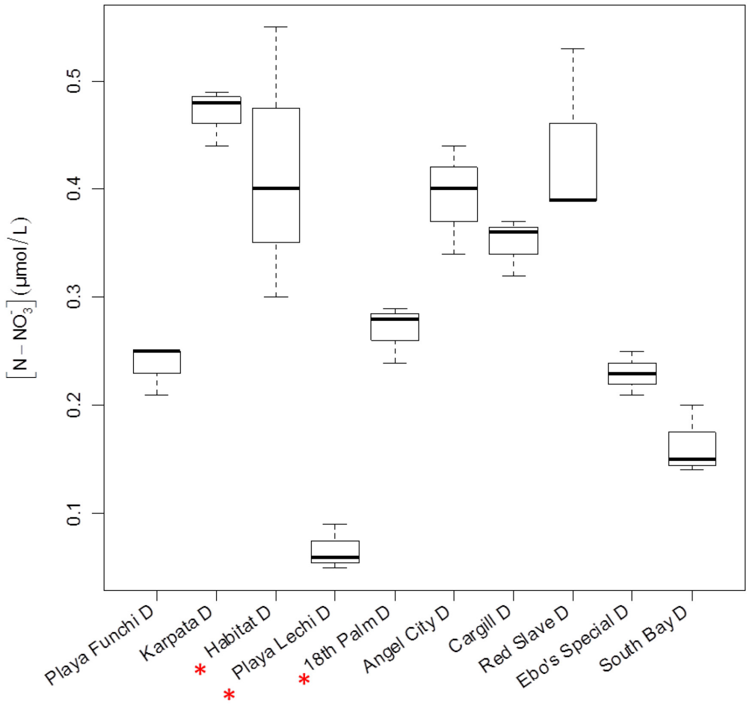


Figure 4 *NO_3 concentrations (µmol/l) at different locations, at deep (-20m) sampling position. * indicate location within sensitive zone = assumed enriched area.*

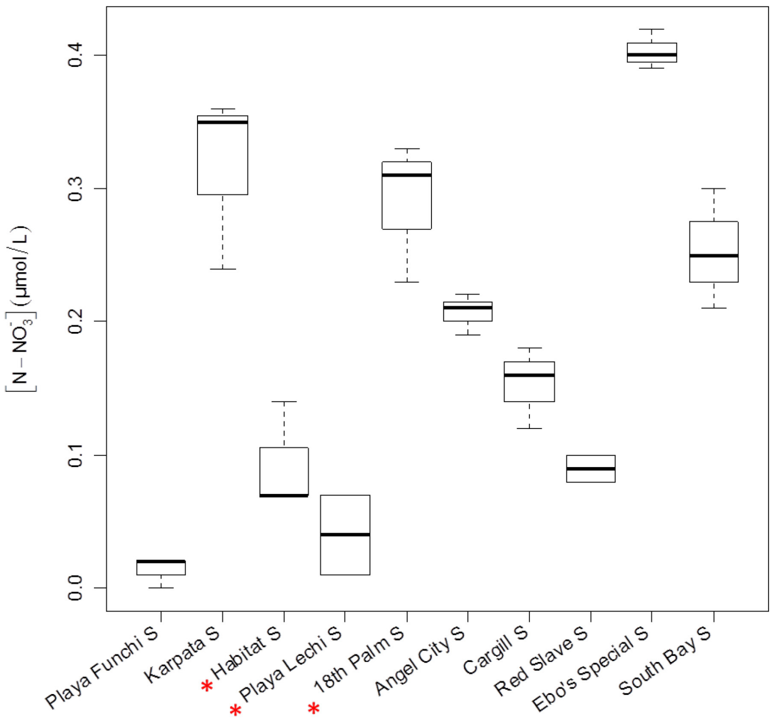


Figure 5 *NO_3 concentrations (µmol/l) at different locations, at shallow (-6m) sampling position. * indicate location within sensitive zone = assumed enriched area.*

Summary of statistical significant difference of N- NO_3 between locations at -20m. Locations in sensitive zone (enriched area) are written in bold:
18th Palm **, Angel City ***, Cargil ***, **Habitat** ***, Karpata ***> **Playa Lechi**
Red Slave > Playa Funchi **, Playa Lechi ***
18th Palm > Playa Funchi ***

Summary of statistical significant difference of N-NO3 between locations at -6m. Locations in sensitive zone (enriched area) are written in bold:
Ebo's Special > **Habitat**, Playa fungi, **Playa Lechi**, Red Slave (all ***)
Angel City and **18th Palm**> Playa Funchi, **Playa Lechi** (***)
Karpata> Playa Funchi***, **Playa Lechi** *** and **Habitat** *
Red Slave > Playa Funchi ***
South Bay> Playa Funchi*** and **Playa Lechi** ***

3.2.3 Nitrite: N-NO_2

In Figure 6 the results for NO_2 are presented as boxplots. N- NO_2 does not vary between deep and shallow sampling depths (see annex 3 for statistics), and therefore no distinction for depth is made in figure 4 and further data analysis. The values are very low, and close to or under detection limit. The box plot therefor show abnormal plots.

No clear deviation was observed between reference locations and the sensitive zone. Lowest concentrations were observed at South bay and Playa Lechi, highest concentrations at habitat and Angel City.

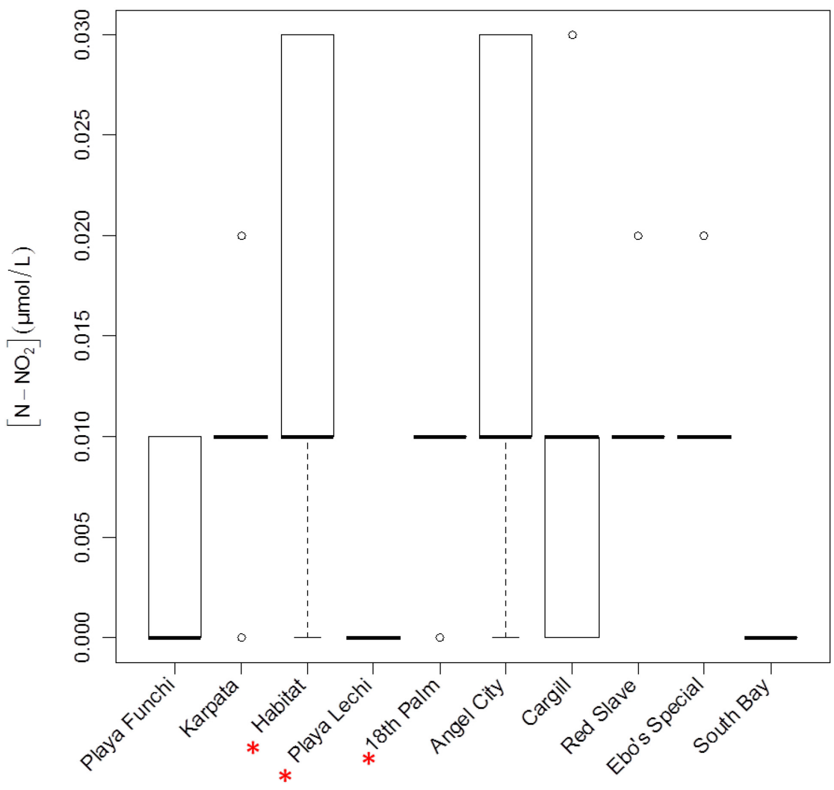


*Figure 6 N-NO2 concentrations at different locations. * indicate location within sensitive zone = assumed enriched area.*

Summary of statistical significant difference of N- NO_2 between locations. Locations in sensitive zone (enriched area) are written in bold:
Karpata > **Playa Lechi****, Souh Bay ***
Habitat > **Playa Lechi*****, South Bay ***
Playa Lechi < **18th Palm**, Angel City, Red Slave, Ebo's Special
South Bay < **18th Palm** **, Angel City ***, Cargil *, Red Slave***, Ebo's Special***

3.2.4 Nitrate+ nitrite: N-NO_x

In Figure 7 the N-NO_x (NO_2+ NO_3) concentrations are plotted in a boxplot. N-NOx does not vary between deep and shallow sampling depths (see annex 3 for statistics) and therefore no distinction for depth is made in figure 5 and further data analysis.

N-NOx consists mostly of N-NO_3, and does not exceed the environmental standard of 1 µmol/l N.
No clear deviation in N-NOx concentration was found between reference locations and locations within the sensitive zone. Playa Funchi and Playa Lechi show lowest N-NOx concentrations, being significantly lower than most other locations except South Bay. Karpata and Red Slave show the highest concentration. For details see the summary on significant differences.

Wieggers (2007) found average concentration of N-NOx of 0,58 µmol/l, with lowest values at Playa Lechi and south bay, and highest at Playa Funchi, Habitat and Red Slave. These differences between locations are not in line with this study, but some similarities occur (e.g for Playa Lechi and Red Slave). In this study, the average concentration is 0,26 µmol/l, being more than half of the concentrations found by

Wieggers. However, large variance applies to these data within and across locations. Temporal variance is likely to affect the concentration of N due to seasonal variance and or daily variance (tidal influence). Therefore, no conclusions can yet be drawn on the observed difference in NOx concentration.

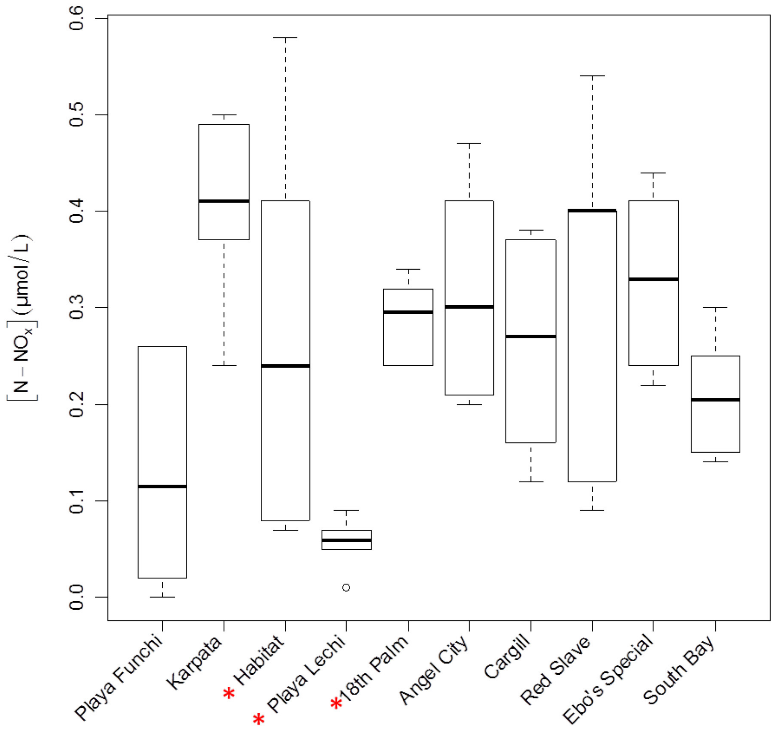


Figure 7 *N-NO$_x$ (NO$_3$+ NO$_2$) in µmol/l at different locations. * indicate location within sensitive zone = assumed enriched area.*

Summary of statistical significant difference of N-NOx between locations. Locations in sensitive zone (enriched area) are written in bold: Playa Funci < Karpata, Habitat, 18th Palm, Angel City, Red Slave, Ebo's Special Playa Lechi< Karpata, Habitat, 18th Palm , angel City, Cargill, Red Slave, Ebo's Special

3.2.5 Dissolved inorganic Nitrogen (DIN)

In Figure 8, DIN concentrations are plotted in a boxplot, and in Figure 9 average DIN values split into NH4 and NOx are presented. Total N does not vary between deep and shallow sampling depths (see annex 3 for statistics) and therefore no distinction for depth is made in figure 6 and further data analysis.

Total nitrogen exceeds the environmental standard at various locations: Habitat, Angel City, Cargill, and Red Slave. No clear deviation between locations in the sensitive zone and in reference areas was observed. Only Habitat is located in the sensitive zone, the other locations lay south of Kralendijk. Red lave and Angel City were assumed to serve as reference locations due to their unexposed position. Cargill might have elevated concentrations due to the outflow of enriched water from the salinity plant.

Wieggers (2007) found average DIN concentrations of 1,50 µmol/l, with lowest concentrations at Playa Lechi, Ebo's Special and Karpata, and highest concentrations at Angel City, Red Slave and 18th Palm. In this study, the average concentration is 1.08 µmol/l ± 0.81 (SD), with lowest concentration found at

Playa Lechi, 18th Palm and South Bay, and highest concentrations found at Angel City, Habitat and Cargill. Similarity between the study is the low concentration at Playa Lechi and high at Angel City, but the low concentration at 18th Palm is clearly deviating among the two studies.

The average DIN in this study is 1.08 µmol (± 0.81), which is almost 30% lower than the values of Wieggers. Structural decrease of DIN is cannot however not be demonstrated yet as both studies are single observation with different methods. Difference can be explained by e.g. seasonality, and Wieggers has not reported the period of sampling to look into this matter.

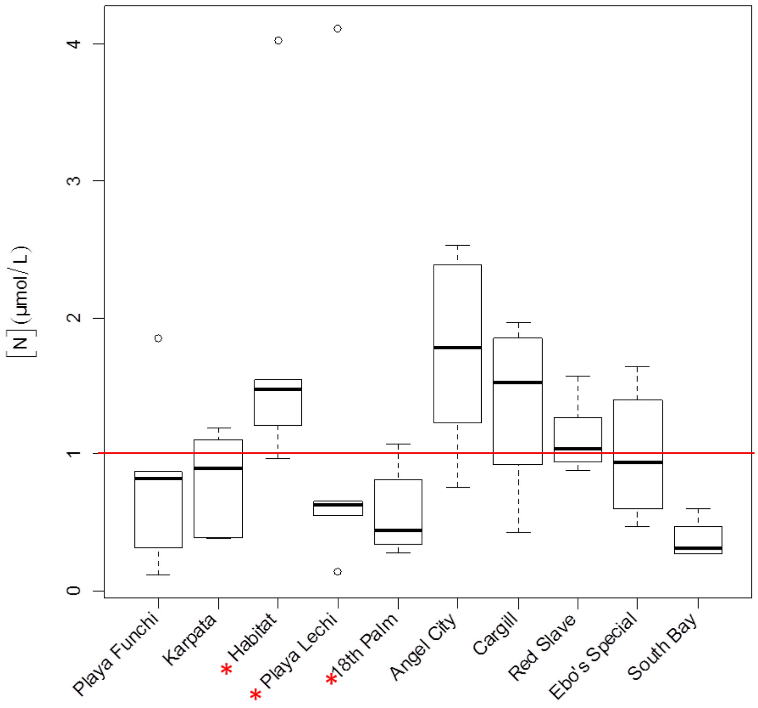


Figure 8 *Total Dissolved Inorganic Nitrogen in µmol/l, at different locations. No distinction between deep and shallow. Samples are pooled, n= 6. Red line at 1 µmol/l represents environmental standard. * indicate location within sensitive zone = assumed enriched area.*

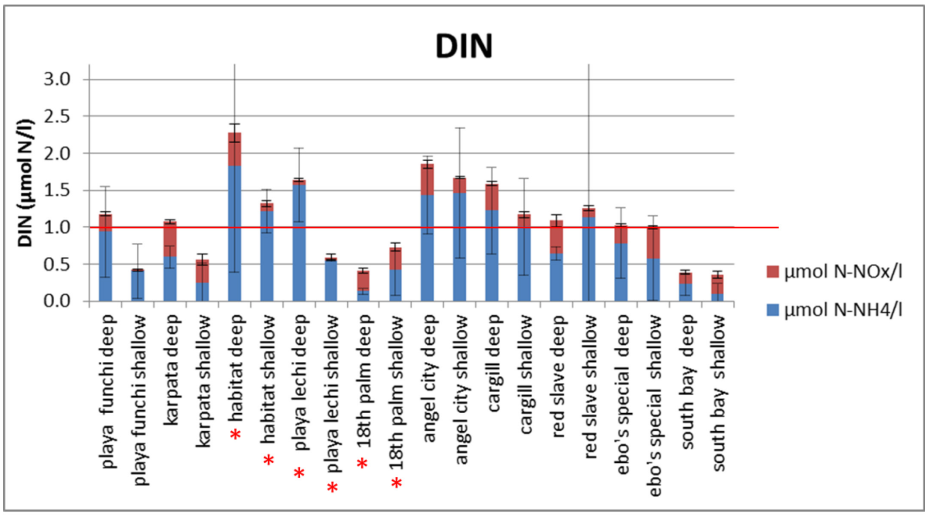


*Figure 9 DIN split into the contributions of N-NOx and N-NH4 including standard deviation. Red line at 1 µmol/l represents environmental standard. * indicate location within sensitive zone = assumed enriched area.*

Summary of statistical significant difference between locations. Locations in sensitive zone (enriched area) are written in bold:
Habitat > South bay **
AC>**18P**** + South Bay *
CAR> SB*
In annex 3 details are provided.

3.2.6 Phosphate: P-PO_4

In Figure 10, P-PO4 concentrations are plotted in a boxplot, and in Figure 11 as average values. P-PO4 concentrations are not structurally influenced by sampling depth (fig. 10; see annex 3 for statistics). Therefore no distinction for depth is made in Figure 10 and further data analysis. The replicates per location and depth showed some variance, and the dataset is corrected for outliers (based on assumptions as described in section 2.8). This correction resulted in the discard of one datapoint: Red Slave S1.

P-PO4 does not exceed the environmental threshold level of 0.1 µmol/l. No clear deviation between locations in the sensitive zone and in reference areas was observed.

