Diet and maximum size of Queen conch (Lobatus gigas) around the island of Curaçao

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Contents

Summary

The main focus of this study was to gather more information about the diet and ecology of the Queen conch (*Lobatus gigas*) in the island of Curaçao and provide background knowledge for future restoration and mariculture programs. The presence of conchs in the Sea Aquarium of Curaçao, where there is practically no poaching or predation, offered a good opportunity to study the diet and length distribution of the conchs under various semi-natural conditions. For this study, samples of conchs and possible items of their diet were collected from 5 natural sites and 6 basins of the Sea Aquarium. The presence of various species of sea mammals (i.e. dolphins, sea lions) in these basins was thought to influence diet composition and therefore the growth rate of the conchs.

Stable isotope analysis of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) was used to test this hypothesis and to determine the main diets of the conchs in every site. This method allows identifying the composition of consumers and estimating the contribution of the different elements of a consumer´s diet. The results showed that in the basins of the Sea Aquarium and in the natural sites where there was human influence, the $\delta^{15}N$ of the conchs and the algae sampled were high compared to the more pristine natural sites. The reason for that might be that the sewage of resorts and the Sea Aquarium discharge close to where the conchs were found. In the Sea Aquarium, the presence of the sea mammals inhabiting the basins could also be the reason for the high levels of $\delta^{15}N$.

The second main goal was to assess if the maximum sizes reached in the Sea Aquarium were larger compared to the wild and, if that was the case, try to relate it to the different diets. The length of this study did not allow for growth measurements. Therefore, the maximum length of the shell and the thickness of the flared lip were used as proxies for local growth conditions. Within the basins of the Sea Aquarium, the largest specimen found had a shell length of 33cm and a lip thickness of 1.2cm. Moreover, adults with flared lips were found in all the basins of the Sea Aquarium, but juveniles were mostly lacking. At most natural sites, however, only juveniles were found and only at Playa Grandi, some specimens with a shell length of more than 28cm and complete developed flared lip were found. The presence of these large specimens suggests that the large sizes reached in the Sea Aquarium are related to the lack of predation (including fisheries) rather than to the diet.

This study underlines that adult Queen conchs in the waters surrounding Curaçao are very rare and appear to occur only in restricted places where there is no poaching of large specimens. Even though specimens of Queen conch were larger and had a different diet in the basins of the Sea Aquarium than those living within more natural waters of Curacao, a causal relationship between growth and diet could not be established. The contrasting size-frequency distributions, finding mostly adults in the Sea Aquarium and juveniles in natural sites, suggest that the high mortality of Queen conch at accessible locations strongly influences the survival and subsequent the length that can be reached. Future restoration of wild populations appears only feasible if juveniles are allowed to reach adult size at which they can reproduce.

1. Introduction

1.1 General information

The Queen conch (*Lobatus gigas,* Linnaeus 1758, known until 2008 as *Strombus gigas*) is a large marine gastropod native to the tropical northwest Atlantic (Brownell & Stevely, 1981). In the 1970s, the demand of Queen conch meat in the Dutch Antilles increased due to tourism, leading to a collapse on the fisheries of this species (Pillsbury, 1985). Not only the meat of the species is valuable, but also the pink shell of the adults with a flared lip that has been used for tools, ballast, building, jewellery and ornamental (Ray et al. 1994). Under natural (unfished) conditions, predation is stronger on small and therefore more vulnerable specimens, and we can see this phenomenon happening in many other species of marine gastropods, for example the Milk conch (*Lobatus costatus*) (Aldana-Aranda & Patiño 1998). Nevertheless, the human predation of Queen conch is focused on the adult population (i.e. shells with flared lip developed), the group that has a greater economical value and that it is becoming rare in nature (Prent, 2013). The Queen conchs with a flared lip are not necessary sexual mature and many of them are caught before their first reproduction cycle (Willemse, 2013).

In 1985, the Queen conch was enlisted as commercially threatened and in 1992 it was included in Appendix II of the CITES *(Convention on International Trade in Endangered Species of Wild Fauna and Flora)* (Prent, 2013). Despite the regulations applied and the several projects to reintroduce juveniles in areas where the conch has been fished out little recovery has been shown (Aldana-Aranda & Patiño 1998). In the present, most of the Caribbean countries have fishery regulations to avoid overfishing and in some areas, for example Bonaire (Dutch Caribbean), fishery on the conch is prohibited (Prent, 2013). Nevertheless, due to the high value of this edible gastropod, the stocks are still heavily exploited, being overfishing the main factor of the population reduction (Prent, 2013). Habitat degradation might also be a factor, especially the loss of nursery habitats close to the shore and changes in algae and phytoplankton composition on which the conch feeds (Stoner, 2003).

1.2 Growth and biology of Queen conch

Lobatus gigas can reach shell lengths of more than 35 cm (Brownell & Stevely, 1981). In the third year of life, the growth of adults in length almost stops and they develop a flared lip (McCarthy, 2007). After this point, the growth is seen in the thickness of the lip (McCarthy, 2007). According to Brownell and Stevely (1981), the estimation of the age can be done using the length of the shell until the third year. Therefore, specimens with a length shorter than 11cm are less than one year (juveniles); between 12 and 17 are around 2 years old (sub-adults or rollers) and larger than 18 cm are at least 3 years old (adults) (Brownell & Stevely, 1981). It has been estimated that at 2,5- 3,2 years the conchs start developing the flared lip and the growth speed of the shell length and meat slows down (Berg, 1976).

The age of first reproduction does not necessarily coincide with the formation of the lip. In fact, most of the studies show that the estimated age of the first reproduction is after the lip is completely formed, when the animals are around 3,5 years old (Appeldorn, 1988). The time needed to develop a flared lip is changeable depending on environmental conditions and diets but studies have shown that in less than 3 months a complete lip can be formed (Randall, 1964). To estimate age after the lip is built is very complicated due to the erosion of the shell. Several studies attempted to do it by measuring the same specimens over time and the results differed significantly depending on the area and the diets of the conchs (Stoner, 1989).

The distribution and abundance of the Queen conch is strongly associated to the presence of the algae they feed on (Stoner & Ray, 1993). Nevertheless, the diet in nature is very variable. This species is described to be mostly herbivorous and depending on the development stage it feeds on different seagrass beds (Littler & Littler, 2000). In their adulthood they have already developed a strong and long snout that allows them to select and take big pieces of macro algae (Stoner & Ray, 1993). They usually live in sandy areas or seagrass meadows (Littler & Littler, 2000). Studies have shown that when available, the adults feed on seagrass as *Thalassia* and macro algae species as *Cladophora sp., Hypnea cervicornis* or *Polysiphonia sp*. ((Danylchuk et al. 2003). They also ingest phylamentous and phytoplankton (Danylchuk et al. 2003). The juveniles seem to prefer phytoplankton and turf algae and when they are sub-adults they start feeding also on seagrass (Pillsbury, 1985). Filamentous turf algae is a general term to describe different species of algae that cover the rocks, coral or sand and that present different morphology (Appeldoorn, 1988). Preferences of the diet of Queen conch might change over the year depending on the physiological needs and the availability of the algae species (Santaella & Aranda, 1994).

The abundance of the conch is also related to the presence of predators (Brownell & Stevely, 1981). Apart from poachers, the Queen conch also has to face natural predators. The most common ones are octopus, hermit crabs, stingrays, spotted eagle rays and different types of sharks as lemon shark or tiger shark (Stoner & Sandt, 1992). Also big species of fish, as hogfish or gray snappers have been reported to prey on conch (Brownell & Stevely, 1981). Moreover, other species of gastropods as apple murex (*Murex pomum*) or the tulip (*Fasciolaria tulipa*) are commonly found eating the meat of conchs (Appeldoorn, 1988).

1.3 Situation of Curaçao

Currently, natural stocks of Queen conch are severely depleted in most of the Caribbean islands, being specially affected by fishing in the Leeward Islands, Curaçao and Bonaire (Hensen, 1983; Theile, 2001). Studies carried out in 2001 showed a density of 111 conchs in a 5 hectares sampling area of Bonaire (Theile, 2005). A study conducted in 2000 in the Bahamas also showed that when the density of conchs is lower than 56 conchs ha^{-1} mating almost never occurred (Stoner & Ray-Culp, 2000). Nevertheless, no surveys have been done to determine the density and the conditions of the populations in the island of Curaçao.

Although in 1992 the conch was included in Appendix II of the CITES it is still commercialized (Prent, 2013). The records show that at the end of the 70s the price of the Queen conch in the islands was $2 per kilogram and in the last 30 years it raised up to $30 per kilogram (Davis, 2005). As the prices keep increasing, the pressure on the few natural stocks of Queen conch has increased (Schweizer & Posada, 2006). Curaçao and Bonaire have a large conch market, with most of the conchs imported legally from Jamaica and Colombia and part illegally, from Venezuela and the Islands themselves (FAO, 2007). Nevertheless, the status of the Queen conch in Curaçao is not well known, as there has not been almost any study conducted in this area (Theile, 2001). Habitat restoration projects and aquaculture programs could help to rebuild the severely depleted natural stocks in the Caribbean (Berg, 1976). As a base for these future projects, more knowledge about the diet and preferences of habitat of this species is needed.

The population present in the basins of the Sea Aquarium of Curaçao offers a good opportunity to compare the diet and maximum length between the wild and a semi-natural system. Workers from the Sea Aquarium had described high densities and larger sizes of the conchs compared to data from natural sites. There are no studies focused on this phenomenon or in the factors that may cause this anomalous growth. The content of the diet of these conchs may be one of the reasons for the fast growth. Furthermore, the maintenance of the inhabitants such as dolphins, sea lions, and large species of fish in the different basins coincides with input of nutrients (i.e. via the faeces) and could therefore positively affect the growth of conchs by influencing the algal growth. Also the remains of the fish used to feed the animals of the basins could contribute to the conch diet.

This project had two main objectives. First, I wanted to assess if there were significant differences in the diet composition of the conchs living in the Sea Aquarium of Curaçao and the ones in the wild. The second goal was to test if the total growth (i.e. length of the shell and lip thickness) reached in the Sea Aquarium facilities was larger compared to the specimens from the wild sites and if so try to relate it to the diet.

To accomplish the first objective, a stable isotope analysis was used. This method has become a very helpful tool to analyse the composition of consumers, their food and therefore to estimate the contribution of the different elements of a consumer´s diet (Philips et al. 2014). For this study, Carbon and nitrogen were used to identify which resource pools support the Queen conch. The ratio of ^{15}N to ^{14}N (i.e. $\delta^{15}N$) shows a gradual enrichment with trophic transfer and can be used to estimate the trophic position of organisms (Phillips et al. 2014). Carbon isotopes ratios (i.e. $\delta^{13}C$) do not show big changes with trophic transfer (Post, 2002) but we used it to identify original sources of the diet of the conch. Consumers normally show an enrichment of 3.4 $^{0}/_{00}$ percentage mass of ^{15}N and of 0.1$^{0}/_{00}$ of ^{13}C compare to their preys (Tilley et al. 2013) and we would expect to find this relationship between the conchs and their diet.

2. Methodology

2.1 Research area

The study was conducted in the island of Curaçao, which is an autonomous country in the Kingdom of the Netherlands. It is situated in the southern part of the Caribbean Sea and it includes the main island and the inhabited island of Klein Curaçao, located at the southeastern part. The sampling was carried out in 5 natural sites and 6 different basins in the Sea Aquarium of Curaçao. The five natural places were: a beach situated in the northeast of Piscadera Bay, CARMABI *Research Station* beach, Playa Grandi, East Point and Klein Curaçao (Figure 1). In the Sea Aquarium, 6 basins were sampled (Figure 2). The basins differed among other things, in the animals they contained: four of them were inhabited by dolphins (West, East, Therapy 1 and Therapy 2 basin), other one contained sea lions and the last one, the Animal Encounter basin, different species of fish. The basins were also provided by a continuous supply and exchange with natural seawater from the adjacent Caribbean Sea (Figure 2) (see Appendix A).

Figure 1. Location of the wild sites sampled in Curaçao. Map from Google Maps

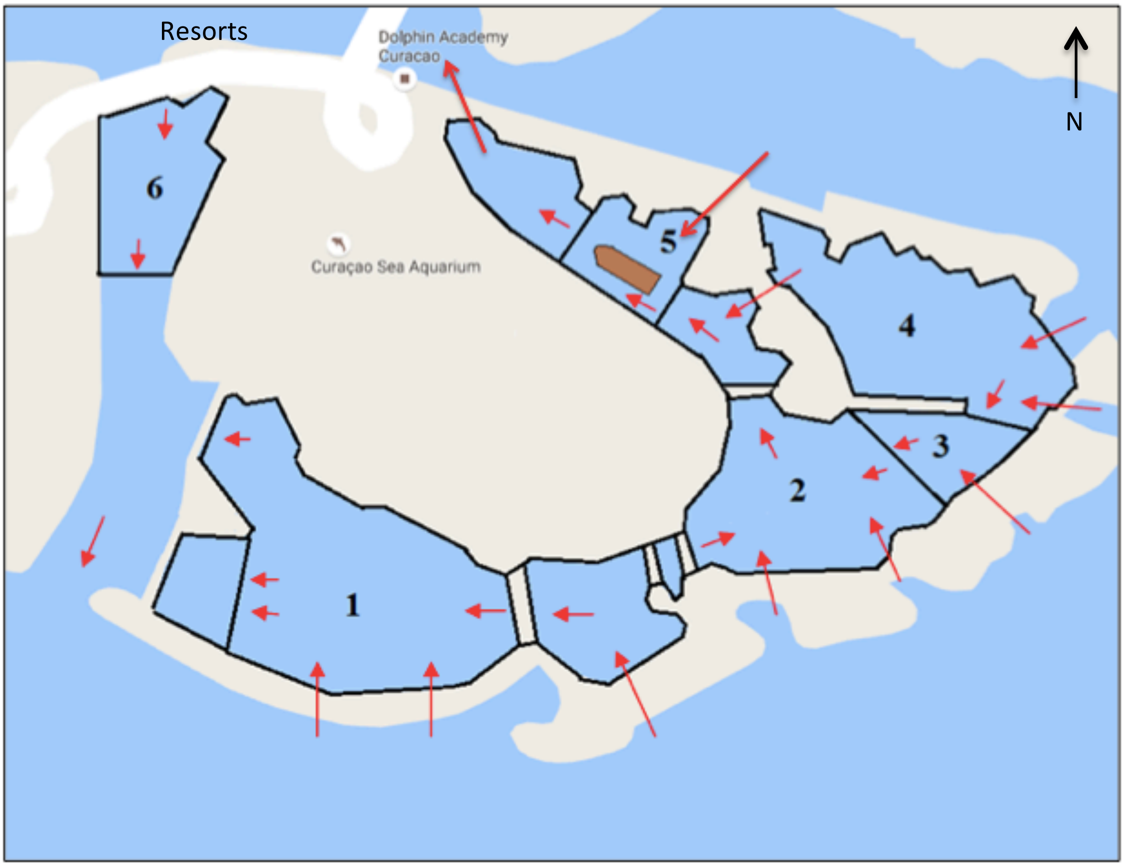

Figure 2. Map of the facilities of the Sea Aquarium. The numbers indicate the different basins: 1 for the West basin; 2 for the East basin; 3 for the Therapy 2 basin, 4 for the Therapy 1 basin; 5 for the Animals Encounter basin and 6 for the Sea lions basin. The red arrows show the direction of the currents. Map from Google Maps

2.2 Sampling

The sampling was conducted during the months of September, October and November. In the 11 sites where the study was carried out (5 wild sites and 6 in the Sea Aquarium), samples of algae and conch were collected. From every site 5 specimens of queen conch were collected except from East Point where only 3 conchs were found. From every algae sampled, 5 replicates were taken. In the Sea aquarium, suspended particulate organic matter (SPOM), fish used to feed the animals and faeces of sea lions were also sampled.

2.2.1Procedure in the Sea Aquarium

The six basins were sampled using the same methodology. Five specimens of Queen conch where collected from each basin, and when possible different size classes were taken (juveniles, sub-adults, adults with and without flared lip). Samples of all the potential food items for the queen conch were collected. This included different species of macro algae, faeces of the inhabitant of the basins (only sea lion samples were possible to obtain), samples of the fishes used to feed the dolphins and sea lions (i.e. herring, capelin and mackerel) and SPOM.

An estimation of the abundance of every lagoon was done. Moreover, all the conchs with a flared lip were measured with a ruler in order to find the largest one for every basin. Finally, a general assessment of every basin was done, noting down relevant characteristics (i.e. currents direction, type and abundance of predominant algae, presence of empty shells, depth and presence of other gastropods as the milk conch) and a photographic report of every site and specimen was taken (See Appendix B, Figure 10).