Wieggers (2007) found average P-PO_4 concentrations of 0.11 µmol/l on Bonaire, with lowest concentrations observed at Playa Lechi, Karpata and Playa Funchi, and highest concentrations at Angel City and 18th Palm. In this study, P-PO_4 average concentration is lower, being on average 0.02 µmol/l (all locations, all depths), being lowest at Playa Lechi, Cargill and Ebo's Special, and highest at Angel City and 18th Palm. Playa Lechi being low at PO4 is a similarity between these studies, and was observed for NH_4 as well.
The average P-PO_4 in this study is much lower than the value of 0.11 µg/l by Wieggers. This difference in amount of PO_4 could be explained by various factors as seasonality, daily/tidal regime and run off of sediments with associated nutrients.

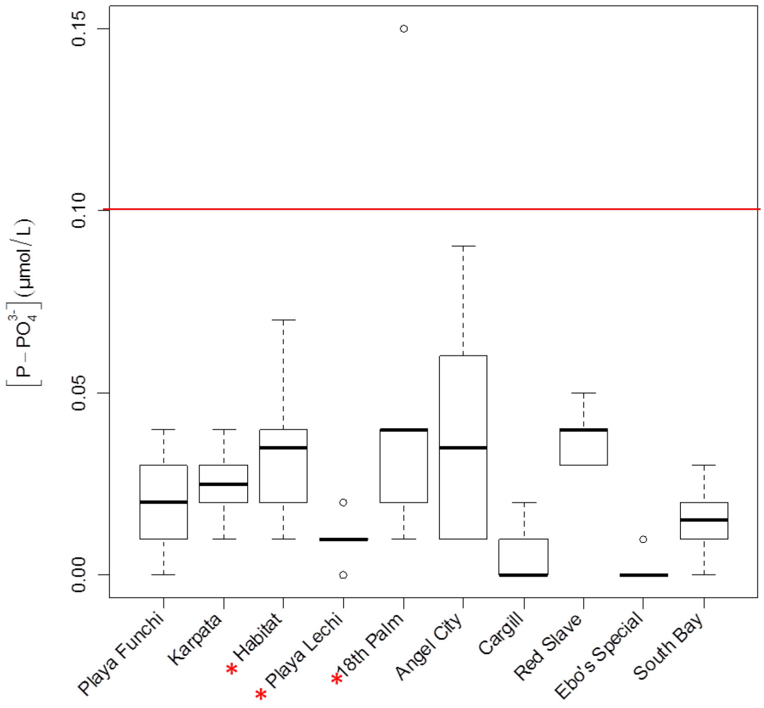


*Figure 10 P-PO4- concentrations µmol/l. Red line represents environmental threshold concentration. * indicate location within sensitive zone = assumed enriched area.*

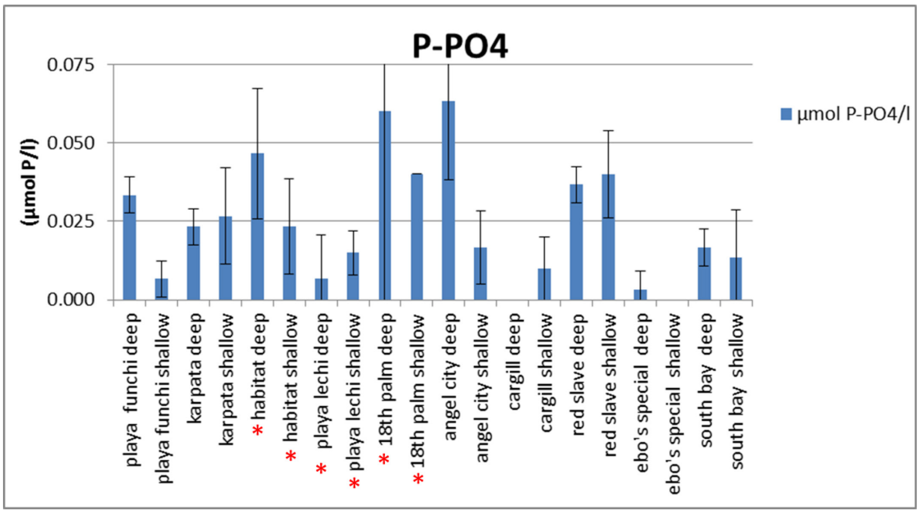


*Figure 11 P-PO4 average values including standard deviation. * indicate location within sensitive zone = assumed enriched area.*

Summary of statistical significant difference between locations. Locations in sensitive zone (enriched area) are written in bold:
Ebo's Special < Playa Funchi**, Karpata***, **Habitat** ***, **18th Palm*****, Angel City *** Red Slave***, South bay *
Cargill < Karpata**, **Habitat*****, **18th Palm*****, Angel City *** , Red Slave***

Some nutrient parameters vary largely (e.g. NO3, NH4) and this variance hampers the determination of a significant difference between locations, and consequently the possibility to track the future decrease of nitrogen. Variance can have been a locations specific feature, or being introduced during sampling, processing of the sample, conditions in the laboratory, conditions during storage and transport (slow freezing of samples and defreezing during transport) and analysis. The monitoring and sample processing protocol was strictly followed, and although there are some "suspect" samples that lay outside the boxplot, only 1 sample was detected as a significant outlier (Red Slave S1).
The slow freezing of the samples could have played a role, but would have resulted in lower ammonium concentrations due to nitrification and higher nitrate concentrations due to reduction. NOx concentrations does not vary that much, and seems not to have influenced the data.

3.3 Faecal bacteria (enterococci)

In Figure 12, Figure 13, Figure 14, and Figure 15 data on enterococci number are presented.
Levels of enterococci, ranged from undetectable to 429 cfu 100 ml^{-1}, with an average for deep sampling depth of 2.7 ± 6.4 cfu 100 ml^{-1} (n =30), an average of 32.1± 89.7 cfu 100 ml^{-1} (n = 30) for shallow samples, and 51.3± 127 cfu 100 ml^{-1} (n = 20) for surface samples (n= sample total). High variance is explained by the large differences between locations.

Depending on the standard, five or six samples out of 80 exceeded the standard (see table 2), based on single observations (all guidelines evaluate on multiple measurements and take a 90 or 95-percentile). The samples which exceed a standard were taken at Habitat (shallow n=3, resp. 324, 254, 306 cfu/100 ml) and Playa Lechi (surface n=2, resp 406 and 429 cfu/100 ml), or Cargill surface (97 cfu/100 ml). None of the deep water samples exceeded a limit or standard. An enterococci level of 185 cfu 100 ml^{-1} is assumed to have a risk of illness factor of 5% (1 in 20 bathers will become ill) for bathing waters (Kay et al., 2004). This value is used by the World Health Organization (WHO), as an indication of unacceptable water quality .

Playa Lechi and Habitat lay within the sensitive zone, both suspect of receiving groundwater outflow to the reef. During sampling, heavy rains took place, and surface run off can have contributed to bacteria run off towards sea. The high numbers at Playa Lechi are most probably a result from surface run off, and the higher number at Habitat at -6 m from groundwater outflow. The higher numbers at Cargill can be explained by surface run off as well.

Enterococci were not detected at Playa Funchi, Red Slave, South Bay and Ebo's Special. It should be taken into account that a positive control test was lacking, and that during the incubation of South Bay and Ebo's Special, the incubator exceeded the prescribed temperature. The effect of this could be a false negative score due to the fact that the bacteria (if present) did not survive the elevated temperature. Although no enterococci are expected at these locations, the conclusions for South Bay and Ebo's Special have to be considered with attention.

Table 3 Number of samples exceeding a standard for enterococci number.

Standard (cfu) (100 ml^{-1})	EU bathing water directive (185) (EEC, 2006)	Caribbean Blue Flag (100) UNEP (2003)	ISO guideline (100) (ISO, 1996)	US EPA (35) US EPA, 1986
Number samples exceeding	5	5	5	6

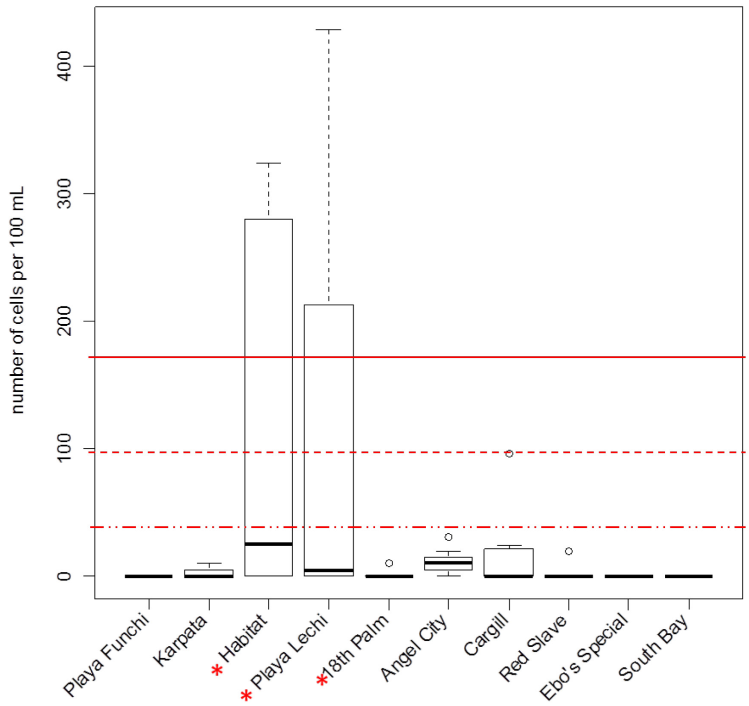


Figure 12 *Enterococci numbers per location (number of samples=8), depth not taken into account. Red line represents the EU bathing water standard (185 cells per 100 ml). Dashed red line represent the (UNEP, 2003) Caribbean blue flag criterium (< 100 cells/ 100 ml). Course dashed line represents the US EPA standard of 35 cells/100 ml. * indicate location within sensitive zone = assumed enriched area.*

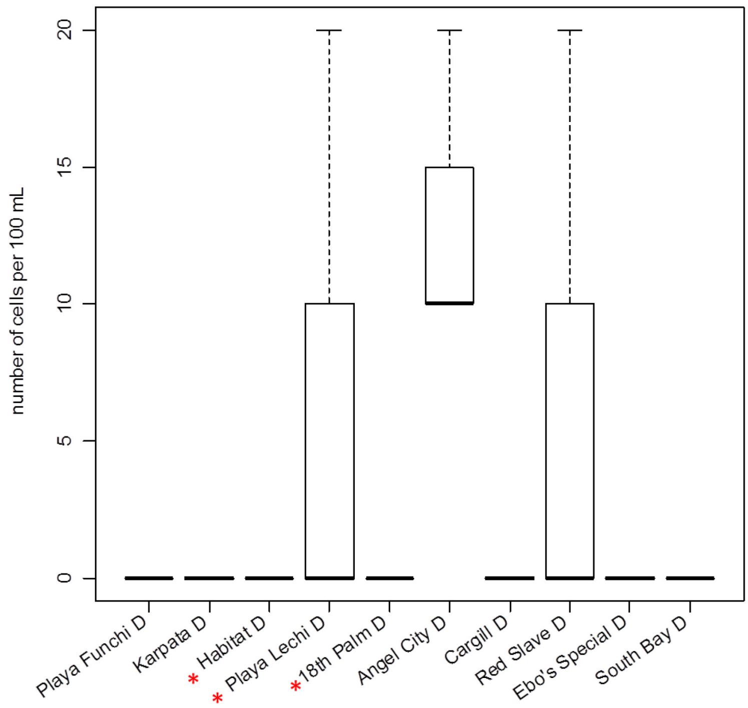


*Figure 13. Enterococci numbers per location at the depth of 20 m (number of samples =3). * indicate location within sensitive zone = assumed enriched area.*

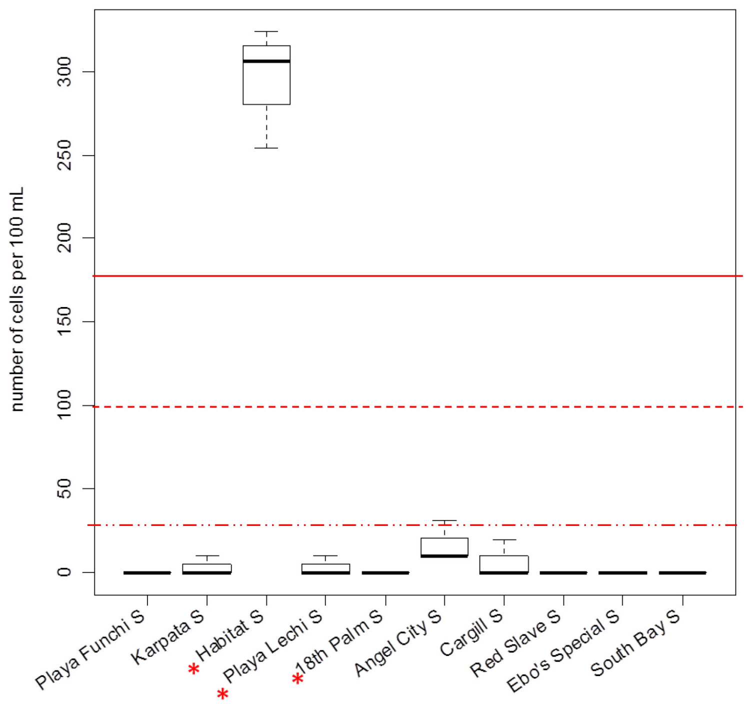


*Figure 14. Average enterococci numbers per location at the shallow depth of 4-6 m (number of samples=3). Red line represents the EU bathing water standard (185 cells per 100 ml). Dashed red line represent the Caribbean blue flag criterium (< 100 cells/ 100 ml). Course dashed line represents the US EPA standard of 35 cells/100 ml. * indicate location within sensitive zone = assumed enriched area.*

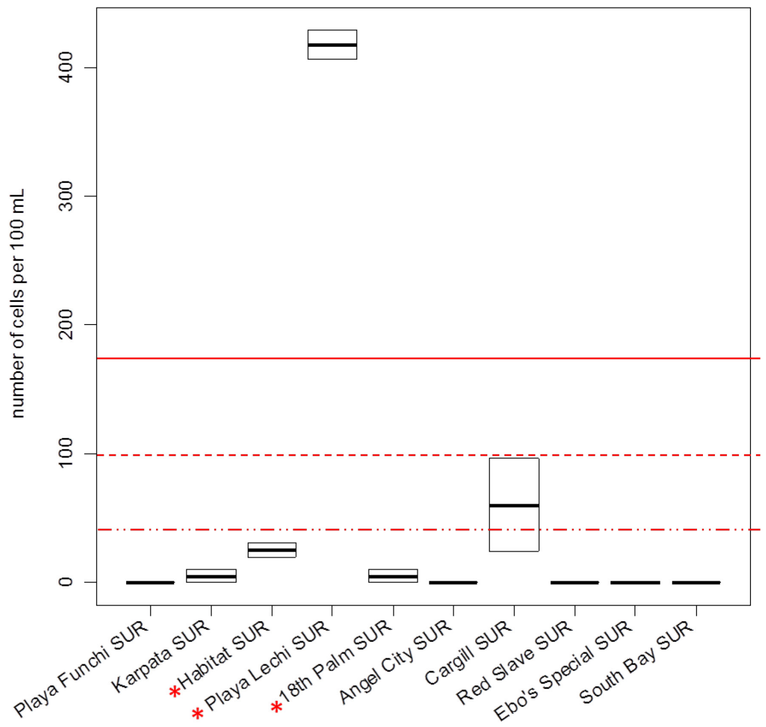


*Figure 15 Average enterococci numbers per location in surface water (umber of samples=2). Red line represents the EU bathing water standard (185 cells per 100 ml). Dashed red line represent the Caribbean blue flag criterium (< 100 cells/ 100 ml). Course dashed line represents the US EPA standard of 35 cells/100 ml. * indicate location within sensitive zone = assumed enriched area.*

3.4 Chlorophyll a

In Figure 16 concentrations of Chlorophyll a are plotted in a boxplot. Chlorophyll a does not vary between deep and shallow sampling depths (see annex 3 for statistics) and therefore no distinction for depth is made in figure 8 and further data analysis.

Chlorophyll a levels do not exceed the environmental standard (0.5 µg/l). A clear geographical difference was observed, but no clear deviation between locations in the sensitive zone and in reference areas. Locations at the south, Red Slave, Angel City and Cargill show lowest chlorophyll a concentrations. Location within the sensitive area, and up north show highest concentrations. Location at Klein Bonaire, Ebo’s Special and South Bay, show intermediate concentrations.

Wieggers (2007) found chlorophyll a concentration between 0,15- 0,22 µg/l, with lowest concentrations at Angel City, Playa Funchi, Ebo’s Special and South Bay. Highest values were found at 18th Palm and Karpata. No similarities are found between these two studies. In this study, chlorophyll a concentrations vary between 0,05 – 0,21 µg/l, and locations with lowest concentrations have 0,06-0,11 µg/l chlorophyll a. Location with highest chlorophyll a concentration in this study show concentrations of 0,13-0,19 µg/l. It is not described when Wieggers took his samples. Seasonal (Venezuelan upwelling, annual or even daily variation of chlorophyll a concentration is most probably an underlying factor of the observed difference and should be further studies before drawing conclusions.

No correlation between chlorophyll a and nutrient concentration is observed (Annex 4). Other, ecological mechanisms such as light and turbidity, and the concentration of silica could be additional explaining factors. These factors are not taken into account in this monitoring. Furthermore weather (wind and wave action resulting in mixing water layers) and tidal regime can be co-factors. All locations were monitored during low tide, with the tide coming in (Annex 1 for details on tide regime).

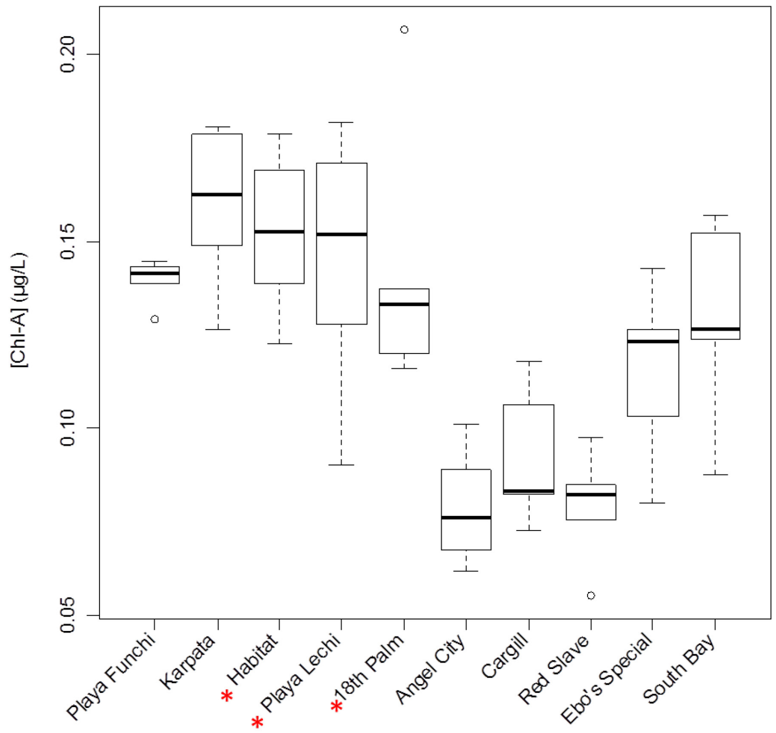


Figure 16 *Boxplot of chlorophyll a data. Red line represents environmental threshold concentration. * indicate location within sensitive zone = assumed enriched area.*

Summary of statistical significant difference between locations: Playa Funchi, Karpata, **Habitat**, Playa Lechi, **18th Palm** > Angel City, Cargill, Red Slave (all ***) Eo special **, South Bay*** > Angel City South Bay > Red Slave ***

3.5 Stable isotope ratios in macro algae

Across depth and locations different species were collected, and the biomass (not weighted) of each collection differed (based on presence). In annex 2 an overview is given of the macro algal species collected per location and depth. Discussing these data, it should be noted that the net biomass of some samples was limited, and that the duplicate measurements of these samples showed large variance. Although samples were cleaned to the best notice, the variance might be attributed to e.g. sand or salt particles weighted with the samples, and contribute to total weight. Furthermore, the total %N of some samples was low, and this could results in a noisy result. The data should be considered with this in mind.

No significant relation with location and/or depth was observed for isotope ratios. This was because the data were single species observations per location, but as well, no trend in the data was observed when data were grouped. In Figure 17 the δ15N ratio (‰) is presented per location (n=variable, mostly based on 2-3 species per depth). In Figure 20 δ15N ratio (‰) is presented per species (n is variable). In

Figure 18 and Figure 19, δ15N ratio (‰) are grouped of resp. *Dictyot*a sp. (*Dictyota pinnatifiada* and *Dictyota pulchella*) and *Halimeda* sp. (*Halimeda opuntia* and *Halimeda coposia*) and plotted per location. These figures show the differences of δ15N per specie per location. Dictyota species show less variance within the samples compared to samples of *Halimeda* species. The variable δ15N values of *Dictyota* and *Halimeda* suggests that these seaweeds may be readily able to utilize whatever form of nitrogen is most available, and that local variance in δ15N should be well indicated.

When all data (all species from all locations) are averaged, the average value is 1.1 ‰ (±1.4). No difference between reference and sensitive zone is observed. Indicated isotopic ratios in macro algae under sewage influence are > 3 ‰ (see report 1 for an overview of cited literature). The average value of the isotope ratio in this study does not indicate that the sampled macro algae have been exposed to relatively large n volumes from sewage.

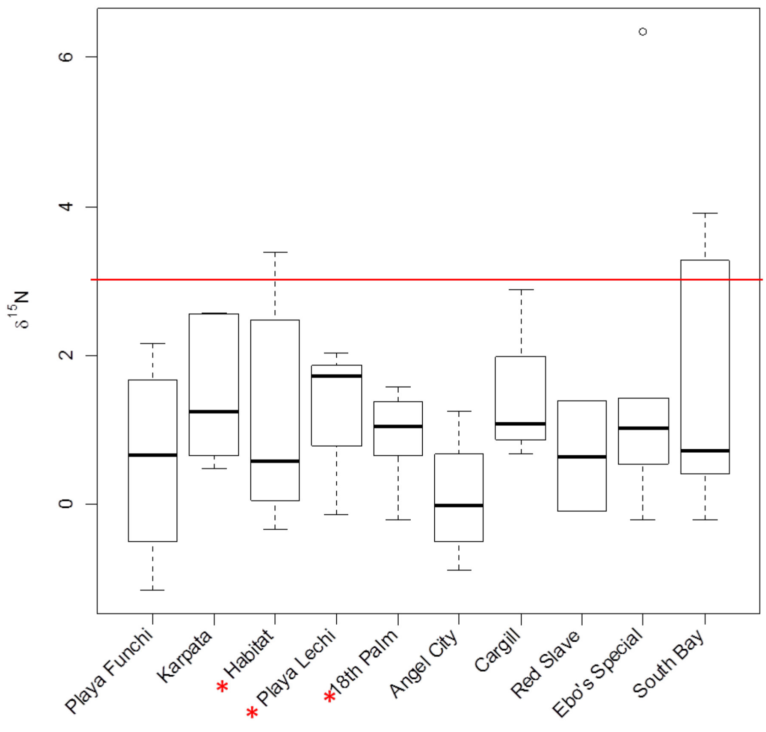


*Figure 17 δ15N isotope values (‰) of at different based on grouped macroalgae. Red line indicates indicator level for sewage related δ15N ‰ (>3‰). * indicate location within sensitive zone = assumed enriched area.*

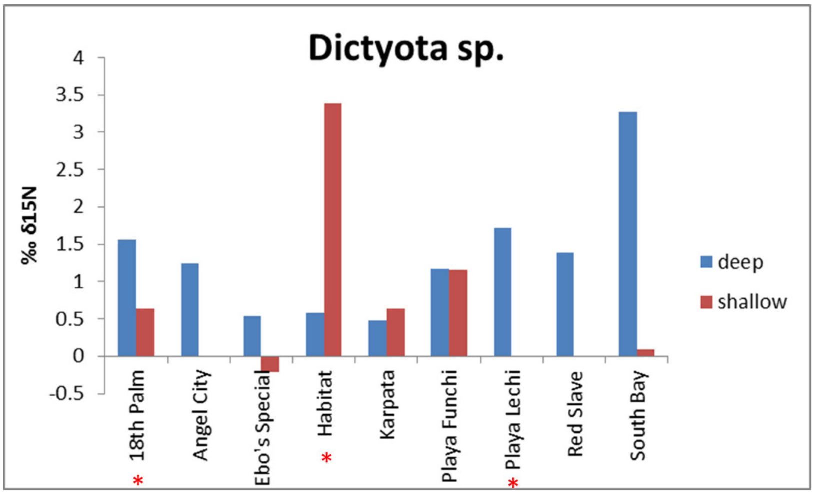


Figure 18 *Isotope ratio δ15N ratio (‰) for Dictyota sp. At different locations and two depths. * indicate location within sensitive zone = assumed enriched area.*

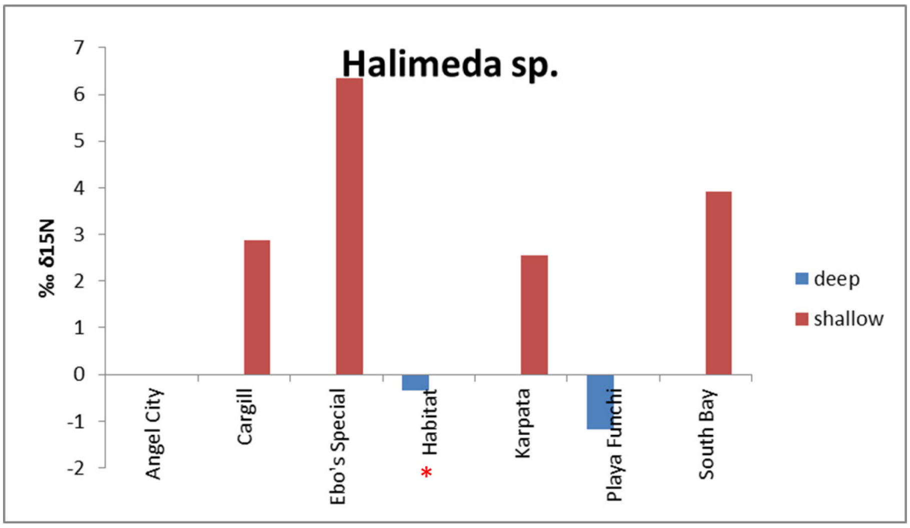


Figure 19 *Isotope ratio δ15N ratio (‰) for Halimeda sp. At different locations and two depths. * indicate location within sensitive zone = assumed enriched area.*

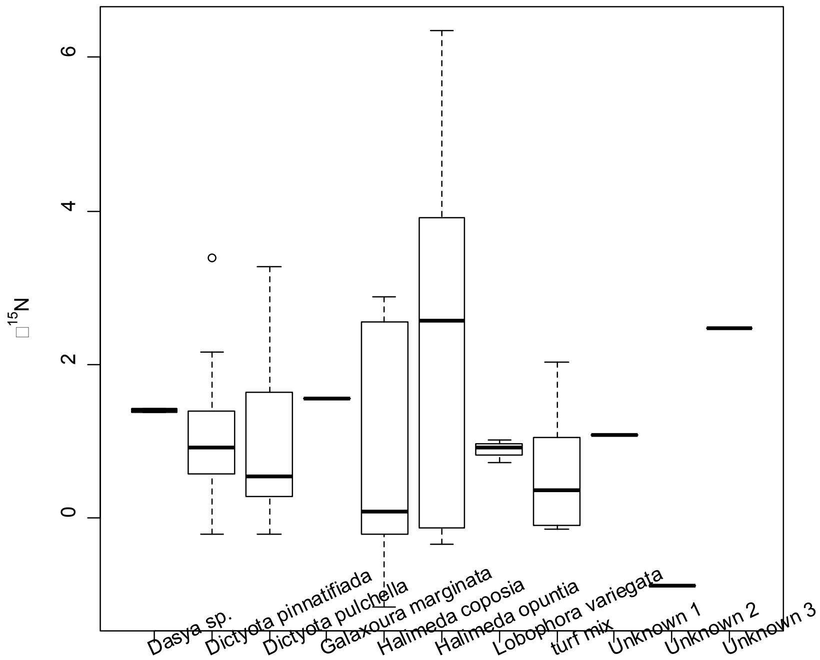


Figure 20 Isotope ratio δ15N ratio (‰) for different species. Locations and depth not included as discriminating factor.

3.6 Dendrograms

In the dendrograms, locations are clustered according to similarity. Locations in the same cluster (on the same "branch" of the "tree") share characteristics, whereas locations in separate clusters (on the different "branches" of the "tree") are more dissimilar. In the dendrogram showing shallow locations (Figure 21) , the southern locations Red Slave, Angel City and Cargill show similarity. Habitat "Shallow" location is "standing" alone, and is clearly deviating from all other locations. No similarity of locations within the sensitive zone (Habitat, Playa Lechi, 18th Palm) is shown (Figure 21), nor a similarity based on e.g. north-south geographical order. Playa Funchi and Playa Lechi show similarity, which mostly can be explained by the NO_3 data.

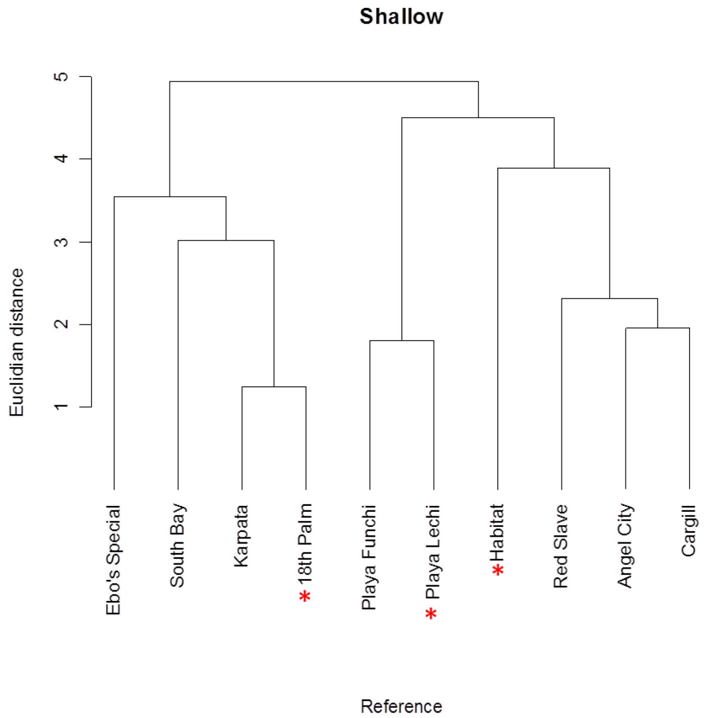


Figure 21 Dendrogram based on "shallow" locations.

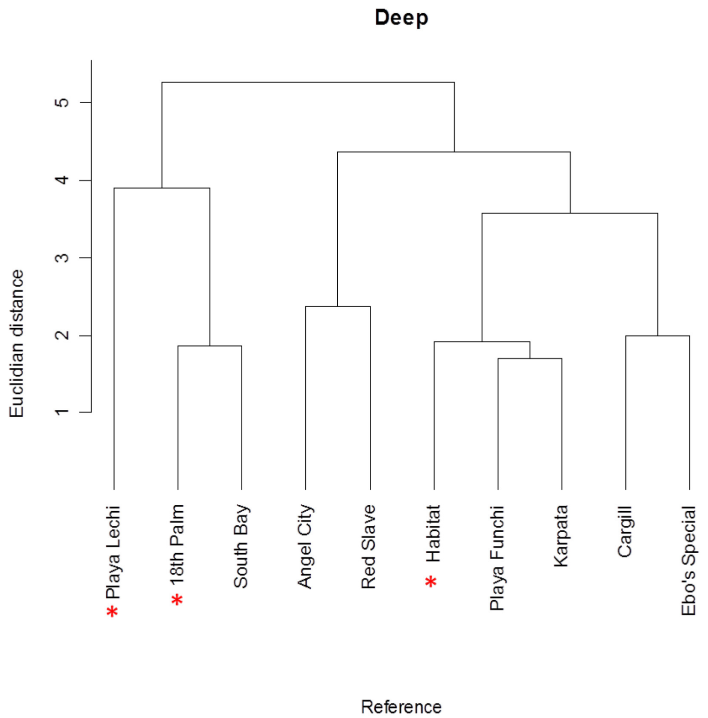


Figure 22 Dendrogram based on "deep" locations.

In the dendrogram showing deep locations (Figure 22) , the southern locations Red Slave and Angel City show similarity, as they did for the shallow locations as well. No similarity of locations within the sensitive zone (Habitat, Playa Lechi, 18th Palm) is shown (Figure 21), nor a similarity based on e.g. north-south geographical order.
In contrast to the observation within the "shallow" dendrogram, Playa Funchi and Playa Lechi show no similarity at the deeper part of the reef.

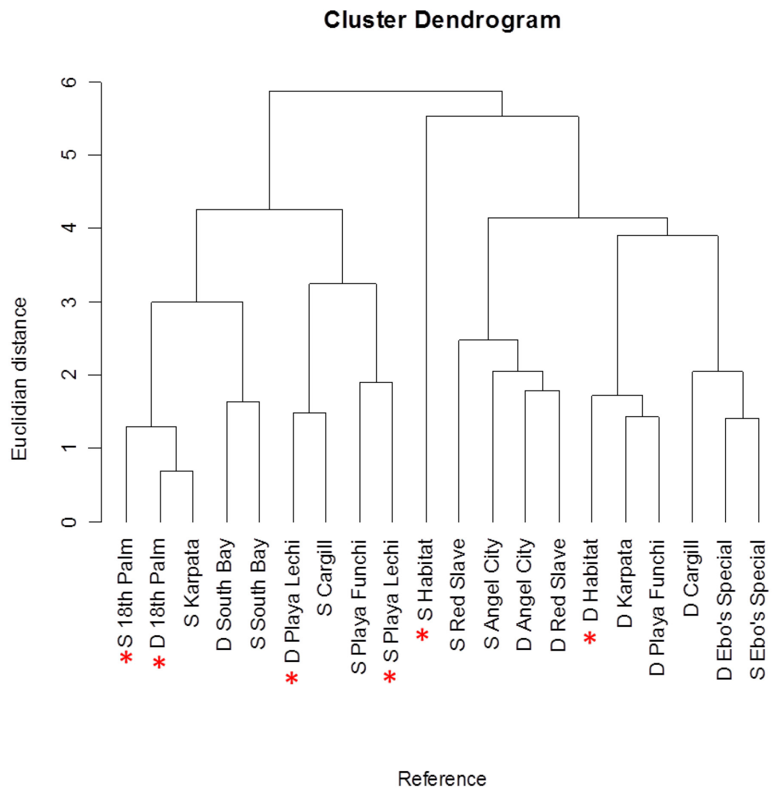


Figure 23 Dendrogram based on all locations (deep + shallow).