2.2.2 Procedure in the wild sites

The sites of Playa Grandi, East Point and Klein Curaçao were chosen considering the possibilities of finding Queen conch. This was based on the assumption that they are suitable areas for the Queen conch with restricted access for fishery and human activities. To sample East Point and Klein Curaçao a boat was needed. The samples of East Point were taken by personnel of the Sea Aquarium in one of their expeditions. The samples taken in front of CARMABI *Research Station* beach were found randomly in the sand plain before the reef. The conchs of Piscadera bay were found piled up in the beach after a big storm. In every site I attempted to collect 5 specimens of Queen conch with different size classes when possible and 5 replicates of every potential food item (i.e. different types of algae).

Also a general assessment of every site was done, noting down relevant characteristics (i.e. type and abundance of predominant algae, depth and presence of empty shells) and a photographic report of every site and specimen was taken (See Appendix B, Figure 11).

2.2.3 Conch sampling

Most of the conchs were collected while SCUBA diving, carried with a net, brought ashore or on board, and placed in a cooler with seawater during the transportation to the lab. In the lab, they were placed in aquaria with flowing seawater. The conchs from Piscadera Bay were found in the beach after a big storm (end of November). Big amounts of material (sand, wood, dead animals, stones, coral, etc.) were dragged out to the beach during the storm and ~ 15 conchs were visible.

After the collection, a number was assigned to each specimen and the following information was written down: time and date of collection, time and date the different measurements were taken, place of collection and number of aquarium in which they were placed.

Some other morphological features were also measured. The length of the shell was measured using a ruler, from the tip of the spine to the distal end of the siphonal canal (Brownell & Stevely, 1981) (Figure 3). Moreover, the relationship between the diameter and height of spines was noted down, the presence or absence of lip. When present, the thickness of the flared lip was also measured following the method of Appeldoorn (1988), using callipers on the flared outer lip, in the posterior part of the shell aperture.

Figure 3. Length of the shell measured from the tip of the spire to the distal end of the siphonal canal.

The sex is only identifiable in sub-adults and adults when the verge (i.e. masculine reproductive organ) is formed (Appeldoorn, 1988). To identify the sex, the conch was hold out of the water for 30 seconds to a minute until the animal was partly hanging out of the shell and the verge was visible in males. After the sex identification, a sample of flesh was taken. A biopsy forceps was used to cut a small portion of muscle tissue. The flesh samples were kept in labelled vials and placed in a freezer (-20ºC). All the conchs were kept in the aquaria for a few days until the wounds from the cuts were almost healed and then placed in the basins.

2.2.4 Benthic Algae sampling

After the conch collection, the algae that could be potential food for the conchs were collected and put in plastic zip bags. In the wild, the samples of algae were taken at the same time as the conch collection. In the Sea Aquarium, the diving time was limited due to the presence of the marine mammals. Therefore, for every basin two dives were needed: one for the conch collection and another one for the algae sampling. The main criterion for the alga sampling was to collect all the species present on a perimeter of 2 meters around the conchs. Moreover, the species that were described in the literature as part of the conch diet were collected. The most important groups of algae described to be in the Caribbean Sea and in the diet of the Queen conch were: turf algae (assemblages of less than 1 cm high stands of filamentous algae and cyanobacteria with small macro-algae), *Dyctiota* (brown algae), *Lyngbya* (Cyanobacteria), *Thalassia* (Seagrass), *Champia parvula* (red algae), *Hypnea* (red algae) and *Cladophora* (green algae) (Figure 4).

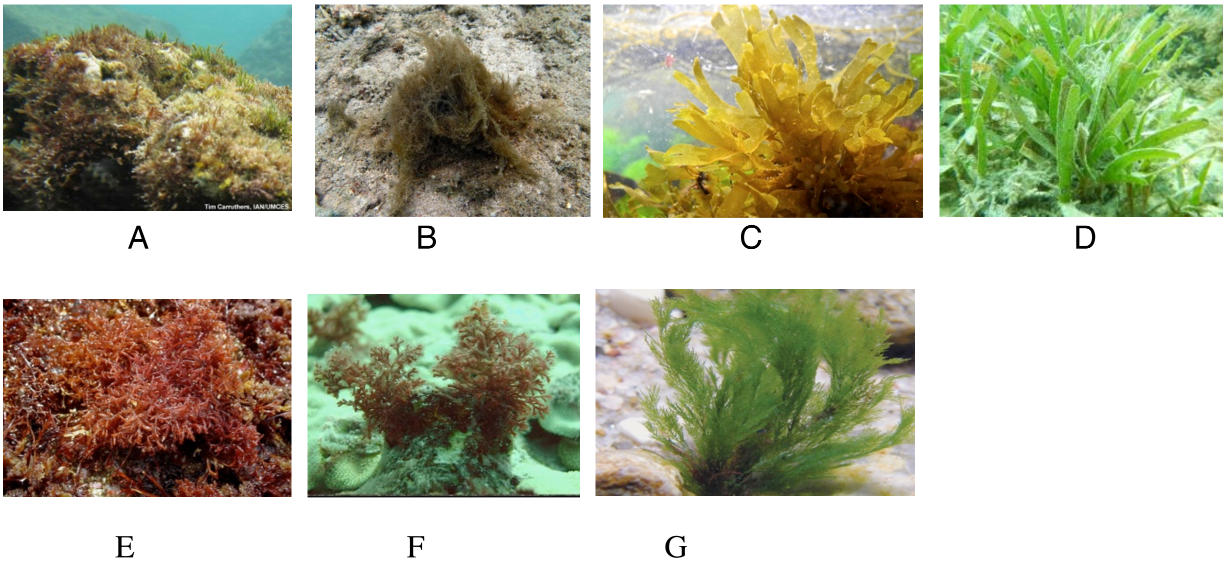

Figure 4. Groups of algae described by the literature to be in the diet of the Queen Conch. A Turf algae, B *Dyctiota*, C *Lyngbya*,D *Thalassia*, E *Champia parvula*, F *Hypne*a and G *Cladophora*. Pictures from www.algaebase.org.

The samples were taken to the lab and from each alga 5 different replicates were taken, washed with filtered seawater, placed in labelled vials and stored in a freezer (-20ºC).

2.2.5 SPOM sampling

For the sampling of suspended particulate organic matter, water of every basin was collected with a bucket (~ 10 litres per basin) and pre-filtered with a 200µm sieve to remove large zooplankton. 25mm filter cartridge with a pre-combusted glass fiber filters (GF/F, pore size ~0.7μm) on a 50 ml syringe was used to filter the water. Per filter 2 litres of seawater were used. The filter was removed with filter tweezers, folded and wrapped in pre-labelled aluminium foil and stored in a freezer (-20 ºC). The 10 litres collected were used to obtain 5 replicates per basin.

2.2.6 Sea lion faeces and fish samples

Samples of sea lion faeces were also taken for the stable isotope analysis. The trainers of the sea lions took the sample from the facility and kept it in a labelled tube in the fridge. The sample was divided into 5 labelled vials that were placed in the freezer (-20ºC). Furthermore, the different species of fish that were used to feed the inhabitants of the basin were sampled (i.e. capelin, herring and mackerel). For this, a piece of the dorsal muscle tissue of every species was cut and distributed in 5 labelled vials that were kept in the freezer (-20 ºC).

2.3 Freeze drying

The freeze-dry technique was used to preserve the samples during the transportation to the Netherlands. All the samples collected (conch flesh, algae, fish flesh, sea lion faeces and the filters) were placed in the freezer (-20ºC) before the freeze-drying. A freeze-drying machine was used at CARMABI *Research Station*. All the vials with the frozen samples were opened placed inside the freeze-drier machine (-52 ºC) and run during 24h. When the process was finished and the samples dehydrated, the vials were closed and kept in an isolated plastic box to avoid the absorption of humidity. The filters were kept in the aluminium foils inside zip bags.

2.4 Stable Isotopes analysis

All the samples were analysed in the facilities of the Research Centre of NIOZ (Netherlands Institute for Sea Research). In table 1 it is shown the ranges of weights for every type of sample and the standards that are used in this method.

Table 1. Weights of the standards and samples needed for the stable isotope analysis.

Type of sample	Weight (mg)
Ace (standard)	0.5-0.8
Urea (standard)	0.3-0.8
Casein (standard)	0.3-0.8
Fish	0.4-0.8
Queen conch	0.4-0.8
Algae	1.5-3
Faeces	0.4-0.8

Every sample was put into a tin cup, weighted on a scale, shaped as a small ball and placed in a plate that contained 94 holes, 70 for samples and 24 for standards.