In the dendrogram showing all locations (Figure 23), the southern locations Red Slave and Angel City show similarity. No similarity of locations within the sensitive zone (Habitat, Playa Lechi, 18th Palm) is shown (Figure 21), nor a similarity based on e.g. north-south geographical order. Habitat shallow is clearly deviating from all other locations.
The dissimilar pattern among the locations illustrates that each location is steered by its own specific (set of) factors. These factors could e.g. rainfall and volume of groundwater outflow, influence of bays

4 Discussion and conclusion

The goal of this coastal monitoring study was to collect baseline water quality data to study the effectiveness of the water treatment facility that is planned to be operational from mid-2012. The water treatment plant will treat water collected from the so-called sensitive area, which is the urbanized area between Punt Vierkant and Hato (Van Kekem et al., 2006). The treatment plant should result in a 70% decrease of nitrogen from septic tanks to the reef (6.5 tonnes) (Van Kekem et al., 2006), and based on specifications by MIC, 2011 a total of 17520-35040 kg of nitrogen (equals 17.5-35 tonnes) will be removed from the sensitive area (based on influent characteristics).

In this discussion, the data are discussed in relation to the observed water quality during this baseline monitoring, the observations in the sensitive zone cq. reference locations, and the potential of the parameters to detect the effectiveness of the treatment plant within the next years.

4.1 Nutrient levels

Nutrients are measured as direct indicator of nutrient status at the reef, and as indirect measure of enriched groundwater outflow to the reef. Direct measurements of water quality, as done in this baseline study, provides information about the condition of the water column at that specific point in time (Cooper et al 2009). This is valuable information, but it should be evaluated in a certain frequency of sampling to account for temporal variability. Nutrient level are sometimes questioned to be a good indicator value for water quality due to the fact that nutrient levels are in a constant flux in reef ecosystems (cannot be measured properly) (Dodds, 2003), and effects of nutrient enrichment is location specific (Szmant, 2002). Nutrient levels above environment threshold levels are, however, very indicative for a potential disturbed situation. Growth rates of algae are significantly affected at nutrient concentrations around the nutrient threshold concentrations (Bell et al., 2007). Relatively small variations (in µg/l) in nutrient concentrations around the threshold concentration, can lead to large changes in the algal growth rates. The relatively small magnitude of the environmental threshold concentrations, in comparison with the nutrient concentrations in wastewater discharges and runoff, means that large nutrient discharges can affect reefs over large distances, and even very small discharges can affect nearby reefs (Bell et al., 2007). These nutrient threshold concentrations are likely to result in eutrophic effects (Bell, 1992), but will depend on both duration or intensity of the stress factor (Cooper et al., 2009). The extent of these two factors are however not quantified in literature.

This study shows that nitrogen levels at some locations (Habitat, Angel City, Cargill, Red Slave) are above environmental threshold concentrations for total inorganic nitrogen, which means that at this level of water quality, the coral reef ecosystem can be seriously affected. PO4 levels did not exceed environmental threshold levels.

No clear relation of water quality at locations in the sensitive zone and at reference locations can be made. DIN levels exceed the levels at so called reference sites (Angel City), and are below the levels at locations within the sensitive zone (Playa Lechi and 18^{th} Palm). A clear relation of water quality between the sensitive zone and reference locations is thus lacking, and could be explained by various aspects:

<u>Location specific oceanographic/weather conditions:</u> besides groundwater outflow of nutrients, each location is related to other factors determining the nutrient status. The importance of temporal variability which can be extreme in coral reef systems is often neglected, leading to potentially inconsistent definition of background environmental conditions (Fichez et al., 2005). Conditions to take into account are e.g. the wind interaction, wave and tide interaction, local eddies and retention from lagunas (e.g 18^{th}

Palm) and the harbor, and the relative position of the location to the direction of the Venezuelan upwelling. These conditions are not defined in this study and not well understood yet to relate them to the results.

Location specific sources of nutrients: Important factor influencing the temporal variation of enriched groundwater is retention time of groundwater in relation to rain events. This can steer variance at a very short time frame of minutes to hours (e.g. the first flush at the beginning of a rain event). Gast et al. (1999) found that enhanced (not significantly higher than reference reef) nutrient concentrations at the town reef on Curacao showed daily patterns, explained by variations sewage discharge. In the study of Gast et al. (1999) rainfall did not correlate with elevated inorganic nutrients, but small scale variation in the flow field of near the point of discharge could cause highly variable concentrations. In the case of Bonaire, the points of discharge are not identified. The correlation with rainfall is not expected as discharge via rainwater comes with so-called first flushes. The first flush contains more nutrients and other components and can be easily missed during monitoring.

Besides sewage enriched groundwater, other nutrient sources are important to consider as well. The relative influence of bay or storm water to the sampling site as e.g. laguna at Plaza for 18th Palm and the harbor, the locations of roois (gullies), point source as flamingo airport (see data Broekgaarden et al., 2011), sediment run off from salina's, and run off from the street (possibly containing nutrients from animal manure). To get grip on this variance, monitoring should be much more fundamental of design, e.g. to detect diurnal variance in order to avoid misinterpretation of data.

Dendrogram figures showed that Habitat is very dissimilar compared to all other locations and a potential source can explain the dissimilar observations at this location. Just north of sampling location "Habitat", the desalinitation plant WEB is located. The water intake is at approximately -6 m and the brine effluent is discharged at the surface, approximately 150-200 meter north of "Habitat". No additional specifications of the brine effluent (quantity and quality) is available for this study. Gacia et al. (2007) studied the environmental effects of brine effluent and found nitrogen levels in the effluent to be 100 times higher than the local seawater. These high nitrogen levels affected seagrass health by altered enzymatic activity.
Although prevailing current is from south to north, and salinity measurements are not different than at other locations, the plant is very likely to influence local water quality aspects near "Habitat" and should be looked into more detail as a potential source of nutrients.
Another explanation for dissimilar results for Habitat is the lack of local treatment of sewage at the resort, and the lack of discharge of effluent to the preliminary treatment plant.

Another point source that should be looked into more detail is near the beerput of Flamingo airport. Based on the quick scan results from Broekgaarden et al. (2011) ammonium concentration in the groundwater is ~1500 mg N/l. The conductivity of 14 ms/cm, indicates that this groundwater is (inter) connected to the coast, and that this excessive ammonium could reach the reef.

Nitrogen balance- organic nitrogen: In this study only inorganic nitrogen was monitored. Organic nitrogen was not taken into account in this study, and local mass balance of nitrogen can be (partly) steered by this unknown part. Broekgaarden et al., (2011) report concentrations of 1420mgN/l of Kjeldahl Nitrogen (consists of NH4 + organic N). However, the NH4 concentration is 1500 mgN/l, suggesting that the organic nitrogen that in groundwater of flamingo airport is not present. At some other locations (e.g. Lagun) NH4 and Kjeldahl N concentrations implicate that organic nitrogen can be a large part of total nitrogen in groundwater. Because organic nitrogen can be transformed into bio available form of nitrogen it is advised to include organic N in future monitoring.

Site specific benthic coverage: Differs largely among the locations (see e.g. data Meesters & Van Beek, in prep). Nutrient dynamics as uptake and excretion by e.g. sponges and fishes can locally affect nutrient concentrations (e.g. Diaz & Ward 1997, Meyer & Schultz 1985). Benthic coverage data, collected by STINAPA are not yet available for this study. Once these data are reported, these should be evaluated regarding specific indicators for eutrophication, and the similarity of locations within the sensitive zone, and outside reference locations. IUCN (2009) assessed some of Bonaire's reefs locations and ranked them according to their overall resilience. In Table 4 this ranking is presented.

Table 4 Resilience rating for Bonaire's reefs by IUCN (2009)

location	**Resilience rating (IUCN, 2009)**
Playa Funchi	Medium
Karpata	High
Habitat	Medium (based on location Cliff = Habitat)
Playa Lechi	Not assessed
18^{th} Palm	Medium
Angel city	Medium
Cargill	Medium
Red Slave	High (based on site Vista Blue which is close by)
Ebo's special	Not assessed
South Bay	High

4.2 Stable isotope ratios in macro-algae

Isotopic values are considered to be a high priority indicator in both long and short term monitoring studies in which anthropogenic nitrogen, as e.g. from sewage run off/effluent (Cooper et al., 2009, Risk et al., 2009).
The average value of the isotope ratio in this study was 1.1 ‰ and showed large variability in location, and species.
Factors which can be of influence of (temporal) variation were considered in order to explore the future potential of this indicator, such as species dependent ratios or seasonality.
The δ15N within macro algae is a time integrated indicator, and one can assume that seasonally dependent growth rates can influence δ15N among seasons. Other studies show, however, that seasonal variance of 15N within macro algae is not likely to occur. Mayr et al. (2011) found seasonal δ15N variability to be small compared to regional differences. Seasonal data collected by Lapointe & Mallin at Bonaire locations most likely will confirm this conclusion. Lapointe collected isotope ratios in March, June, and October 2006, January, March, and July 2007 and February 2008. Looking into the draft results of Lapointe & Mallin, it seemed that these values were not high on average, local and species specific values could be near the ratio of 3‰. Seasonality as a significant factor was not analysed in this study. If data of Lapointe & Mallin do not show strong seasonal variance, it is not likely that during another season, the isotope values are higher, and that the indicator is applicable to indicate sewage as a nitrogen source during a specific season.
Species specific δ15N were not detected and based on these data, no advise for upcoming monitoring in this respect can be provided.

Regarding variance in terms of spatiality, the ratios were not higher within the sensitive zone compared to reference locations. At a few locations, Habitat, South Bay, Ebo's Special δ15N ratios (‰) were above 3 (up to 6.3). However, two out of these three locations are 'reference' locations, and not under direct influence of sewage. Another 15N source has thus to be present. The wetland characteristics (salinas and

anoxic conditions) could be steering deviating N15. Using locations at Klein Bonaire for isotopic reference sites to compare with locations under sewage stress is therewith not possible.
Based on this observation, locations at Klein Bonaire should be rejected as a reference location for monitoring of nutrient status of Bonaire.

Indicated isotopic ratios in macro algae under sewage influence are > 3 ‰ (see report 1 for an overview of cited literature). The average value of the isotope ratio in this study was 1.1 ‰ and does not indicate that the sampled macro algae have been exposed to relatively large sewage volumes. Other nutrient sources might be equally or more important than sewage. It could be considered to monitor δ15N in the groundwater in order to explain the low values. As δ15N levels are strongly related to N source values, the major N source(s) for a given water mass must be known and characterized. This is especially important when comparing different water masses. Along developed coastlines with e.g. addition of inorganic fertilizer with low δ15N values will complicate any search for a sewage signal (Risk et al., 2009). Analysing δ15N in groundwater should be considered in next monitoring in order to explain the low ratio found in this study.

4.3 Chlorophyll a

Chorophyll a was used as an indicator for primary production. All locations showed chlorophyll a concentrations below the environmental threshold level of 0,5 µg/l. In this baseline study, chlorophyll a data showed clear geographical difference, but no clear deviation between locations in the sensitive zone and at reference areas.
As primary producers (algae) can respond to nutrient influx very quickly (minutes to hours), especially given the high light intensity, the indicator chlorophyll a can vary within this same short time span. The temporal and spatial conditions affecting nutrient availability (discussed in previous section) are thus relevant to fully understand the dynamics regarding this indictor as well. In addition, light attenuation and turbidity, and iron concentration steer algal biomass, but were not included in this monitoring study. These steering factors should be taken into account in future monitoring to interpret chlorophyll a data. The 500 ml sample was enough to detect chlorophyll a in expected concentrations, but if possible should be as much as possible to be able to detect any variance. Storage and transport of chlorophyll a samples are both very critical aspects. Any defreezing or temperature rise can alter the concentrations significantly (pers. comment M. van der Weiden). It is unknown what the effects of the local storage and transport have been. If possible, direct analysis of field samples at .g. CIEE by use of a fluorometer is suggested as good alternative.

4.4 Faecal bacteria (enterococci)

Enterococci are a proxi for monitoring faecal bacteria and human waste in marine waters. Faecal bacteria can enter the coastal water by leaking septic tanks into the groundwater, or via run-off of storm water.

At three locations bacteria numbers exceeded environmental standards, of which two lay within the sensitive zone. Surface run off is the most likely factor for elevated numbers at Playa Lechi and Cargill. The variability in the bacteria count show that spatial heterogeneity is common, but as these samples were taken during rain events, temporal variability should be taken into account in upcoming studies. More locations at the surface at more time intervals across the different seasons should be taken into account to account for temporal and spatial variability. The relation with surface run-off quality, and groundwater quality is a factor to include as well to account for the different sources. As faecal bacteria by means of enterolert tests are a representative and practical (easy and fast to perform) indicator for water quality, this indicator should be included in future monitoring.

An additional note is that a positive control was not taken into account during this monitoring, and should be integrated within next monitoring.

4.5 Transport of samples

Taking into account the logistic difficulties, we consider the transport of samples succeeded in terms of the actual possibilities. However, transport is still accompanied with risk of defreezing and thus quality loss.

For next monitoring, the volume that needs to be transported can be much better estimated by previous experience, than was before the monitoring of November 2011. Dry-ice volumes needed to transport these volumes are now better to estimate, and can be ordered in advance.

For future monitoring, the most practical manner is to analyze samples at the island. If methods are suitable enough, and quality is assured, this option should be considered. Benefits are no risk of defreezing samples during transport resulting in noisy data, and the ability to include more samples/higher frequency of sampling.

4.6 Conclusions

The study of November 2011 leads to the following preliminary conclusions:

- Benthic surveys were not included in this study, and add largely to a whole ecosystem assessment on eutrophication. In upcoming research this should be included.
- Based on nutrient levels, in the south and in one location in the sensitive zone a eutrophic status was observed. The other locations did not have nutrient levels harming the development of a healthy coral reef, based on nutrient concentrations alone. Nutrients levels are however in a constant flux, and data should be considered in an ecosystem context.
- Enriched groundwater with nutrients from sewage is not the only source of nutrients. Other sources as nutrients from the salt pans in the south and from brine near Habitat probably add to the eutrophic status at these locations. Furthermore percolation and surface run off from Salinas and stormwater via roois are probably a source of nutrients as the isotope values at the other locations are low too.
- Monitoring in the coastal zone alone, will not provide adequate indication of the effectiveness of the treatment plant. Monitoring in the coastal zone is effective to detect areas at risk, and to detect long term changes in overall water quality (= so called "surveillance monitoring").
- Monitoring in the coastal zone should be supported by additional so called "investigative monitoring" at the sources to quantify the relative contribution of each of these sources in order to be able to discuss additional measures.

Above mentioned conclusions need to be considered using additional monitoring. Based on a one time monitoring activity no definite conclusions are possible related to the treatment plant.

5 Recommendations for monitoring

Based on the results from the baseline monitoring of November 2011, it was concluded that water quality monitoring in the coastal zone of Bonaire alone, will not give a decisive answer on the question if the treatment plant is effective enough to restore and maintain the ecological quality of Bonaire's reef. We suggest to focus in the future on both surveillance monitoring and investigative monitoring.

"*Surveillance" monitoring* in the coastal zone will be needed in order to identify areas at risk, determine long-term changes in water quality, and to evaluate environmental risk assessment.
Additional monitoring should be directed to measurements and evaluation of the quantity and quality of the sources. This " *investigative monitoring"* will be used to establish causal relations. In relation to the effectiveness of the treatment plant, it is advised to direct "investigative" monitoring to:

- quantity and quality of the influent and effluent of the Water Treatment Plant
- quantity and quality of other sources of nutrients
- groundwater quality

5.1 Surveillance monitoring

5.1.1 Indicators

<u>General water quality parameters</u>
General water quality parameters should in upcoming monitoring be extended with pH, dissolved oxygen, turbidity and light attenuation to support interpretation.

<u>Nutrients</u>
Based on nutrient levels alone, a clear observation of eutrophic locations due to sewage enriched groundwater in the sensitive zone is lacking (except for "Habitat"). A decrease of enriched groundwater due to the installation of the treatment plant might therefore not be detected by nutrient indicators in the coastal zone.

Furthermore, the variability of nutrient level in the baseline study of November most probably hampers the detection of a significant decrease over time due to diurnal and seasonal factors. The 70% nitrogen decrease due to the installation of the treatment plant is significant, but probably variable in time, and furthermore not the only source of nitrogen.

Based the above, nutrient levels are not the best indicator in the scope of the treatment plant study, and nutrient concentrations cannot be used alone to determine water quality and its effect on the coral reef ecosystem. However, nutrients still stand as a key parameter within environmental surveys (Fichez et al. 2005) as they indicate general water quality. Nutrient concentrations as indicators should therefore still be considered for inclusion in future monitoring in the coastal zone of Bonaire to detect water quality as such, and to detect any <u>potential</u> trend over time, related to nutrient status in general.

As already discussed, many factors steer local variance of nutrients. It is advised to increase the understanding of local nutrient dynamics on the reef (see frequency section below).

A quick statistical power analysis on data of November reveals that a minimum number of 3 samples per sampling location is advised in order to be able to detect a 70% decrease of NO4 and NO3 concentrations (see annex 6). It can be questioned if the effectiveness of the treatment plant can be detected even by this number of samples. The power is estimated based on a 70% decrease of the total

N from all sources together, which is not true (other sources contribute to the total Nitrogen balance, such as the salt company, run off, and percolation from Salinas). Therefore, the surveillance monitoring is advised to include at least a minimum of triplicate nutrient samples.

Adding silica, ferrous iron and total nitrogen, and total phosphor to the set of nutrients would help to interpret chlorophyll data, and adding organic nitrogen as a parameter would help to understand the total nitrogen balance. The total suit of nutrients to be analyzed is advised to include: NH_4, NO_2, NO_3, PO_4, Total P, and organic nitrogen (Kjehldahl N).

Isotope ratios

Isotope ratios in macro algae are good indicators to detect sewage influence and are widely accepted as such. In the monitoring of November 2011, the isotope values were low (avg ~ 1.1) being considered as background values, and not indicative for sewage. Furthermore, the variance among species and locations was high. Combining these observations with the nutrient results indicating eutrophic status of the reef at some locations, but not specifically within the sensitive zone, it can be assumed that other sources must be equally or more dominant source of nutrients then sewage alone.
We consider the isotope ratios too variable and not high enough to monitor a clear trend in the upcoming monitoring which adds to answering the research questions. Furthermore, it turned out that the sampling and processing of samples is very time consuming before conducting the actual analysis.
In this respect, is advised to discard this indicator in future monitoring.

Chlorophyll a

Chlorophyll a concentration in the water column is an indicator of for primary production. Since the results show clear differences between locations, chlorophyll a should be included in upcoming monitoring, and attention should be on co-factors steering chlorophyll a. Analysis of chlorophyll a at CIEE laboratory should be considered to minimize effects of freezing and storage conditions

Bacteria

Surface water bacteria numbers in this study show that storm water might be a source of bacteria. Detection and quantification of bacteria in various sources (groundwater and surface run off/storm water including animal wastes) should be included in order to be establish the relation of numbers of bacteria in the coastal zone to the treatment plant (= investigative monitoring). Besides the relevance to indicate the effectiveness of the treatment plant, this indicator is of high value to indicate bathing water quality. If the standard for bathing water is exceeded, appropriate measures at the true source(s) should be taken. This indicator should thus be included in upcoming surveillance monitoring to pinpoint risk locations. Based on the low variability in the previous dataset and , we propose to reduce the number of replicates to one or two. The inclusion of positive and negative control samples is recommended at each analysis.

Benthic indicators

Integration of these water quality data with benthic survey data is considered to be a priority. Within the benthic monitoring and analysis of data, the following clear indicator for nutrient enrichment aspects should be evaluated, such as the distribution and density of bio eroders (e.g sponges), *Diadema*, and bivalves. Additional ideas are included in section 5.3.

5.1.2 Locations

In the monitoring of November 2011, 10 locations were visited. These locations were on forehand classified as (potentially) influenced by the treatment plant, or as (relative) references. Results showed, however, no clear difference between these classified locations.

Reference locations pointed out at forehand did not show clear "reference" data. Due to the short stretch of Bonaire's coastline, and sources varying in type, quantity and quality, and local influence of natural variation (upwelling, eddies, etc) reference locations are hard to define in this monitoring.

Locations of Klein Bonaire, South Bay and Ebo's special, seemed to be influenced by wetland dynamics (unknown wetland processes in anoxic conditions), and are therefore, not the best references to include in future monitoring. To get an overview of local variance and general water quality these locations are interesting to include, but do not specifically add to regarded the research question related to the treatment plant.

If the study would only focus on the effectiveness of the treatment plant, more locations, e.g. Front Porch, within the sensitive could be included to potentially detect more local trends within this area. To get grip on the variability in the sensitive zone, locations could be added, e.g. Front Porch. Synchronization of locations already included in the benthic monitoring of STINAPA is advised.

Depth was only for NO3 a discriminating factor, but in general does not add much extra information. It could therefore be considered to exclude 1 depth from future monitoring.

5.1.3 Frequency

Seasonal and diurnal dynamics (and thus variance) in nutrient availability is common at reef systems. Factors steering this seasonal variance are e.g wet and dry season, dynamics in regional upwellings, atmospheric pressure, biannual tidal regime, and irregular discharge in quality and quantity.
It is advised to get insight in the diurnal and seasonal variance of the nutrient availability to be able to pinpoint the best season(s), time and frequency for surveillance monitoring.

Diurnal variance can vary between locations, and a pragmatic choice has to be made to get insight of diurnal variability at some of the locations. E.g. variation at 2 locations in the sensitive zone- 18th Palm and Habitat, and a location in the south (e.g. Cargill) and in the north (e.g. Karpata). Diurnal variance should be monitored in wet and dry season as variable availability can occur (Gast et al 1999). Based on the specification in Table 5 the total costs for analysis would be ~4.3 KE, excluding sampling, lab and reporting.

Table 5 Costs overview for a diurnal sampling

Locations	**Depths**	**triplicate**	**Frequency based on 4 hour interval**	**Total nr samples per season**	**Costs (60 EUR per sample)**
4	1	3	6	72	~4320 KE

Seasonal variance should cover at least the wet and in the dry season, but it advised to include more time points that only two per year to get grip on variance during the year in order to pinpoint a best "monitoring season". In Dutch monitoring program for the Water Framework Directive, a minimum of 4 times a year is prescribed to monitor nutrient status (Faber et al 2011) for surveillance monitoring. This frequency reflects the season in which the parameters are expected to be highest. The background data to pinpoint such a season in Bonaire is lacking and a solid advise on frequency and season can therefore not yet be provided. For this moment, an adaptive strategy is thus proposed.

Based on DOC-data of van Duyl, (personal communication, manuscript in press) obtained in Bonaire, seasonality was shown to be a significant factor to consider in monitoring. The study of Van Duyl showed higher concentrations of DOC in the dry season compared to the wet season. Explanations are discussed in the manuscript, and possibly atmospheric conditions or bi annual tidal regime steer percolation/outflow from the land to the reef. When adopting this assumption, the dry season (May/June) should at least be included as season in which the most pronounced effects could be detected. This has to be evaluated in the scope of future monitoring. Based on obtained data monitoring should be further adapted.

Table 6 Cost estimate for analysis of 3 types of indicators, at one depth, taking into account replicates (triplo and duplo).

indicator	**depths**	**replicates**	**samples per location**	**costs per location**	**Costs per 10 locations**
nutrients	1	3	3	€ 180	
chlorophyll	1	3	3	€ 21	
bacteria	1	2	2 (+ controls)	€ 48	
				€ 225	**€ 2250**

Costs for analysis might be reduced when some nutrient samples are analysed at CIEE. Costs estimate is not yet received, nor the conditions for external guests and accuracy specifications of analysis.

5.1.4 Field and lab time

In November 2011 the field work and processing of field samples covered the following days:
Day 1: preparation of field gear
Day 2-8: field sampling and sample processing
Day 9-10: macro-algal sample processing, cleaning and packing and storage of material.

The ten locations were sampled in six days, processing of samples in total 8 days. In the morning 2 locations were sampled, and depending on logistics and time needed in the field to collect samples , around midday samples could be processed in the laboratory.
Processing of water samples of 1 location with two depths sampling, could be conducted by 1 person in 4.5 hours time in the afternoon (see Table 7). After processing of the water samples, the water bottles had to be rinsed according to protocol for sampling the next day. This took another 1 hour. When processing samples of 2 locations, ~8 hours in the laboratory is needed. This set up results in long working days (>12 hours including field assistance). .

Table 7 Overview of processing time per activity in November 2011

Processing	**Nr samples**	**Time (minutes)***
Nutrients	10 (20 ml each)	50
Enterolert prep	12	60
Enterolert reading	12	5
Chloropyll	6 (500 ml)	45
Marco algae	6	90
rinsing	12	30
Total		**280 (4.5 hours)**

*: 2 depth, triplo processing

<u>Upcoming monitoring: three locations per day instead of two, one depth instead of two</u>
As considered, macro-algae do not need to be included in upcoming monitoring. One depth instead of two depth sampling, will significantly reduce lab time (not by halve due to start up time). Nutrient

samples for organic nitrogen should be included. This set up results in an altered processing time (Table 8). Following this sampling program, a maximum of 3 field locations per day can be sampled by STINAPA. For 10 locations four field days are needed instead of five-six as was the case in November 2011.

Table 8 Processing time in the laboratory for 1 location and 3 locations

Processing	**Nr samples**	**Time (minutes)**	**3 locations (minutes)**
Nutrients	3 (20 ml each)	20	60
Enterolert prep	4	20	60
Enterolert reading	2	5	15
Chlorophyll	3 (500 ml)	20	60
rinsing	6	15	45
Total		**80**	**240**

This set up has the following consequences:

- IMARES fieldwork protocol prescribes to be in the field with a minimum of 2 persons. According to SCUBA prescriptions, a minimum of two divers is requested. One additional person on shore is recommended. After field work, one field person could assist in lab to be able to process the samples in 1 day (= 2nd lab person)
- Samples need to be returned to the lab directly after sampling to start processing (but this will results in some logistic inefficiency which has to be taken into account).
- A set up of four full days covering three locations a day, results in a minimal need of two persons on site. Benefits are that IMARES needs less days on Bonaire (~ -2).

The option of including students in this monitoring is considered. Benefits of including students to this project is that they can explore e.g. more locations, more indicators, at more time intervals etc. which is not always possible in a budget restricted project. However, students are not always available, and quality of the data cannot be quarantined as students are in training, and cannot be hold fully responsible for project results. Furthermore, guidance of students takes time as well which should be taken into account to the project.

5.2 Investigative monitoring

5.2.1 Monitoring of the treatment plant and groundwater

Sampling water at the reef, the relation with groundwater outflow is monitored indirectly. More direct assessment of treatment plant effectiveness are to monitor influent and effluent, and to monitor groundwater quality. The influent and effluent quality should be monitored to detect the actual nitrogen reduction. This should be registered together with influent volumes (e.g. per sub-area), in order to calculate the total sewage reduction in the western coastal area. The net nitrogen volume will not end up in the reef, and this contributes directly to a more resilient ecosystem, as human waste and the risk of correlated diseases will be reduced, and the eutrophic state will decrease.
Groundwater quality should be monitored to detect any decrease of nutrient discharge in the sensitive zone towards the coast, and to account for retention and release of absorbed nutrients. Groundwater quality monitoring near Flamingo airport (Broekgaarden et al., 2011) should be conducted in more detail. If this area is connected to the treatment plant, a hotspot is cleared.

Influent and effluent

The investigative monitoring is considered to be equally important as surveillance monitoring. Investigative monitoring enables a more direct assessment of the effectiveness of the treatment plant in the actual reduction of nitrogen emission . By combining measured nutrient concentrations and volumes of influent and effluent of the treatment plant (e.g. per sub-area), the total nutrient reduction in the western coastal area can be calculated. The net nitrogen volume will not end up in the reef, and this contributes directly to a more resilient ecosystem.

The following parameters are advised to include:

- Total nitrogen (kjeldahl)
- NH4, NO3 and NO2
- Bacteria

Bacteria samples should be processed within 24 hours and analysis can be performed by CIEE using Enterolert test kit (costs mentioned in previous section). Kjeldahl samples can be stored at ambient temperature. NH4, NO2 and NO3 samples should be processed shortly after sampling, and kept frozen until analysis (up to 6 months).

The nutrient sampling and analyses can most probably be analyzed by the treatment plant facility or at any commercial laboratory.

Monitor frequently a 24 hours integrated sample, depending on the variability of the influent and effectiveness of the plant.
Due to seasonal variability (e.g. low/high tourist season), a year-round sampling regime is advised.

5.2.2 Groundwater

Groundwater quality should be monitored to detect the groundwater outflow quality, and to detect any decrease of nutrient discharge in the sensitive zone towards the coast. Groundwater quality monitoring should be conducted in more detail near hotspot areas such as Flamingo airport and Cargill area.

The parameters to be analyzed in groundwater are

- Total nitrogen (Kjeldahl)
- NH4, NO3 and NO2
- Bacteria
- Conductivity
- Temperature
- Ureum
- D15N
- Labelled tracer (optional)

See details for nutrients and bacteria in previous section.
Conductivity and temperature data contribute to the understanding of mixing of water.
In a pilot area, a labeled tracer could be added to the groundwater/sewage (e.g. via septic tank) and traced via the wells. Retention time, and dilution of the groundwater could then be estimated via modeling. D15N could be added to the parameter list to understand the relatively low d15N values in the coastal zone.

Locations:

Groundwater wells in sensitive zone, Cargill area*, flamingo airport and reference zones**.

*Cargill area is suspect to be a source of nutrients and should be quantified in order to quantify the relative contribution to the groundwater outflow in the sensitive zone.
** reference zones are hard to identify. Should be regarded as "relative" reference.

A "screen" of wells along the coast, and some land inward should be considered to account for variability in groundwater outflow due to the "karst characteristics" of the soil. Expert groups working in the field of groundwater quality monitoring e.g. WUR BWA, Deltares or Grontmij, could further advise on this matter.

Frequency:
Monitor frequently, depending on the variability of the discharge. Discharge is unknown. Take into account rainwater events. Sampling some locations during rain-events and outside rain-events on an (two) hourly basis for 24 hours is advised to get grip on the diurnal variability.

Cost estimate:
See analysis costs for nutrients and bacteria in previous section. *For ureum, d15N and labeled tracer monitoring no estimate is given.* Conductivity can be measured on site during sampling, and has no additional costs.

The draft monitoring wells-water program proposes to include 32 wells in the program. Based on this number, an preliminary cost overview for the analysis of parameters is drafted. This does not include lab and field technician and lab fee costs. In Table 9 cost estimates are provided for wells regarding nutrient and bacteria analysis. In Table 10 two cost estimates are provided for two scenarios. Both type of scenario's are advised, but number of wells and frequency can be discussed. Based on these two scenarios, approximately **20 KE** is needed to get an indication of groundwater quality and its variation. This is excluding lab/field technician fee, and excluding reporting costs. Field assistance might be provided by the treatment plant personnel who will be trained for sampling and analysis. If analysis is performed in the treatment plant facility, then lower costs for nutrient analysis is foreseen.

Table 9 Overview of estimated costs for nutrient and bacteria analysis per sampling per well.

per sampling	nutrients	bacteria	Total costs
per well	40	6	€ **46**
32 wells	1280	192	€ **1472**

Table 10 Overview of estimated costs for nutrient and bacteria analysis for 2 scenarios.

	nutrients	bacteria	Total costs
e.g. two hourly basis for 24 hours at 4 wells	1920	288	€ **2208**
e.g. regular frequency of once month one year	15360	2304	€ **17664**

5.2.3 Nitrogen balance other sources

The effectiveness of the treatment plant on the reef quality should be studied in relation to other nutrient sources. To get grip on the relative contribution of nutrient input from sewage compared to other sources, the quality and quantity of nutrients contributed by other sources should be monitored as well. At some locations, DIN concentrations exceed the environmental threshold levels, but no clear relation with sewage stress was yet identified. This study has indicated that other sources must contribute to

total nitrogen at the reef. Suspect source to be relatively large is the Salt company Cargill (based on high DIN), and storm water run-off (including sediment) via Salinas and roois (based on bacteria).
Nutrient enrichment by Cargill and sediment and storm water runoff via e.g. roois and Salinas should thus be quantified. Important factors to consider are the temporal as spatial variability within these two sources, and first study should be focus on "hotspots" areas as Salina Di Vlijt and roois in the sensitive zone. Nutrient enrichment via groundwater near the airport (Broekgaarden et al., 2011) is another aspect to quantify.

(Ongoing) student projects could very well give first answers to this matter and should be evaluated in this perspective. Depending on the results, future study should be conducted, together with other expert groups or consultants in the field of sediments/soil and or (geo-) hydrology.

5.3 Synchronization and support of research at STINAPA

Benthic composition monitoring
In this water quality research, bio indicators were advised to include next to nutrient analysis. Benthic monitoring The comparison of responses from a composite of bio-indicators will provide the most useful information on the status and trends of reef ecosystems. As the extent of water-quality degradation increases, so does the scale at which the responses are manifested, and the time taken for the system to return to its previous state when the stressors are removed (Cooper et al., 2009).

Exposure to a low-level stress will first appeal a response at the e.g. colony level, such as coral brightness. As stress increases, either in terms of duration or intensity, responses at the population and community level may become evident through reduced juvenile densities, changes in the community structure, through e.g. the loss of susceptible species, or increased macro-algal abundances. Response time is, therefore, a critical criterion that underpins bio-indicator selection in any environmental monitoring. Moreover, changes in water quality such as elevated nutrient concentrations and turbidity decreases coral brightness, while sedimentation stress increases brightness (through bleaching) on upward facing surfaces (Cooper et al., 2009). Benthic indicators most relevant information in combination with information on stresses.

STINAPA already monitors the benthic composition on annual basis via AGRRA protocol. Benthic indicators such as bio-eroders could be monitored specifically in this benthic monitoring of STINAPA. Historic and future data, stored in video and/or photo material, could be processed in the scope of this specific subject, and specific indicators if not yet included, might be added to the general suit of parameters.
However, time needed to process the large quantity of data is enormous and STINAPA staff has limited time. This hampers the process of analyzing and reporting data, and subsequently is a missing link in assessment of the water quality and its effect on coral reef quality. Support in the processing of these data could be constructive to all kinds of management questions, including the treatment plant facility. An idea is to make the raw data available to student projects in order to help the analysis and reporting of the results.

Light and motion project vs Remote sensing
In the " light and Motion" project of the university of Southern California, approximately twenty moorings, rising from the ocean floor to a depth of 5m below the surface, are envisioned along the developed Bonaire coast. Sensors are attached to the mooring lines at three depths to measure the intensity of various colours of sunlight that penetrate to depth. By comparing readings scientists can identify harmful contaminants in the water. These sensors take and record data every 8 minutes, and consequently researchers are able to see trends. These trend data can be used to evaluate the

effectiveness of efforts to reduce contaminants, such as nutrient reduction by the planned treatment plant, and to identify changes on Bonaire.