2.4.2 Decarbonation

SPOM

The samples of SPOM needed to follow a different preparation process due to the content of inorganic carbon. The filters were decarbonized before the analysis in order to obtain the isotope carbon values of the sample without the signal of particulate inorganic carbon (e.g. $CaCO_3$). All the filters, except the ones corresponding to the West lagoon of dolphins, were cut in half and placed in a silver boat. A. To analyse the ones of the West lagoon, the whole filter was used due to the low content in organic carbon. As in this process acid was used, the samples were placed in silver cups instead of tin cups. The silver boats were placed in a desiccator with concentrated hydrochloric acid gas (37%) dissolved in water for 24h. A drop of demineralized water was added to every sample to make the penetration of the acid more efficient. After that, they were dried in an oven at 60ºC for another day.

Benthic Algae

After running a first stable isotope analysis, some of the samples of the algae showed very positive values for the carbon isotope ratio, probably due to inorganic carbon contamination. To obtain the real isotope values for the algae, these samples were also decarbonized. For that, concentrated hydrochloric acid (37%) was added to the samples and shaken so that most of the sample reacts with the acid. The tubes with the samples and the acid were placed in a table shaker during 8 hours and after that, the entire sample is expected to have reacted with the acid. The samples were then centrifuged and the acid was pipetted out. A few drops of demineralized water were added to the samples, mixed again in the table shaker, centrifuged and the remain liquid was pipetted out. This process was repeated several times until there was no acid in the samples. To test this, an indicator paper was used. Finally, the samples were freeze-dried during 12 hours.

Once the samples were decarbonized and freeze-dried, each sample was transferred to a tin cup and weighed. Afterwards the tin cups were closed with tweezers, shaped as a ball and placed in a measuring plate.

2.4.3 Organic Elemental Analyzer

When a plate was full, the 70 samples and the standards were placed for 24 hours in an *Organic Elemental Analyzer* to run the stable isotope analysis. This machine analyses the isotopic composition of organic material. For this study, the analysis was run for carbon and nitrogen. The process consists in a conversion of the samples to gasses by combustion and then separation of the pure gases (CO_2 and N_2). The gasses go then through an isotope ratio mass spectrometer and the composition of the samples is calculated relative to the standards (acetanilide, urea and casein). The results obtained after this process are expressed as parts per thousand differences from the standards:

$$\delta X = (Rsample/Rstandard - 1)\ x1000$$

In this equation X is ^{15}N and ^{13}C; R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$; and the δ represents the ratio of ^{15}N and ^{13}C relative to their lighter isotopes ^{14}N and ^{12}C of the samples and therefore an elevated δ indicates a higher proportion of the heavy isotopes (Hobson et al. 1994).

2.5 Statistical analysis

I used the program *RStudio* to run all the statistical analysis. First, the data were tested for normality. Analysis of variance (ANOVA) was used to compare the isotope composition of the conchs within the Sea Aquarium. This same test was run for the wild places. Moreover, a one-way ANOVA was also used to see if there were significant differences in the $\delta^{15}N$ and $\delta^{13}C$ values of the Sea Aquarium compare to the wild. If this test showed a significant difference, a Tukey´s test was run to see between which sites there was a significant difference. A *T-test* was used to see if there was significant difference in the average and the maximum lengths of the wild conchs and the conchs in the Sea Aquarium.

3. RESULTS

In this study a total of 60 living conchs were found and analysed for stable isotopes composition: 37 conchs were from the different basins of the Sea Aquarium and 23 from the wild sites (see Appendix C, Table 5). The isotope values of the SPOM of the different basins, the fish and the faeces of the sea lions are summarized in table 6 (see Appendix C). In the wild, seven different types of algae were collected while in the Sea Aquarium 9 were thought to be possible sources for the conch diet (see Appendix C, Table 7).

3.1 Stable isotope composition

The means and standard deviations of the Queen conch isotope composition of the different sites were calculated (Table 2). The ANOVA test did not show significant differences in $\delta^{13}C$ of conchs between the wild and the Sea Aquarium (see Appendix C, table 8), but it showed significant differences in the $\delta^{15}N$ (see Appendix C, Table 9). The Tukey´s test showed a significant difference in the means of $\delta^{15}N$ of the conchs from the Sea Aquarium and the ones from the wild (see Appendix C, Table 10). Figure 5 shows 3 different colours that represent the different groups of conchs according to the ANOVA and Tukey´s analysis. One of the groups includes all the means of the basins of the Sea Aquarium with the exception of the Sea Lion facility that shows a higher $\delta^{15}N$ mean. The means of the wild places could be grouped in 2 different sets, one with CARMABI, Piscadera and Sea Lion basin; and the second one, with Playa Grandi, East Point and Klein Curaçao.

Table 2. Mean and standard deviations of $\delta^{15}N$ and $\delta^{13}C$ of the Queen conchs sampled in the 5 wild sites and the 6 basins of the Sea Aquarium.

Description	Mean $\delta^{15}N$	St dev. $\delta^{15}N$	Mean $\delta^{13}C$	St dev. $\delta^{13}C$
CARMABI	8.44	1.35	-14.21	0.82
Piscadera	10.54	0.40	-13.84	0.74
Klein Curaçao	2.42	0.27	-11.88	0.51
Playa Grandi	3.42	0.94	-13.51	1.60
East Point	1.64	0.11	-11.30	0.41
Animal Enc.	6.56	0.53	-13.53	0.64
East basin	5.51	0.47	-13.47	0.67
Sea lion	8.92	1.28	-12.09	1.41
Therapy 1	5.99	0.16	-13.87	0.51
Therapy 2	5.54	0.35	-13.71	0.80
West basin	5.20	0.47	-14.08	0.35

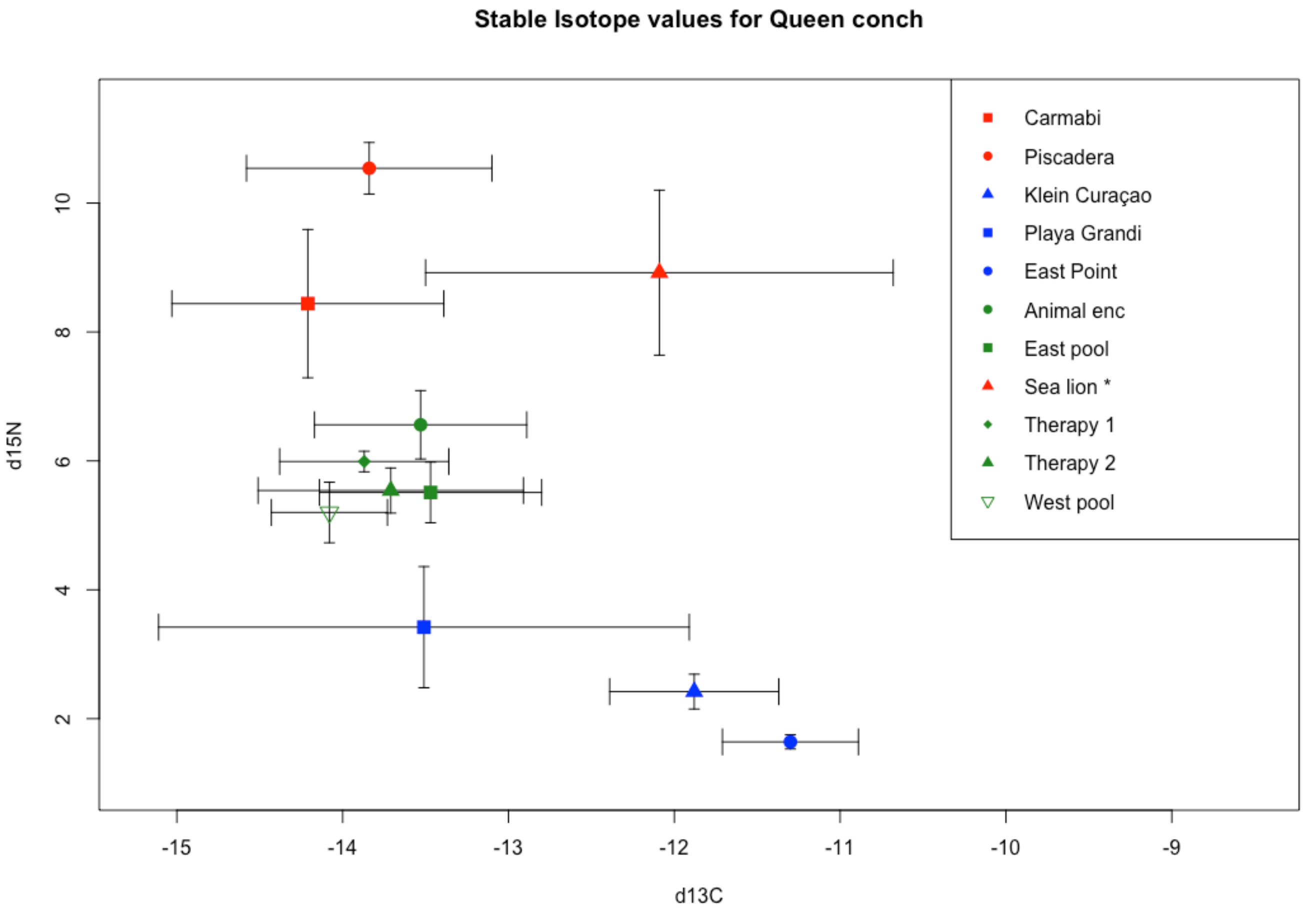

Figure 5. Bivariate plot of means and standard deviations of $\delta^{13}C$ vs. $\delta^{15}N$ values from tissue samples of all the Queen conchs collected. The different colours show the three different groups that were identified according to Tukey's test. The asterisk points out the high value in $\delta^{15}N$ of the Sea Lion basin.