The "Light and Motion" project measures the effect of nutrients in the water that can lead to excessive algae growth which can choke the reef to death. It will also measure dissolved organic matter, a by-product of animal and human waste which can have the same effect.

In the scope of remote sensing research and application for management purposes, these results can be regarded as valuable " ground-truth" data. A collection of ground-truth data enables calibration of remote-sensing data, and aids in the interpretation and analysis of what is being sensed via satellite images. By combining these data with satellite images on the coastal water of Bonaire, a so called-ground-truthing can be established, and potentially back casting (on future and historic data satellite maps) can be performed in order to detect effectiveness of the treatment plant on a longer, potential costs effectiveness and finer scale then possible by field measurements.

It should be tried to make these data available for e.g. GIS student projects.

6 Data management

Collected data at the BES islands are hardly accessible, and consequently a common knowledge base is lacking. In order to contribute to a shared knowledge base and common base for discussion on spatial issues on the islands, data need to be freely accessible. Many data are being collected, especially on biological aspects, and a univocal structure to these manage data is essential. Besides biological data, environmental data (e.g. on water quality) are monitored. The management of data and their storage in a standard format, including clear metadata descriptions is further explored in this section.

Multiple options exist to unlock BES data to users at various research institutes, ministries, NGO's and other interested parties.

Aspects discussed are metadata formats and their applicability, portal - and database options (including status and accessibility).
Basic assumption is that for unlocking the water quality data no new portal will be set up, and only already existing portals are considered, such as:

- Informatiehuiswater
- Informatiehuis Marien
- Seadatanet
- DONAR
- Portal WUR

Each of these portals is briefly described in the following paragraphs.

6.1 Informatiehuiswater (IHW)

Informatiehuis Water is a collaboration between "Interprovinciaal Overleg", "Rijkswaterstaat Waterdienst" and "Het Waterschapshuis", and provides a portal on the collection and unlocking of information regarding water quality. It is responsible for the exchange of information between water managers in order to facilitate all aspects of water policy (evaluation, execution, preparation). Besides surface water quality, groundwater quality is specifically integrated in this portal.

Informatiehuis Water uses the Aquo standard as a metadata format, and as database standard. This open standard is used within the policy of Kaderrichtlijn Water, and contributes to an open and easy access and exchange of data/information. Informatiehuis Water implements the EU guideline INSPIRE, and the spatial data management is in this respect compatible with EU guidelines. Dutch marine data under the Water Framework Directive are covered within the data collection. It is yet unknown whether data for areas oversees can be included.

6.2 Informatiehuis marien

InformatiehuisMarien (IHM) is a common initiative by the ministries of IenM, EL&I and Defense to collect information on ecology, soil and water quality, and to make this information available to different parties involved in North Sea area. It has to be discussed within the responsible departments whether data of BES islands will be included.

At this moment, IHM is busy defining its scope, and as it shows, this scope is restricted to monitoring data and information related to answer policy questions related to the sea. Its aim is to provide an easy access to actual and reliable data and information for ministries. Optional there will be a portal for third parties. It is intended to have a first draft of the IHM operational (Stolte et al., 2011)

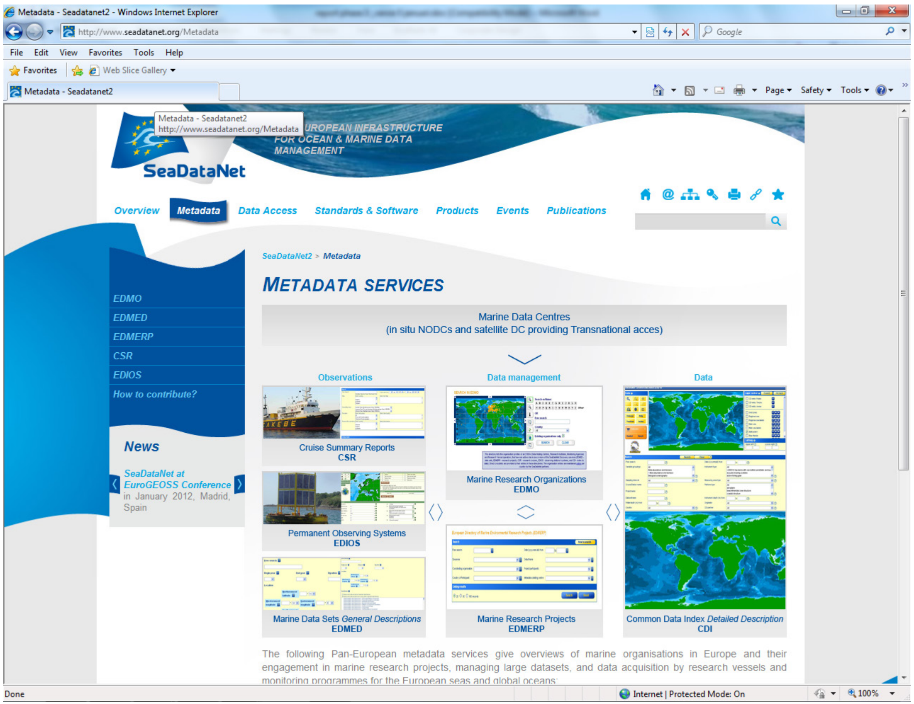
According to the draft memo on data policy (De Haas et al., 2011), the quality requirements are to synchronise with international (ISO) standards for metadata and e.g. INSPIRE guidelines. These specifics are described under SDN as well.

At the moment IHM is not yet operational, and the organization of IHM is unclear. To sort provisionally, the metadata of current and upcoming monitoring can be described according to ISO standards as Aquo or SeadataNet (SDN).

6.3 Seadatanet (SDN)

SeaDataNet (SDN) brings together a unique group of major institutes and marine data centres from countries bordering the North-East Atlantic, and its adjacent seas.
National Oceanographic Data Centres, Designated National Agencies for international data exchange and Satellite Data Centres represent the backbone of the marine data and information infrastructure. They are skilled in management and added value services on a wide range of marine information and data, in physical oceanography, marine biology and marine chemistry and currently manage the data management structure of several research programmes.

SDN can be accessed via http://www.seadatanet.org/Overview



SeaDataNet has developed an efficient distributed Marine Data Management Infrastructure for the management of large and diverse sets of data deriving from in-situ and remote observation of the seas and oceans. European professional data centres, active in data collection, constitute a Pan-European network providing on-line integrated databases of standardized quality.

The on-line access to in-situ data, meta-data and products is provided through a unique portal interconnecting the interoperable node platforms constituted by the SeaDataNet data centres.

The development and adoption of common communication standards and adapted technology ensure the platforms interoperability. The quality, compatibility and coherence of the data issuing, is assured by the adoption of standardized methodologies for data checking.

Since 2010 IMARES has begun to describe available marine and oceanographic datasets with metadata in order to search IMARES data in an easy way. These data are collected in a European context and made accessible via SeaDataNet. The Dutch wing of SeaDataNet is coordinated by NODC.

SeaDataNet is working on a Common Data Index (CDI), which is very practical to find and to retrieve oceanographic (meta)data, e.g. via MIKADO software. This CDI complies with ISO 19115 standard for metadata. It is expected that during project "SeaDataNet II" the CDI and metadata will meet the standards of the EU INSPRE guideline.

The CDI of SeaDataNet works with a standard geographic coverage codes and instrument and parameter vocabulary. The geographical coverage code for Bonaire or BES island is included via the code "Caribbean sea", and specifications can be included in the free text area.

This means that SeaDataNet is ready for use as a portal for BES data.

Parameters include physical, biological and oceanographic parameters, and nutrients are included in the parameter category.
Chlorophyll, bacteria counts and isotope values in biota are not yet available as input parameters. These might potentially be added in cooperation with the administrator, but is not yet sure.

SDN acts as a portal, and data are not stored at a SDN server. Data are stored at the source and SDN contains the links to these sources.

We think that SDN is a good portal to unlock BES data as it uses ISO standards, has a large international support among marine institutes and governments, and facilitates with ready to use software and infrastructure in order to retrieve data.

The remaining aspect is to prepare a source to which SDN can link to. This could be the WUR portal, or any other open source at the WD.
Additional data on groundwater and sediment quality and quantity data that are assumed to be collected in future monitoring are not likely to be included in SDN.

6.4 DONAR/Waterbase

In the database DONAR (Data Opslag Natte Rijkswaterstaat) physical, chemical and biological data collected via the program "Monitoring Waterstaatkundige Toestand des Lands (MWTL)" are stored. With "Waterbase", a web-application, these water quality data can be obtained. Data can be retrieved in figures or as numbers, via excel or text format. Stolte et al (2011) described that DONAR will not be supported in future, and that a successor system will be proposed. A vision on this matter is described in the data policy memo of RWS. As the status of DONAR is unclear at the moment, we consider this option as less relevant to unlock BES data. Therefore, metadata and other specifications of DONAR and Waterbase are not further discussed.

6.5 WUR portal Dutch Caribbean Biodiversity monitoring

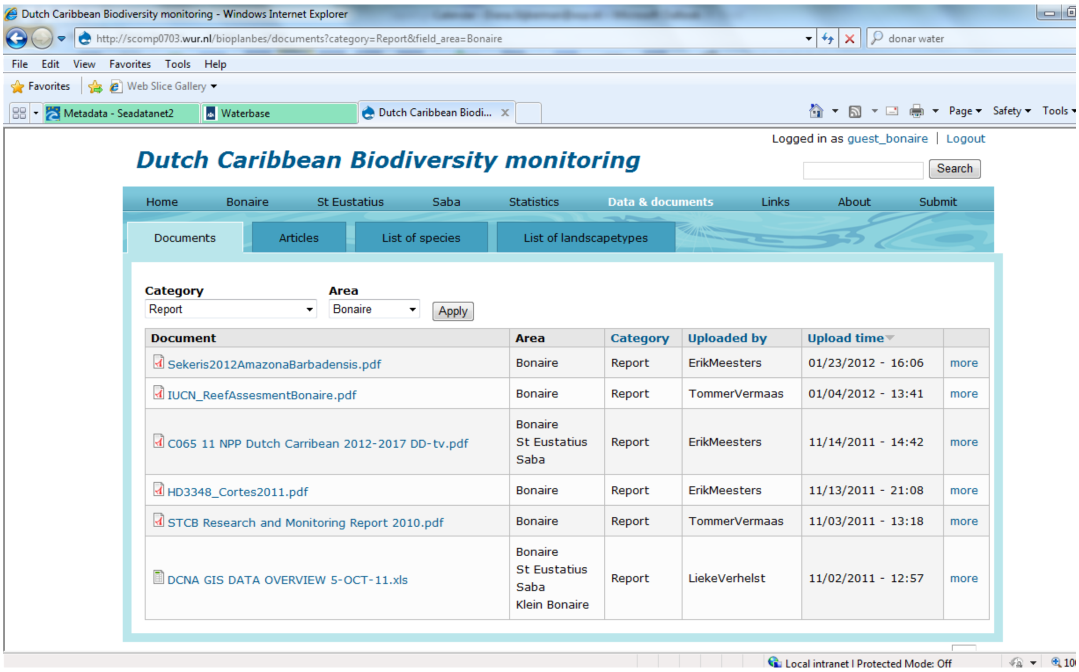
Within Wageningen UR, (IMARES and Centrum GeoInformatie) an initiative was taken to set up a knowledge portal in order to manage data efficiently (incl. data protection), to share data, and to facilitate spatial visualization of the collected and stored data. The aim is to construct the portal in such a way that relevant information is easily accessible, in order to contribute to the reporting obligations of international treaties and nature conservation on the islands. Organisations at the islands are involved in the organisation of the portal, and have expressed their willingness to contribute.

This initiative started in 2011, and the preliminary structure is build, and includes first data sets and reports. The initiative is funded by the Ministry of Economic Affairs, Agriculture and Innovation. The location of the portal is http://scomp0703.wur.nl/bioplanbes/

No guidelines on metadata or other protocols are yet defined.

The data till date are mainly related to biological inventories, both terrestrial and marine. Water quality data can be added to the structure via the administrator or acquired upload authorisation via Alterra.

This portal is considered to be of high relevance for the dissemination of water quality data as it is accepted on the BES islands, and will be used to store all relevant information, varying from data to reports. In time, this portal will cover most of the information, and will be known as such.
From that respect it is advised to integrate water quality information in this portal to disseminate the information in the most pragmatic manner.



Picture 6 Screen view of the WUR portal containing Dutch Caribbean monitoring data on biological features.

6.6 Recommendation

It is advised to further explore (and contribute to) the development of the WUR portal on BES data and use the ISO standard by SDN to describe metadata (via e.g. MIKADO software).
The WUR portal provides the opportunity of storing all BES data (including(ground) water quality, source information,…) in a format of choice. Excel tables and figures, including the reports can be uploaded, and could, for the time being, be suitable enough to disseminate the data.
If chosen to describe the monitoring and data with a metadata format prescribed by international standards, in time, the (meta) data could be synchronised with any other system.

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8 Quality Assurance

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.

9 Justification

Report C028/12
Project Number: 430.51096.01

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

Approved: E.M. Foekema
Researcher

Signature:

Date: March 15th 2012

Approved: F.C. Groenendijk, MSc.
Head of Department

Signature:

Date: March 15th 2012

ANNEX 1 General information

Information water parameters

location		depthcode	depth (m)	date	time	T (°C)	salinity (ppt)	coordinates	coordinates	wheather
PF	Playa Funchi	D		14-nov	9:30	29	37.1	12.28241	-68.413872	rainy night
		S	6	14-nov		29.5	36.73			
		surface		14-nov		28.8	36.72			
KAR	Karpata	D	20	14-nov	11:15	29	36.91	12.219472	-68.351767	sunny
		S	7	14-nov		29	36.4			
		surface		14-nov		29	36.42			
Hab	Habitat	D	20	13-nov	13:00		36.6	12.174259	-68.290114	rainy
		S	5.5	13-nov			36.4			
		surface	x	13-nov			x			
PL	Playa Lechi	D	20	13-nov	11:15	29	36.73	12.157600	-68.279815	rainy night+ day
		S	5	13-nov		29	36.76			
		surface		13-nov		28.9	36.5			
EP	18th Palm	D	20	17-nov	8:00	29	36.95	12.138600	-68.276257	rainy
		S	6	17-nov		29	36.75			
		surface		17-nov		28.5	36.8			
AC	Angel City	D	20	11-nov	8:00	28.9	36.65	12.103475	-68.287190	sunny
		S	5	11-nov		29	36.7			
		surface		11-nov		28.8	36.65			
CAR	Cargill	D	20	16-nov	8:00	29	36.7	12.070457	-68.280288	sunny
		S	?	16-nov		28	37.04			
		surface		16-nov		28.2	36.7			
RS	Red slave	D	20	11-nov	11:00	29	x	12.026512	-68.251207	sunny
		S	7	11-nov		29	x			
		surface		11-nov		29.9	36.88			
SB	South Bay	D	20	15-nov	9:00	29	36.84	12.149662	-68.320325	rainy
		S	4	15-nov		29	36.9			
		surface		15-nov		28.7	36.75			
ES	Ebo's special	D	20	15-nov	10:00	29	36.84	12.165718	-68.319320	rainy
		S	4.5	15-nov		29	36.74			
		surface		15-nov		28.9	36.75			

Tidal regime November 11- November 17th 2011

2011-11-11 6:28 AM AST Sunrise
2011-11-11 12:47 PM AST 0.57 meters High Tide
2011-11-11 6:05 PM AST Sunset
2011-11-11 10:56 PM AST 0.26 meters Low Tide
2011-11-12 6:29 AM AST Sunrise
2011-11-12 1:23 PM AST 0.57 meters High Tide
2011-11-12 6:05 PM AST Sunset
2011-11-12 11:42 PM AST 0.25 meters Low Tide
2011-11-13 6:29 AM AST Sunrise
2011-11-13 2:01 PM AST 0.57 meters High Tide
2011-11-13 6:04 PM AST Sunset
2011-11-14 12:23 AM AST 0.24 meters Low Tide
2011-11-14 6:30 AM AST Sunrise
2011-11-14 2:44 PM AST 0.56 meters High Tide
2011-11-14 6:04 PM AST Sunset
2011-11-15 12:57 AM AST 0.24 meters Low Tide
2011-11-14 6:30 AM AST Sunrise

2011-11-14 2:44 PM AST 0.56 meters High Tide
2011-11-14 6:04 PM AST Sunset
2011-11-15 12:57 AM AST 0.24 meters Low Tide
2011-11-15 6:30 AM AST Sunrise
2011-11-15 3:32 PM AST 0.55 meters High Tide
2011-11-15 6:04 PM AST Sunset
2011-11-16 1:26 AM AST 0.24 meters Low Tide
2011-11-16 6:30 AM AST Sunrise
2011-11-16 4:25 PM AST 0.54 meters High Tide
2011-11-16 6:04 PM AST Sunset
2011-11-17 1:47 AM AST 0.25 meters Low Tide
2011-11-17 6:31 AM AST Sunrise
2011-11-17 5:23 PM AST 0.51 meters High Tide

ANNEX 2 Macro-algal species collected

Macroalgae species and codes per location.

Location	depth	speciesno	code	speciesname
Playa Funchi	D	1	PF D1	Dictyota pinnatifiada
Playa Funchi	D	2	PF D2	Halimeda copiosa
Playa Funchi	D	3	PF D3	UNKNOWN4
Playa Funchi	S	1	PF S1	Dictyota pulchella
Playa Funchi	S	2	PF S2	Dictyota pinnatifiada
Playa Lechi	D	1	PL D1	Dictyota pulchella
Playa Lechi	D	2	PL D2	turf mix
Playa Lechi	S	1	PL S1	turf mix
Habitat	D	1	hab D1	turf mix
Habitat	D	2	hab D2	Halimeda opuntia
Habitat	D	3	hab D3	Dictyota pinnatifiada
Habitat	S	1	hab S1	Dictyota pinnatifiada
Habitat	S	2	hab S2	UNKNOWN1
Red Slave	D	1	RS D1	Dictyota pinnatifiada
Red Slave	S	1	RS S1	turf mix
Angel City	D	1	AC D1	Dictyota pinnatifiada
Angel City	D	2	AC D2	UNKNOWN2
Angel City	S	1	AC S1	Halimeda copiosa
Angel City	S	2	AC S2	Halimeda opuntia
South Bay	D	1	Sb D1	Lobophora variegata
South Bay	D	2	Sb D2	Dictyota pulchella
South Bay	S	1	Sb S1	Dictyota pinnatifiada
South Bay	S	2	Sb S2	Halimeda opuntia
South Bay	S	3	Sb S3	Dictyota pulchella
Ebo's special	D	1	ES D1	Dictyota pulchella
Ebo's special	D	2	ES D2	Dasya sp.
Ebo's special	D	3	ES D3	Lobophora variegata
Ebo's special	S	1	ES S1	Halimeda opuntia
Ebo's special	S	2	ES S2	Dictyota pulchella
CARGILL	D	1	CAR D1	turf mix
CARGILL	D	2	CAR D2	UNKNOWN 3
CARGILL	S	1	CAR S1	Halimeda copiosa
Karpata	D	1	KAR D1	Lobophora variegata
Karpata	D	2	KAR D2	Galaxaura marginata
Karpata	D	3	KAR D3	Dasya sp.
Karpata	D	4	KAR D4	Dictyota pinnatifiada
Karpata	S	1	KAR S1	Halimeda copiosa
Karpata	S	2	KAR S2	Dictyota pinnatifiada
Karpata	S	3	KAR S3	Halimeda opuntia
18th Palm	D	1	18P D1	Dasya sp.
18th Palm	D	2	18P D2	Dictyota pulchella
18th Palm	S	1	18P S1	Halimeda copiosa
18th Palm	S	2	18P S2	Dictyota pinnatifiada

ANNEX 3 Results two-way ANOVA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Signif. codes
Enteros (#/100 ml)						
Location	9	42.777	4.753	4.2541	0.000217	***
Depth	2	8.022	4.0112	3.5902	0.033023	*
Residuals	67	74.857	1.1173			
WNH4 (µmol N-NH4/l)						
Location	9	1.4555	0.161724	5.0287	9.36E-05	***
Depth	1	0.0743	0.074295	2.3101	0.1352	
Residuals	47	1.5115	0.03216			
WNO2 (µmol N-NO2/l)						
Location	9	0.83462	0.092735	6.7735	3.66E-06	***
Depth	1	0.06984	0.069845	5.1015	0.02859	*
Residuals	47	0.64347	0.013691			
WNO3 (µmol N-NO3/l)						
Location	9	0.60206	0.066896	6.0124	1.44E-05	***
Depth	1	0.20062	0.200621	18.031	0.000102	***
Residuals	47	0.52294	0.011126			
WNOX (µmol N-NOx/l)						
Location	9	0.62645	0.069606	6.2185	9.85E-06	***
Depth	1	0.19871	0.198708	17.7525	0.000113	***
Residuals	47	0.52608	0.011193			
WPO4 (µmol P-PO4/l)						
Location	9	1.01231	0.112478	7.4816	1.09E-06	***
Depth	1	0.01422	0.014219	0.9458	0.3358	
Residuals	47	0.7066	0.015034			
Chl-a in monster (µg/L)						
Location	9	0.079491	0.008832	11.6356	2.37E-09	***
Depth	1	0.000079	7.92E-05	0.1043	0.7482	
Residuals	47	0.035676	0.000759			
DIN						
Location	9	0.74604	0.082893	3.8136	0.001127	**
Depth	1	0.07212	0.072123	3.3181	0.074888	.
Residuals	47	1.0216	0.021736			
d15N						
Location	9	11.21	1.2455	0.4892	0.8655	
Depth	1	5.695	5.6955	2.2369	0.1496	
Macroalgae	10	13.679	1.3679	0.5373	0.8445	
Residuals	21	53.468	2.5461			

Results post hoc Tukeys test

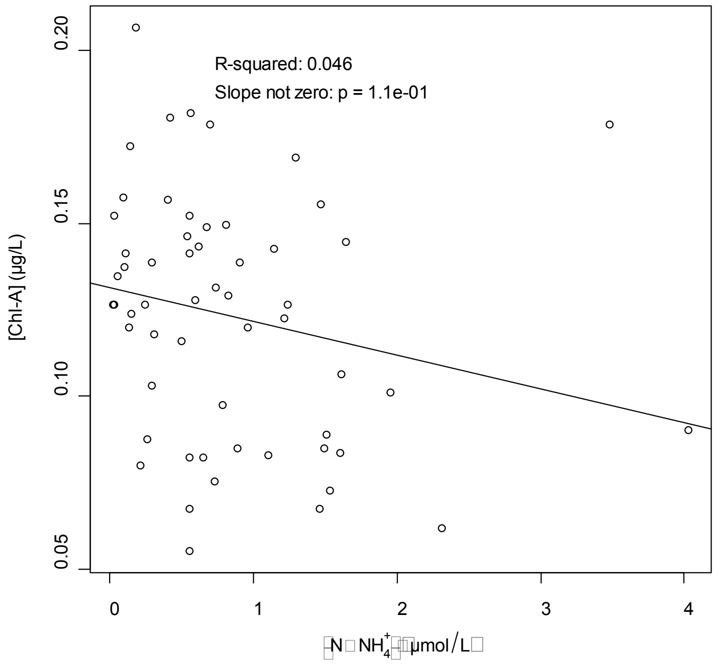
NH4 and NO2 not relevant, NOx is same as NO3 results.

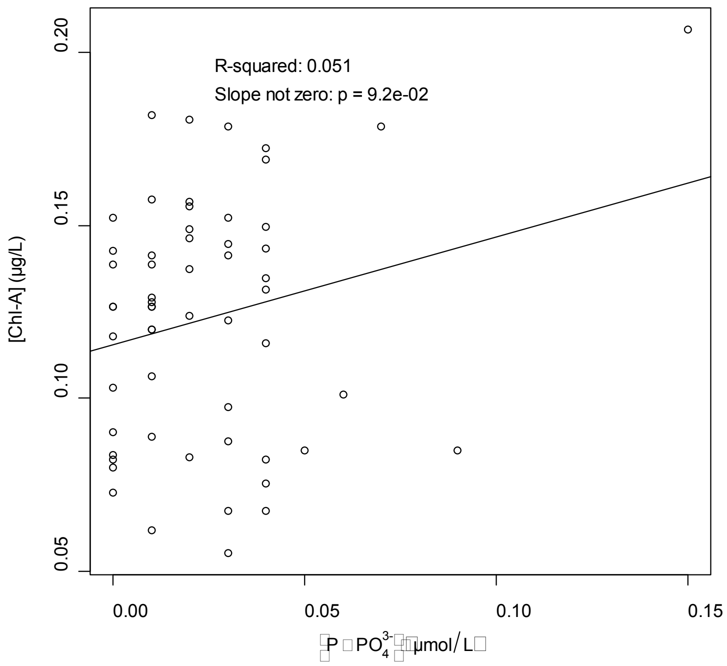
		Enteros				NO3				PO4				Chl-a			
code 1	**code 2**	**diff**	**lwr**	**upr**	**p adj**	**diff**	**lwr**	**upr**	**p adj**	**diff**	**lwr**	**upr**	**p adj**	**diff**	**lwr**	**upr**	**p adj**
Angel City D	18th Palm D	1.890	-0.029	3.810	0.059	0.071	-0.124	0.265	0.996	0.058	-0.287	0.404	1.000	-0.086	-0.165	-0.007	0.021
Cargill D	18th Palm D	0.000	-1.920	1.920	1.000	0.048	-0.146	0.243	1.000	-0.438	-0.784	-0.092	0.003	-0.093	-0.172	-0.014	0.008
Ebo's Special D	18th Palm D	0.000	-1.920	1.920	1.000	-0.028	-0.223	0.167	1.000	-0.333	-0.679	0.013	0.071	-0.024	-0.103	0.055	1.000
Habitat D	18th Palm D	0.000	-1.920	1.920	1.000	0.078	-0.116	0.273	0.989	0.021	-0.325	0.367	1.000	-0.003	-0.082	0.076	1.000
Karpata D	18th Palm D	0.000	-1.920	1.920	1.000	0.107	-0.087	0.302	0.839	-0.049	-0.395	0.297	1.000	0.017	-0.062	0.096	1.000
Playa Funchi D	18th Palm D	0.000	-1.920	1.920	1.000	-0.023	-0.218	0.171	1.000	-0.012	-0.357	0.334	1.000	-0.009	-0.088	0.070	1.000
Playa Lechi D	18th Palm D	0.705	-1.215	2.625	1.000	-0.215	-0.410	-0.020	0.018	-0.227	-0.573	0.118	0.591	-0.032	-0.111	0.047	0.989
Red Slave D	18th Palm D	0.705	-1.215	2.625	1.000	0.091	-0.104	0.286	0.955	-0.001	-0.347	0.344	1.000	-0.109	-0.188	-0.030	0.001
South Bay D	18th Palm D	0.000	-1.920	1.920	1.000	-0.086	-0.281	0.109	0.972	-0.082	-0.428	0.264	1.000	-0.013	-0.101	0.076	1.000
Cargill D	Angel City D	-1.890	-3.810	0.029	0.059	-0.022	-0.217	0.173	1.000	-0.497	-0.842	-0.151	0.000	-0.007	-0.086	0.072	1.000
Ebo's Special D	Angel City D	-1.890	-3.810	0.029	0.059	-0.099	-0.294	0.096	0.910	-0.391	-0.737	-0.045	0.014	0.062	-0.017	0.141	0.286
Habitat D	Angel City D	-1.890	-3.810	0.029	0.059	0.008	-0.187	0.202	1.000	-0.037	-0.383	0.308	1.000	0.084	0.004	0.163	0.029
Karpata D	Angel City D	-1.890	-3.810	0.029	0.059	0.037	-0.158	0.231	1.000	-0.107	-0.453	0.239	1.000	0.103	0.024	0.182	0.002
Playa Funchi D	Angel City D	-1.890	-3.810	0.029	0.059	-0.094	-0.289	0.101	0.939	-0.070	-0.416	0.276	1.000	0.078	-0.002	0.157	0.060
Playa Lechi D	Angel City D	-1.186	-3.105	0.734	0.789	-0.286	-0.481	-0.091	0.000	-0.286	-0.632	0.060	0.217	0.054	-0.025	0.133	0.511
Red Slave D	Angel City D	-1.186	-3.105	0.734	0.789	0.020	-0.175	0.215	1.000	-0.060	-0.406	0.286	1.000	-0.023	-0.102	0.056	1.000
South Bay D	Angel City D	-1.890	-3.810	0.029	0.059	-0.157	-0.352	0.038	0.252	-0.141	-0.486	0.205	0.988	0.074	-0.015	0.162	0.205
Ebo's Special D	Cargill D	0.000	-1.920	1.920	1.000	-0.077	-0.271	0.118	0.991	0.105	-0.240	0.451	1.000	0.069	-0.010	0.148	0.150
Habitat D	Cargill D	0.000	-1.920	1.920	1.000	0.030	-0.165	0.225	1.000	0.459	0.113	0.805	0.002	0.090	0.011	0.169	0.012
Karpata D	Cargill D	0.000	-1.920	1.920	1.000	0.059	-0.136	0.254	1.000	0.389	0.044	0.735	0.014	0.110	0.031	0.189	0.001
Playa Funchi D	Cargill D	0.000	-1.920	1.920	1.000	-0.072	-0.267	0.123	0.996	0.427	0.081	0.772	0.005	0.084	0.005	0.163	0.026
Playa Lechi D	Cargill D	0.705	-1.215	2.625	1.000	-0.264	-0.458	-0.069	0.001	0.211	-0.135	0.557	0.712	0.061	-0.018	0.140	0.308
Red Slave D	Cargill D	0.705	-1.215	2.625	1.000	0.042	-0.152	0.237	1.000	0.437	0.091	0.783	0.003	-0.016	-0.095	0.063	1.000
South Bay D	Cargill D	0.000	-1.920	1.920	1.000	-0.135	-0.329	0.060	0.504	0.356	0.010	0.702	0.038	0.081	-0.008	0.169	0.110
Habitat D	Ebo's Special D	0.000	-1.920	1.920	1.000	0.107	-0.088	0.301	0.846	0.354	0.008	0.700	0.040	0.021	-0.058	0.100	1.000
Karpata D	Ebo's Special D	0.000	-1.920	1.920	1.000	0.136	-0.059	0.330	0.491	0.284	-0.062	0.630	0.225	0.041	-0.038	0.120	0.893
Playa Funchi D	Ebo's Special D	0.000	-1.920	1.920	1.000	0.005	-0.190	0.200	1.000	0.321	-0.025	0.667	0.095	0.015	-0.064	0.094	1.000
Playa Lechi D	Ebo's Special D	0.705	-1.215	2.625	1.000	-0.187	-0.382	0.008	0.072	0.105	-0.240	0.451	1.000	-0.008	-0.087	0.071	1.000
Red Slave D	Ebo's Special D	0.705	-1.215	2.625	1.000	0.119	-0.076	0.314	0.708	0.331	-0.014	0.677	0.073	-0.085	-0.164	-0.006	0.024
South Bay D	Ebo's Special D	0.000	-1.920	1.920	1.000	-0.058	-0.253	0.137	1.000	0.251	-0.095	0.596	0.421	0.011	-0.077	0.100	1.000
Karpata D	Habitat D	0.000	-1.920	1.920	1.000	0.029	-0.166	0.224	1.000	-0.070	-0.416	0.276	1.000	0.020	-0.059	0.099	1.000
Playa Funchi D	Habitat D	0.000	-1.920	1.920	1.000	-0.102	-0.297	0.093	0.888	-0.033	-0.378	0.313	1.000	-0.006	-0.085	0.073	1.000
Playa Lechi D	Habitat D	0.705	-1.215	2.625	1.000	-0.294	-0.488	-0.099	0.000	-0.248	-0.594	0.097	0.437	-0.029	-0.108	0.050	0.996
Red Slave D	Habitat D	0.705	-1.215	2.625	1.000	0.012	-0.182	0.207	1.000	-0.022	-0.368	0.323	1.000	-0.106	-0.185	-0.027	0.001
South Bay D	Habitat D	0.000	-1.920	1.920	1.000	-0.165	-0.359	0.030	0.189	-0.103	-0.449	0.243	1.000	-0.010	-0.098	0.079	1.000
Playa Funchi D	Karpata D	0.000	-1.920	1.920	1.000	-0.131	-0.326	0.064	0.555	0.037	-0.309	0.383	1.000	-0.026	-0.105	0.053	0.999
Playa Lechi D	Karpata D	0.705	-1.215	2.625	1.000	-0.323	-0.517	-0.128	0.000	-0.179	-0.524	0.167	0.897	-0.049	-0.128	0.030	0.691
Red Slave D	Karpata D	0.705	-1.215	2.625	1.000	-0.017	-0.211	0.178	1.000	0.047	-0.298	0.393	1.000	-0.126	-0.205	-0.047	0.000