For every location, the possible food sources were also analysed. In figure 6 the $\delta^{15}N$ and $\delta^{13}C$ of the conchs and all the food items analysed from the wild sites are shown. Figure 7 shows the same information for every facility sampled in the Sea Aquarium. In the wild, 6 different types of algae were collected and it seems that the Queen conch eats mainly 2 of them, turf in most of the sites and *Lyngbya* in Klein Curaçao. In the Sea Aquarium 9 different types of algae were analysed. Five of those algae seem to be the main diet of the conchs in the different basins. In some of the facilities different species of *Cladophora* (assigned with letter F in figure 7) were sampled but species identification was not possible. The dimensions and the amount of fish used to feed every basin were calculated (see Appendix A, table 4). The results of the isotopic composition of faeces, fish and SPOM are shown in figure 9 (Appendix D).

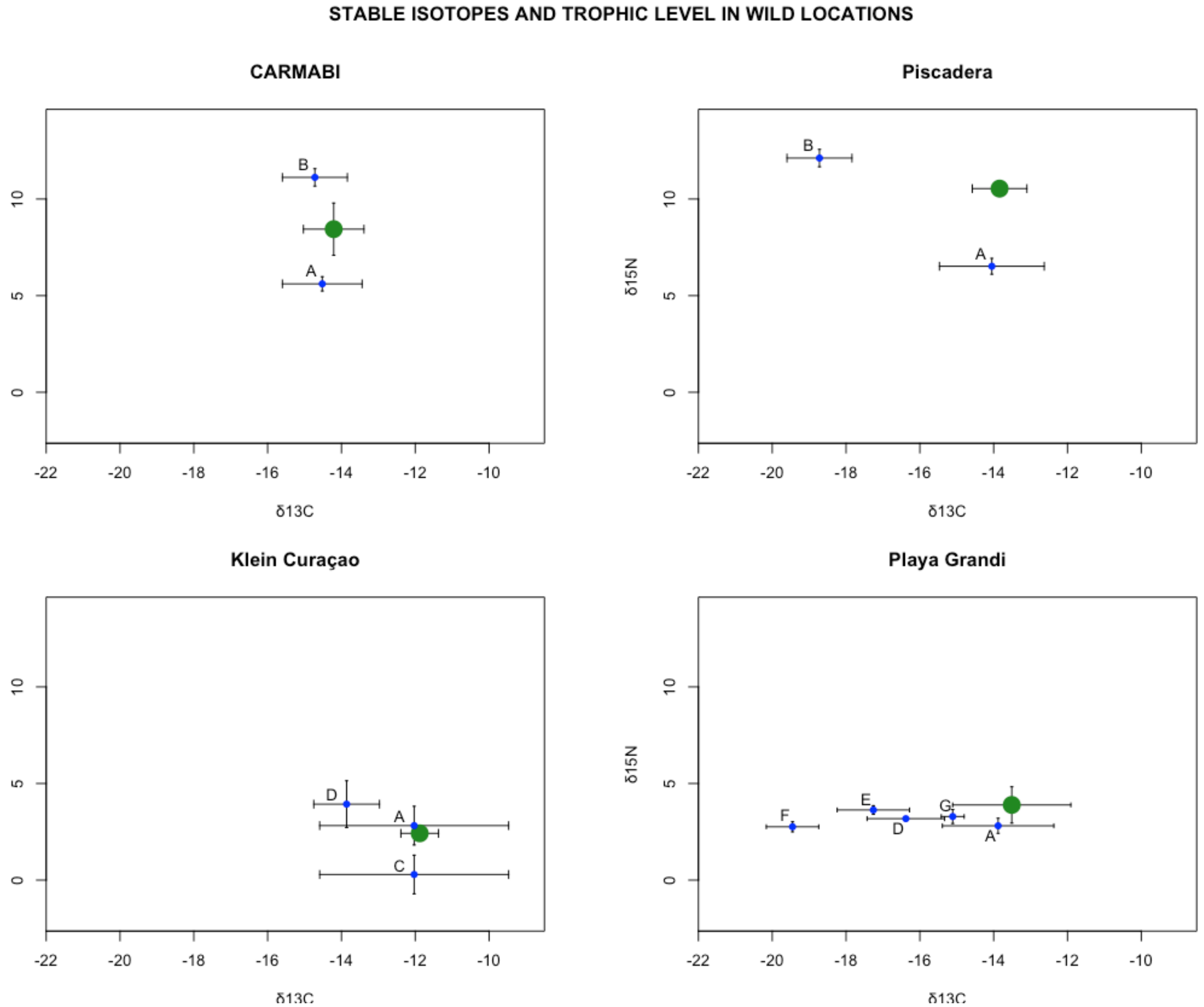

Figure 6. Bivariate plots of means and standard deviation of $\delta^{13}C$ vs. $\delta^{15}N$ values from tissue samples of Queen conchs (green dot) and the different types of algae (blue dots) collected in the wild. The different algae are assigned with letters: A for Turf algae; B for *Dictyota*; C for *Lyngbya*; D for *Thalassia*; E for *Champia parvua*; F for *Cladophor*a; and G for *Halophila*.