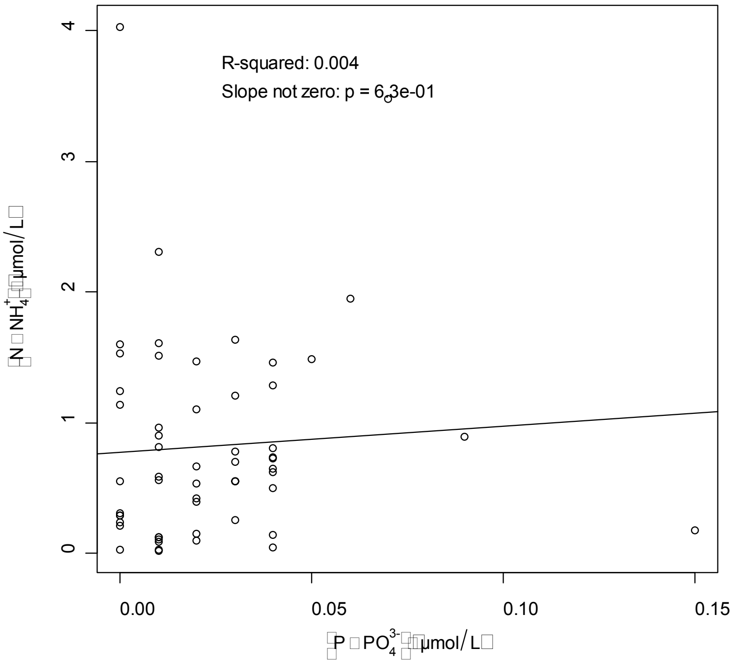
		Enteros				NO3				PO4				Chl-a			
code 1	**code 2**	**diff**	**lwr**	**upr**	**p adj**	**diff**	**lwr**	**upr**	**p adj**	**diff**	**lwr**	**upr**	**p adj**	**diff**	**lwr**	**upr**	**p adj**
South Bay D	Karpata D	0.000	-1.920	1.920	1.000	-0.194	-0.388	0.001	0.053	-0.033	-0.379	0.312	1.000	-0.030	-0.118	0.059	0.999
Playa Lechi D	Playa Funchi D	0.705	-1.215	2.625	1.000	-0.192	-0.387	0.003	0.057	-0.216	-0.561	0.130	0.677	-0.023	-0.102	0.056	1.000
Red Slave D	Playa Funchi D	0.705	-1.215	2.625	1.000	0.114	-0.081	0.309	0.767	0.010	-0.335	0.356	1.000	-0.100	-0.179	-0.021	0.003
South Bay D	Playa Funchi D	0.000	-1.920	1.920	1.000	-0.063	-0.258	0.132	0.999	-0.070	-0.416	0.275	1.000	-0.004	-0.092	0.085	1.000
Red Slave D	Playa Lechi D	0.000	-1.920	1.920	1.000	0.306	0.111	0.501	0.000	0.226	-0.120	0.572	0.601	-0.077	-0.156	0.002	0.061
South Bay D	Playa Lechi D	-0.705	-2.625	1.215	1.000	0.129	-0.066	0.324	0.577	0.145	-0.200	0.491	0.983	0.019	-0.069	0.108	1.000
South Bay D	Red Slave D	-0.705	-2.625	1.215	1.000	-0.177	-0.372	0.018	0.113	-0.081	-0.427	0.265	1.000	0.097	0.008	0.185	0.020
Ebo's Special S	18th Palm S	0.000	-1.920	1.920	1.000	0.065	-0.130	0.259	0.999	-0.447	-0.793	-0.101	0.002	-0.032	-0.111	0.047	0.988
Habitat S	18th Palm S	4.139	2.219	6.059	0.000	-0.185	-0.380	0.009	0.078	-0.067	-0.413	0.278	1.000	0.029	-0.050	0.109	0.995
Karpata S	18th Palm S	0.593	-1.327	2.512	1.000	0.016	-0.179	0.210	1.000	-0.054	-0.400	0.292	1.000	0.025	-0.054	0.104	0.999
Playa Funchi S	18th Palm S	0.000	-1.920	1.920	1.000	-0.482	-0.676	-0.287	0.000	-0.236	-0.582	0.109	0.524	0.011	-0.068	0.090	1.000
Playa Lechi S	18th Palm S	0.593	-1.327	2.512	1.000	-0.317	-0.535	-0.099	0.000	-0.101	-0.488	0.286	1.000	0.041	-0.038	0.120	0.893
Red Slave S	18th Palm S	0.000	-2.146	2.146	1.000	-0.185	-0.403	0.033	0.181	-0.003	-0.389	0.384	1.000	-0.048	-0.136	0.041	0.860
South Bay S	18th Palm S	0.000	-1.920	1.920	1.000	-0.024	-0.219	0.171	1.000	-0.203	-0.549	0.143	0.764	-0.009	-0.088	0.070	1.000
Cargill S	Angel City S	-1.267	-3.187	0.653	0.680	-0.050	-0.245	0.145	1.000	-0.119	-0.465	0.227	0.998	0.046	-0.033	0.125	0.765
Ebo's Special S	Angel City S	-1.972	-3.892	-0.052	0.037	0.123	-0.072	0.318	0.660	-0.350	-0.695	-0.004	0.045	0.047	-0.032	0.126	0.750
Habitat S	Angel City S	2.167	0.247	4.087	0.012	-0.127	-0.322	0.068	0.602	0.030	-0.315	0.376	1.000	0.108	0.029	0.187	0.001
Karpata S	Angel City S	-1.379	-3.299	0.540	0.515	0.074	-0.121	0.269	0.994	0.044	-0.302	0.389	1.000	0.104	0.025	0.183	0.002
Playa Funchi S	Angel City S	-1.972	-3.892	-0.052	0.037	-0.423	-0.618	-0.229	0.000	-0.139	-0.484	0.207	0.989	0.090	0.011	0.169	0.013
Playa Lechi S	Angel City S	-1.379	-3.299	0.540	0.515	-0.259	-0.476	-0.041	0.007	-0.003	-0.390	0.383	1.000	0.120	0.041	0.199	0.000
Red Slave S	Angel City S	-1.972	-4.118	0.174	0.113	-0.127	-0.345	0.091	0.774	0.095	-0.292	0.482	1.000	0.031	-0.057	0.120	0.997
South Bay S	Angel City S	-1.972	-3.892	-0.052	0.037	0.034	-0.161	0.229	1.000	-0.105	-0.451	0.240	1.000	0.070	-0.009	0.149	0.134
Ebo's Special S	Cargill S	-0.705	-2.625	1.215	1.000	0.173	-0.022	0.367	0.136	-0.231	-0.577	0.115	0.566	0.001	-0.079	0.080	1.000
Habitat S	Cargill S	3.434	1.514	5.354	0.000	-0.077	-0.272	0.117	0.991	0.149	-0.197	0.495	0.978	0.062	-0.017	0.141	0.291
Karpata S	Cargill S	-0.112	-2.032	1.808	1.000	0.124	-0.071	0.319	0.648	0.162	-0.183	0.508	0.952	0.057	-0.022	0.136	0.421
Playa Funchi S	Cargill S	-0.705	-2.625	1.215	1.000	-0.373	-0.568	-0.179	0.000	-0.020	-0.366	0.326	1.000	0.043	-0.036	0.122	0.847
Playa Lechi S	Cargill S	-0.112	-2.032	1.808	1.000	-0.209	-0.427	0.009	0.073	0.115	-0.271	0.502	1.000	0.074	-0.005	0.153	0.093
Red Slave S	Cargill S	-0.705	-2.851	1.441	1.000	-0.077	-0.295	0.141	0.997	0.214	-0.173	0.600	0.836	-0.015	-0.104	0.073	1.000
South Bay S	Cargill S	-0.705	-2.625	1.215	1.000	0.084	-0.111	0.279	0.978	0.013	-0.332	0.359	1.000	0.024	-0.055	0.103	1.000
Habitat S	Ebo's Special S	4.139	2.219	6.059	0.000	-0.250	-0.445	-0.055	0.003	0.380	0.034	0.726	0.019	0.061	-0.018	0.141	0.305
Karpata S	Ebo's Special S	0.593	-1.327	2.512	1.000	-0.049	-0.244	0.146	1.000	0.393	0.047	0.739	0.013	0.057	-0.022	0.136	0.437
Playa Funchi S	Ebo's Special S	0.000	-1.920	1.920	1.000	-0.546	-0.741	-0.351	0.000	0.211	-0.135	0.557	0.712	0.043	-0.036	0.122	0.860
Playa Lechi S	Ebo's Special S	0.593	-1.327	2.512	1.000	-0.382	-0.599	-0.164	0.000	0.346	-0.040	0.733	0.126	0.073	-0.006	0.152	0.099
Red Slave S	Ebo's Special S	0.000	-2.146	2.146	1.000	-0.250	-0.468	-0.032	0.012	0.445	0.058	0.831	0.011	-0.016	-0.104	0.073	1.000
South Bay S	Ebo's Special S	0.000	-1.920	1.920	1.000	-0.089	-0.284	0.106	0.963	0.244	-0.102	0.590	0.467	0.023	-0.056	0.102	1.000
Karpata S	Habitat S	-3.546	-5.466	-1.627	0.000	0.201	0.006	0.396	0.037	0.013	-0.332	0.359	1.000	-0.005	-0.084	0.074	1.000
Playa Funchi S	Habitat S	-4.139	-6.059	-2.219	0.000	-0.296	-0.491	-0.101	0.000	-0.169	-0.515	0.177	0.933	-0.019	-0.098	0.060	1.000
Playa Lechi S	Habitat S	-3.546	-5.466	-1.627	0.000	-0.132	-0.349	0.086	0.726	-0.034	-0.420	0.353	1.000	0.012	-0.067	0.091	1.000
Red Slave S	Habitat S	-4.139	-6.285	-1.993	0.000	0.000	-0.217	0.218	1.000	0.065	-0.322	0.451	1.000	-0.077	-0.166	0.011	0.152
South Bay S	Habitat S	-4.139	-6.059	-2.219	0.000	0.161	-0.034	0.356	0.215	-0.136	-0.481	0.210	0.992	-0.038	-0.117	0.041	0.939
Playa Funchi S	Karpata S	-0.593	-2.512	1.327	1.000	-0.497	-0.692	-0.302	0.000	-0.182	-0.528	0.163	0.881	-0.014	-0.093	0.065	1.000
Playa Lechi S	Karpata S	0.000	-1.920	1.920	1.000	-0.333	-0.550	-0.115	0.000	-0.047	-0.434	0.340	1.000	0.016	-0.063	0.095	1.000

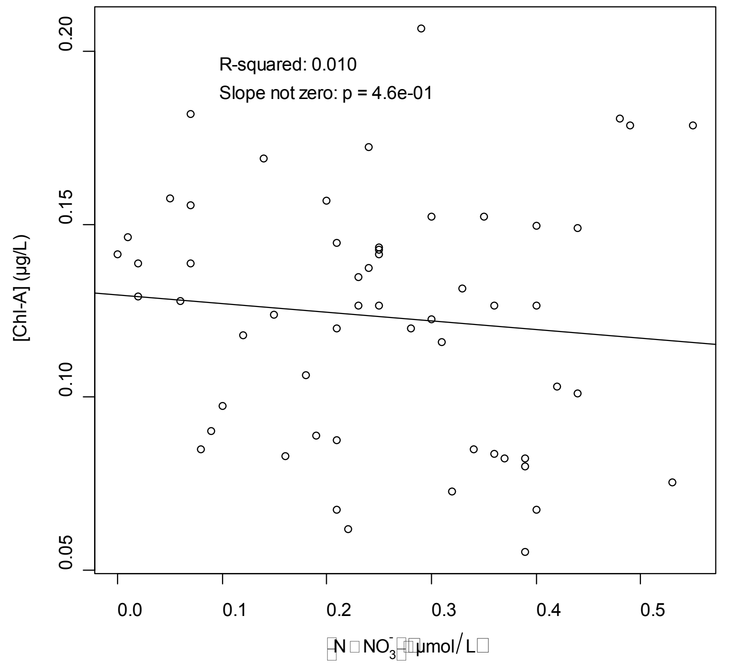
		Enteros				NO3				PO4				Chl-a			
code 1	code 2	diff	lwr	upr	p adj	diff	lwr	upr	p adj	diff	lwr	upr	p adj	diff	lwr	upr	p adj
Red Slave S	Karpata S	-0.593	-2.739	1.554	1.000	-0.201	-0.419	0.017	0.101	0.051	-0.335	0.438	1.000	-0.072	-0.161	0.016	0.227
South Bay S	Karpata S	-0.593	-2.512	1.327	1.000	-0.040	-0.235	0.155	1.000	-0.149	-0.495	0.197	0.978	-0.033	-0.113	0.046	0.982
Playa Lechi S	Playa Funchi S	0.593	-1.327	2.512	1.000	0.165	-0.053	0.382	0.350	0.135	-0.251	0.522	0.998	0.030	-0.049	0.109	0.993
Red Slave S	Playa Funchi S	0.000	-2.146	2.146	1.000	0.296	0.079	0.514	0.001	0.234	-0.153	0.620	0.724	-0.058	-0.147	0.030	0.583
South Bay S	Playa Funchi S	0.000	-1.920	1.920	1.000	0.457	0.263	0.652	0.000	0.033	-0.312	0.379	1.000	-0.019	-0.098	0.060	1.000
Red Slave S	Playa Lechi S	-0.593	-2.739	1.554	1.000	0.132	-0.107	0.370	0.837	0.098	-0.325	0.522	1.000	-0.089	-0.177	0.000	0.048
South Bay S	Playa Lechi S	-0.593	-2.512	1.327	1.000	0.293	0.075	0.510	0.001	-0.102	-0.489	0.285	1.000	-0.050	-0.129	0.029	0.662
South Bay S	Red Slave S	0.000	-2.146	2.146	1.000	0.161	-0.057	0.379	0.388	-0.200	-0.587	0.186	0.895	0.039	-0.049	0.127	0.973

ANNEX 4 Correlation parameters

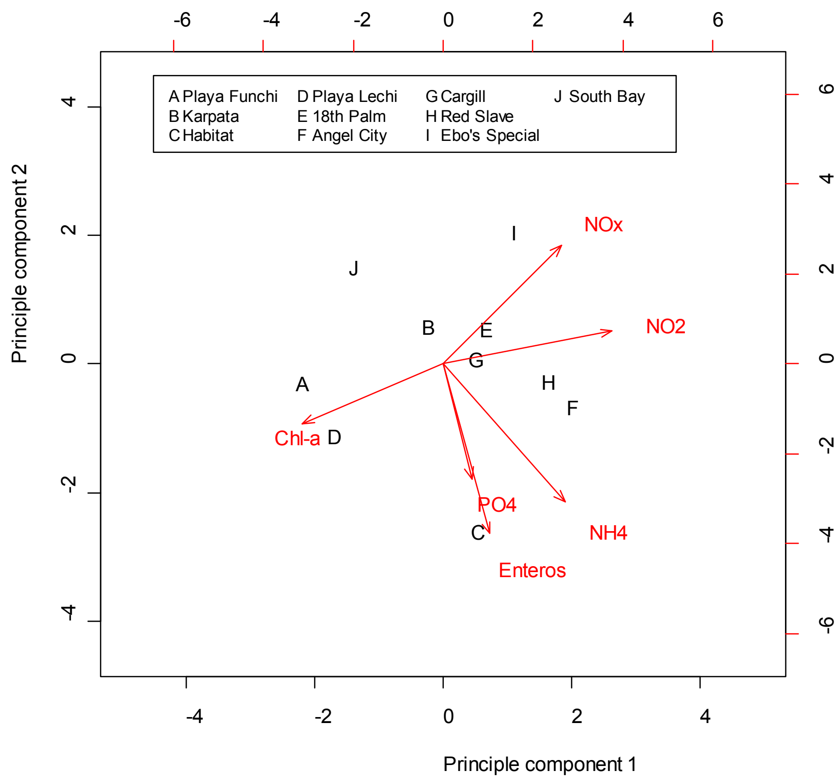


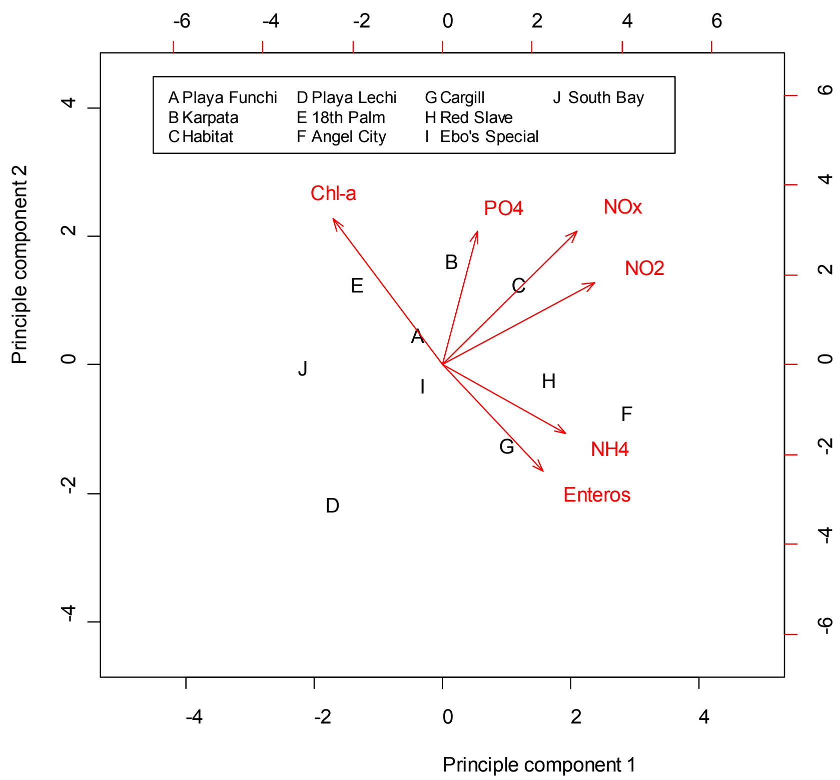



R-squared: 0.004
Slope not zero: p = 6.3e-01
0.00
0.05
0.10
0.15
0
1
2
3
4


R-squared: 0.010
Slope not zero: p = 4.6e-01
[Chl-A] (µg/L)
0.0
0.1
0.2
0.3
0.4
0.5
0.05
0.10
0.15
0.20

ANNEX 5. Principle component analysis





ANNEX 6. Balanced one-way analysis of variance power calculation

Power analyses have been implemented and executed in R version 2.12.2 (The R Foundation for Statistical Computing, Vienna).
Effect size is the variable input parameter and represents the decrease of total nitrogen. Various options were calculated (f= 0.3, f= 0.5 and f=0.7).

Input:	
k = 40	(number of groups: 10 locations x 2 depths x 2 time intervals)
f = 0.3	(effect size)
sig.level = 0.05	(significance level)
power = 0.8	
Output:	
n = 8.280926	(required number of replicates)

Input:	
k = 40	(number of groups: 10 locations x 2 depths x 2 time intervals)
f = 0.5	(effect size)
sig.level = 0.05	(significance level)
power = 0.8	
Output:	
n = 3.468127	(required number of replicates)

Input:	
k = 40	(number of groups: 10 locations x 2 depths x 2 time intervals)
f = 0.7	(effect size)
sig.level = 0.05	(significance level)
power = 0.8	
Output:	
n = 2.177391	(required number of replicates)