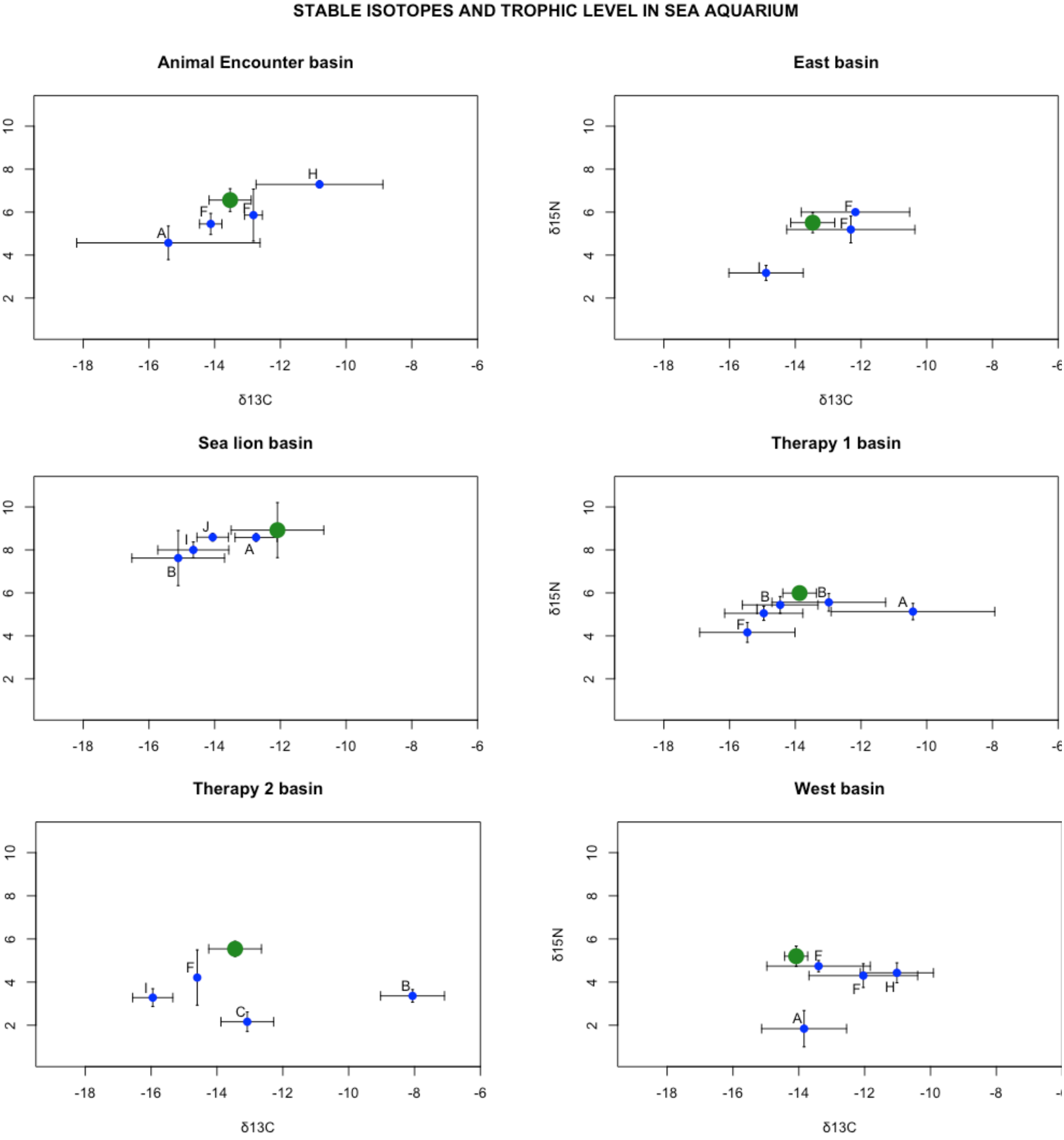

Figure 7. Bivariate plots of means and standard deviations of $\delta^{13}C$ vs. $\delta^{15}N$ values from tissue samples of Queen conchs (green dot) and the different types of algae (blue dots) collected in the different basins of the Sea Aquarium. The different algae are assigned with letters: A for Turf algae; B for *Dictyota*; C for *Lyngbya*; E for *Champia parvua*; F for *Cladophor*a; G for *Halophila;* H for *Hypnea*; I for *Ceramium byssoideum;* and J for *Syringodium*.

3.2 Length comparison

The length of all the conchs sampled in this research was measured and descriptive information was collected from all of them (Appendix C, table 11). Moreover, the average length was calculated for every site (table 3). In table 3 the abundance (number of conchs per basin), maximum length found in every site and the presence or absence of flared lip in the largest specimens is also shown. The results show that in all the basins of the Sea Aquarium, adults with flared lips were present. However, in the wild only adults with flared lip were found in Playa Grandi. In the rest of the wild sites, the conchs sampled were juveniles or sub-adults.

A comparison of the average sizes and the maximum length between the wild and Sea Aquarium was done, and the *t-test* showed a p-value of 0.0138 and 0.0107 respectively indicating that the average and the maximum size was significantly larger in the Sea Aquarium facilities than in the wild sites. Figure 8 shows the average and maximum lengths of the conchs from the wild sites and from the Sea Aquarium basins. The largest sizes were found in the Sea Aquarium facilities (East and West basin) and in Playa Grandi. The conchs with the thickest flared lip were also found in the basins of the Sea Aquarium. In the West and East basin some specimens showed lips thicker than 1 cm. The largest conch found in Playa Grandi had a lip 0.5 cm thick. The smallest specimens were found at CARMABI *Research Station* beach, East Point and Piscadera. In these places all the conchs collected were juveniles and the average and maximum lengths were very similar.

Table 3. Summary of abundance (conchs per basin), average and standard deviation, maximum length and flared lip thickness of the largest specimens found in every site sampled in this study.

Location	Abundance (Approx.)	Av. Length cm	Std dev	Max. Length cm	Lip thickness cm
CARMABI	-	9.38	1.48	11.5	Absent
East Point	-	9.11	0.42	9.4	Absent
Klein Curaçao	-	16.4	3.53	20.2	Absent
Piscadera	-	9.94	0.86	11.3	Absent
Playa Grandi	-	22.76	8.11	28.9	0.5
East basin	250	20.45	3.48	31	1.1
Sea lion basin	70	20.38	6.13	26	0.9
Therapy 1 basin	100	24.54	0.65	27	0.9
Therapy 2 basin	100	20.61	3.91	25.5	0.7
West basin	150	19.02	6.31	33	1.2
Animal encounter	60	23.88	1.65	25	0.8

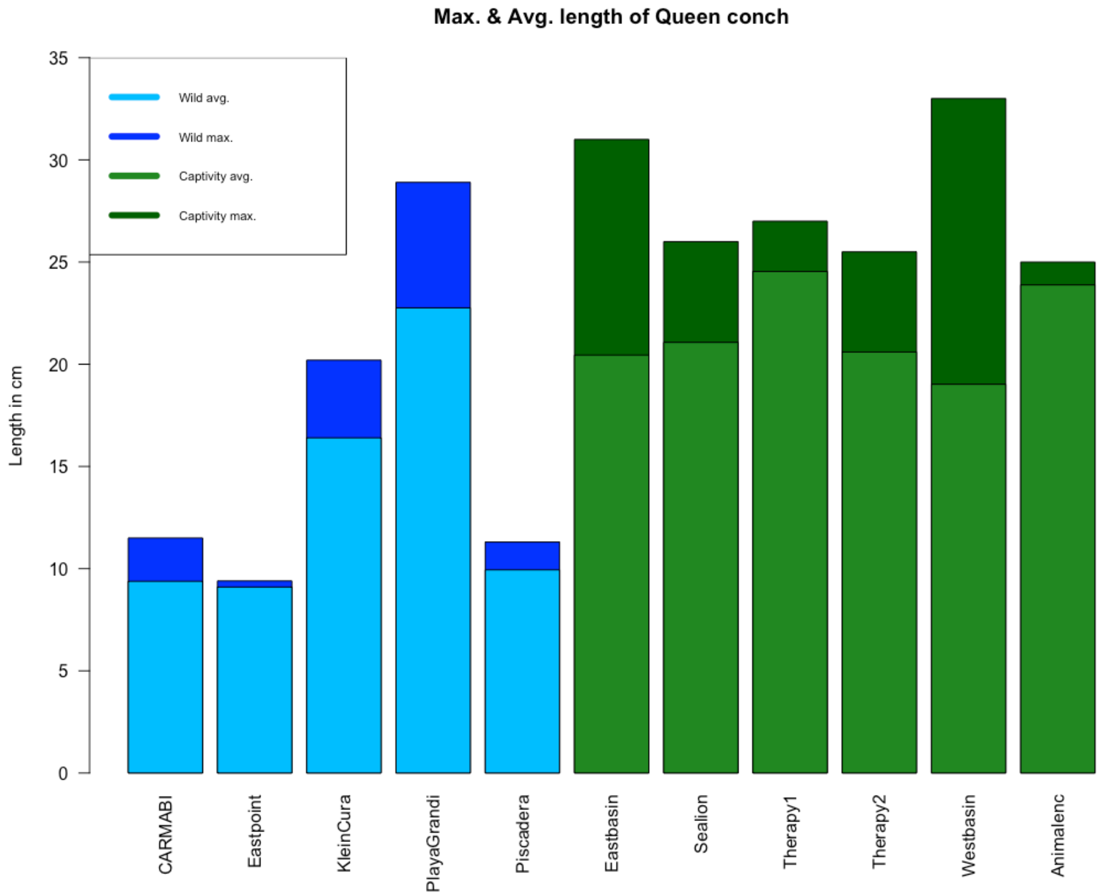

Figure 8. Average length (cm) (lighter blue and green) and maximum length (cm) (dark blue and green) of *Lobatus gigas* for every location. Blue colours are used for wild sites and green colours for Sea Aquarium basins.

4. Discussion

This study was focussed on the diet and maximum length of *Lobatus gigas* in the island of Curaçao. It was an explorative study to identify the main diet of the Queen conch in the wild and in the Sea Aquarium and analyse if there were substantial differences. The results of this study also give an insight in the differences in length of the conchs in the wild and in the Aquarium and the possible explanations for that.

4.1 Diet

In this research we focussed on the variation in stable isotopes of carbon and nitrogen of the Queen Conch and its potential food sources in the wild and the Sea Aquarium of Curaçao. The results showed that the $\delta^{15}N$ of the conchs differed significantly between the wild and the enclosure sites. The values found in Klein Curaçao, Playa Grandi and East Point, the most pristine sampled locations, are the values we could expect in natural environments. Other studies showed that stable isotope values of the Queen conch in the wild should be between 2-4 for $\delta^{15}N$ and 10-15 for $\delta^{13}C$ (Tilley et al. 2013). Our results for the natural places that are not affected by human activities agree with these ranges. On the other two natural places, CARMABI *Research Station* beach and Piscadera, the values of $\delta^{15}N$ were notably high compared to the previous ones. This could be explained by the intense human use of the area. Around the sampled sites there were resorts, restaurants and several apartment complexes as well as the outflow of sewage and waste contaminated water through the outlet of the inland Piscadera Bay. All the waste carries high amounts of ammonia and ammonium that is transformed by bacteria to nitrites and nitrates and taken up by primary producers (Peterson & Fry, 1987). As a consequence, the $\delta^{15}N$ in the algae and the conchs sampled in this area show higher values than at other wild sites.

In the Sea Aquarium the conchs also showed high $\delta^{15}N$ values, especially in the sea lion basin. As in Piscadera Bay, close to the facilities of the Aquarium there are several resorts and restaurants that might have an influence in the nitrogen input in the facilities. Additionally, the sewage of the Sea Aquarium itself discharges nearby the sea lion facility. As we can see in figure 2, the sea lion basin is the closest one to the resorts area and the water that enters this facility comes from that direction. Moreover, depending on the currents, sometimes all the water from the dolphin pools and the animal encounter go through the sea lion basin before discharging into the sea. This water also may carry nutrients and the waste of the other animals that may influence the $\delta^{15}N$ of the organisms in the sea lion facility.

The results of the isotope composition of the algae showed the same pattern as the conchs, having higher values of $\delta^{15}N$ in the Sea Aquarium, CARMABI and Piscadera bay compared to the pristine places. In the wild 6 different species of benthic algae were collected and analysed. In CARMABI, Piscadera and Playa Grandi turf algae was sampled and it was evident that this alga is an important component of the diet of the conchs here. In CARMABI and Piscadera the difference in $\delta^{15}N$ values between the conch and turf algae is ~ 3.4 $^0/_{00}$ and for the carbon isotopes ~ 0.2-0.3 $^0/_{00}$. This suggests that in these two locations turf algae is the main source in the diet of the conchs. Moreover, all the conchs sampled in those places were juveniles and it is known that turf alga is a very important part of the diet of this age group (Pillsbury, 1985; Santaella & Aranda, 1994). In Playa Grandi the difference in $\delta^{15}N$ between the conchs and the turf algae is smaller than 3.4 $^0/_{00}$, and probably there is another source of food with a lower value in nitrogen isotopes that was not sampled. In Klein Curaçao it seems that the main source in the diet of the conchs is *Lyngbya*. This is a genus of cyanobacteria that can fix nitrogen in anaerobic conditions. This is probably the reason for the low values in $\delta^{15}N$ of the conchs in this site. Other studies have also shown that turf and *Lyngbya* are among the most common food sources consumed by the Queen conch in the wild (Lott, 2000).

In the Sea Aquarium, a total of nine different algae were analysed and 5 of them seem to be in the diet of the conchs. In some facilities like the West basin or the Therapy 2 basin the results showed that there are two main compounds in the diet of the conchs, *Lyngbya* and Turf algae comparable to the wild. In the rest of the facilities it seems that the conchs have a more varied diet and they do not feed mainly on one species of alga. The results also suggest that there might be some other items in the diet that were not sampled, as the values of the $\delta^{15}N$ of the algae analysed are significantly lower than 3-4 $^0/_{00}$ in the $\delta^{15}N$ than the values of the Queen conch (Appendix C, Table 7). Moreover, the results of the SPOM, fish and faeces (see Appendix D, figure 9) showed that these items are not directly in the diet of the conch. Nevertheless, the presence of the animals (i.e. dolphins, sea lions and fish of the animal encounter) might be also a reason for the high values in the $\delta^{15}N$ of the algae and therefore they might indirectly influence the composition of the conchs in the Sea Aquarium. The remains of the fish and the faeces are probably assimilated and mineralized by other organisms and inorganic nutrients may end in the algae that the conchs eat. The very high values of the SPOM results could also show the signal of the fish remains and the faeces that might have been in the water sampled.

4.2 Length

The second main goal of this research was to compare the sizes of the conchs in enclosure and in the wild. By looking at the length of the shell, the age of the conchs can be estimated until the flared lip is developed (Brownell & Stevely, 1981).

The basins of the Sea Aquarium showed a high abundance of conchs. The conchs were found dispersed around the basins but also in clusters of 5-10 conchs. This has being described in other studies as a social behaviour of this species (Schweizer & Posada, 2006; Stoner & Lally, 1994). The highest abundances were in the East basin and the

West basin, with estimated amounts of 250 and 150 individuals per basin respectively. Furthermore, the largest sizes and the thickest flared lips were also found in these facilities. In all the basins, the conchs showed sizes of sub-adults and adults with and without flared lip. However, juveniles were very rare and only a few of them were found. This could indicate that the reproduction of the Queen conch in the Sea Aquarium is not successful. There might be various reasons for that, for instance that the egg masses are disturbed and/or eaten by other animals e.g. dolphins in the basins, or that mating is not viable. Nevertheless, there are no studies that analyse this phenomenon.

The average and largest sizes found in the wild were significantly smaller compared to the ones in the Sea Aquarium. In the wild places of CARMABI and East Point, the abundance of conchs was very low. The conchs found in these areas were all juveniles (~1 year old) and the biggest specimen was 11.5cm long. This might indicate that these areas have good conditions for a successful recruitment. Nevertheless, larger empty shells with a hole were found and the presence of these holes indicates that the conchs were poached and thrown back into the sea (Lott, 2000). In these areas, particularly in CARMABI and Piscadera, fishing is an important activity. The target group for this market is the adults with a flared lip and the juveniles are not poached. In Klein Curaçao and Playa Grandi the abundance was higher and sub-adults and adults were also present. In Playa Grandi, specimens with flared lip were found and the largest conch had a similar size compared to the largest individuals in most of the basins of the Aquarium. Moreover, dead shells with thick flared lips (~1-1.5 cm) were found in Playa Grandi. This suggests that the difference in sizes in the wild and the enclosure is mostly related to the poaching in the wild rather than the diet.

The main reason for the large sizes reached in the Sea Aquarium seems to be the lack of poaching and predation. The presence of large specimens in Playa Grandi, where the access is limited and poaching probably restricted, supports this idea. Moreover, it seems that different food sources are similarly attractive to the conchs and the uptake rate could be related to the accessibility of the different species of algae. The presence of the species of algae that are described to be the preferable ones for the Queen conch and the purported high nutrient concentrations in the water of the basins could also influence the growth of the conchs. Nevertheless, due to the length of this study, a causal relationship between growth and diet could not be proven.

5. Conclusion and recommendations

The aim of this study was to get a better understanding of the diet and size distribution of the Queen conch in the island of Curaçao. This study showed that even though there are common species in the diet of the conchs in the wild and in the Sea Aquarium, the food of the Sea Aquarium is enriched in $\delta^{15}N$ compared to the pristine places. This is also reflected in the $\delta^{15}N$ of the conchs. In the basins of the Sea Aquarium there were a higher variety of algae species that are described to be in the diet of the Queen conch compare to the wild sites sampled. Agreeing with this, the results showed that the diet of the conchs of the Sea Aquarium was more varied than the conchs of the wild. These findings suggest that different food sources might be similarly appealing, and that the uptake rate might be related to the availability rather than to possible differences in quality of the food source.

The presence of some specimens of more than 28 cm and thick flared lips in Playa Grandi suggests that the main reason for the success of the conch in the Aquarium is the lack of poaching and predation. Even though specimens of Queen conch were larger and had a different diet in the basins of the Sea Aquarium than the ones found in the wild, a causal relationship between growth and diet could not be established. The contrasting size-frequency distributions, finding mostly adults in the Sea Aquarium and juveniles in the more natural sites, may suggest that the high mortality of Queen conch at places with human activities strongly influences the survival and subsequent the length that the conchs can be reach.

As this research showed and other studies have pointed out, the abundance of adults of *Lobatus gigas* in the wild is very low and restricted to areas where the access to human activities is limited (Stoner et al. 2012). More restoration projects that protect conchs from poachers are needed in order to rebuild the populations in the Caribbean Sea. Aquaculture programs could also help to avoid overfishing of the wild stocks. This research was too short to link the different diets to differences in growth. Nevertheless, more studies that analyse this relationship would be very useful for future mariculture and restoration initiatives.

Acknowledgements:

The focus of this thesis was on the diet and maximum growth of the Queen conch (*Lobatus gigas*) in the island of Curaçao. It was conducted as a part of my Master of Marine Science in Utrecht University and guided by Katja Phillippart and Fleur van Duyl. This project also counted with the help of the research centre of NIOZ and the Treub found (Ministery of Economic Affairs). I would like to thank all the great people that made this project possible:

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Virginia Sánchez, Utrecht 2017

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Appendix A- Description of the studied area

Description of Sea Aquarium facilities

Four of the facilities (Figure 3) contain common bottlenose dolphins (*Tursiops truncatus*). The number of dolphins in each pool is changeable, being the East basin the one with more animals. The dolphins are fed with capelin (*Mallotus villosus)*, herring (*Clupea harengus*, Linnaeus 1758) and mackerel (*Scomber scombrus*, Linnaeus 1758). Different types of fish that are fed with capelin and herring inhabit the Animal encounter basin. The sea lions (*Zalophus californianus*) facility contains 2 sea lions that are mostly fed with herring and capelin as well. A summary of the characteristics of every basin is shown in table 4.

Table 4. General characteristics of the facilities of the Sea Aquarium

Basin	Size basin (m^3)	N of animals	Herring (kg/day)	Mackerel (kg/day)	Capelin (kg/day)
West pool	7909	4 dolphins	6	6	24
East pool	6888	8 dolphins	12	12	48
Therapy 1	9370	5 dolphins	7.5	7.5	30
Therapy 2	2500	3 dolphins	4.5	4.5	18
Sea Lion	2310	2 sea lions	2	0	8
Animal encounter	969	Stingrays, grouper, tarpoons	2.5	2.5	10

<u>West basin</u>

In the west basin most of the water enters from the East and moves westwards to a channel direct to the sea. This water has already passed through other pools so the nutrient content might not be very high. Nevertheless, there are 2 direct entrances of seawater in the south part of the rock barrier that delimits the basin. The centre of the basin is the deepest part (5 meters) and it is mostly covered by sand that it is surrounded by two big patches of turf and green algae.

<u>East basin</u>

In the East basin the water comes mostly through the rock barrier. The currents also bring water from the western basins and from the Therapy areas. This water mixes in the East basin and moves towards the north of the Aquarium. The pool is around 3-4 meters deep with some shallower waters near the fences. The bottom is very sandy with high abundance of green and red algae in the middle of the basin and close to the fences.

Therapy basins

The therapy basins are used for therapeutic purpose and they contain trained dolphins for these activities. Both basins are very similar, situated one next to the other. The water enters through the south and east border in the first one and through the south in the second one. The currents move this water to the northwest to join the East pool and the channel that discharge in the Animal Encounter facility. The first basin is bigger, and deeper (5.5m max depth). The bottom is mostly covered with green and red algae in the borders of the basin and with turf and green algae in the sand patches that cover the middle area. The second pool is smaller with an average depth of 4 meters. It has a large area covered by rocks with red and green algae predominantly and in the middle there is a sand plain with turf algae.

Sea lion basin

The sea lion facility is located in the northwestern part of the Sea Aquarium. The seawater comes from the channel that delimits the Sea Aquarium in the north, goes through the sea lion basin and exits to the sea again. It is one of the shallowest facilities in the sea aquarium with a big rocky area less than one-meter depth.

Animal encounter

The animal encounter basin is a facility that contains different types of fish as a goliath grouper (Epinephelus itajara), tarpons (Megalops atlanticus), permits (*Trachinotus falcatus*), common snooks (*Centropomus undecimalis*), horse eyes jacks (*Carranx latus*) and crevalle jacks (*Caranx hippos*) and stingrays. The animals are fed every day with herring and capelin. The seawater enters from the south and moves northwards to the big channel that surrounds the north part of the Aquarium. The average depth is 3m and the bottom of the basin was rebuilt a few years ago and covered with rocks and big blocks of stones. The predominant algae are red and green species covering the stone blocks under the boat and close to the fences, and turf algae in the shallower areas.

2.1.2 Description of wild sites

Piscadera bay and CARMABI *Research Station* beach

Piscadera bay is an inland bay located at the southern part of Willemstad (capital of Curaçao). The area was traditionally used for fishing, being now and important recreational part of Curaçao, with hotel resorts and restaurants. There are still fishery activities in the area, although it is decreasing over time. The first chosen site was in a small beach at the west side of Piscadera Bay (Figure 9). There is a sand plain before the reef drop starts with different types of algae, being predominant green and turf algae.

The second site was in the sand plain in front of the CARMABI *Research Station* beach (Figure 8). The predominant alga of the site was turf algae, covering all the rocks, sand and wood of the piers.

Figure 9. Location of the 2 sample sites in Piscadera bay. Map from Google Maps.

<u>Playa Grandi</u>

Playa Grandi is a small bay located in the north coast of Curaçao. The surrounding area is used for shooting training of the army and therefore the recreational activities and entrance is restricted. Due to the strong easterly trade winds that generate high waves, wastes and dead algae are washed ashore and accumulate on the bay. The water is very murky, with sandy bottom covered by big patches of different types of algae, being predominant green and red algae. Most of the bay has shallower waters, with an average depth of 2 meters.

<u>East Point</u>

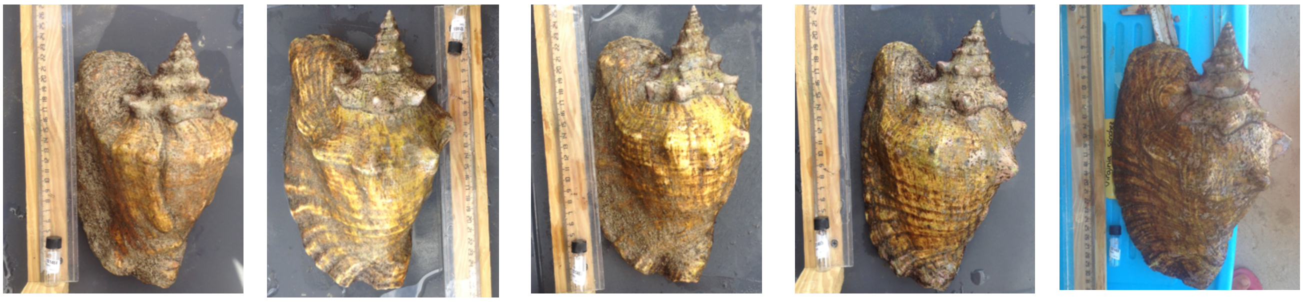
East point refers to the eastern part of the island. A large area of this land is private and therefore the access for tourists or local people is very restricted. Awa di Oostpunt at East Point is situated in the Eastern part of the island. It is a calm basin with crystal clear water where almost any human activities. There is almost no fishery in this area because it is very close to the north coast where the water is too rough to fish. The ecosystem in this part of the island is very pristine and well conserved. There are some areas with long sand plains before the reef drop, and some other areas where the reef starts in the first meters after the shoreline.

<u>Klein Curaçao</u>

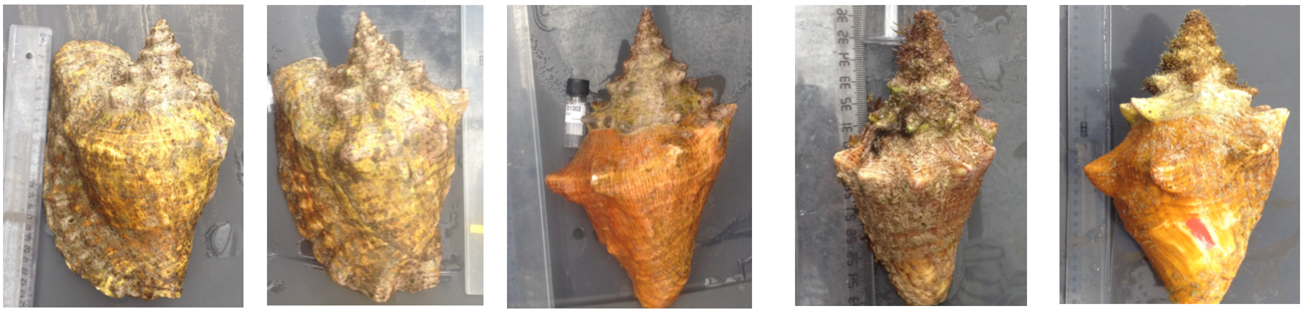
This island, of 1.7 square kilometres, is situated at 28km southeast of the main island. It is uninhabited and the main activity is recreational tourism during the day. The sampling point was located at the west part of the island where large plains of sand are abundant before the reef drop. The plain was 3 meters deep and the bottom was covered with different species of green and brown algae.

Appendix B- Photographic report

A



B



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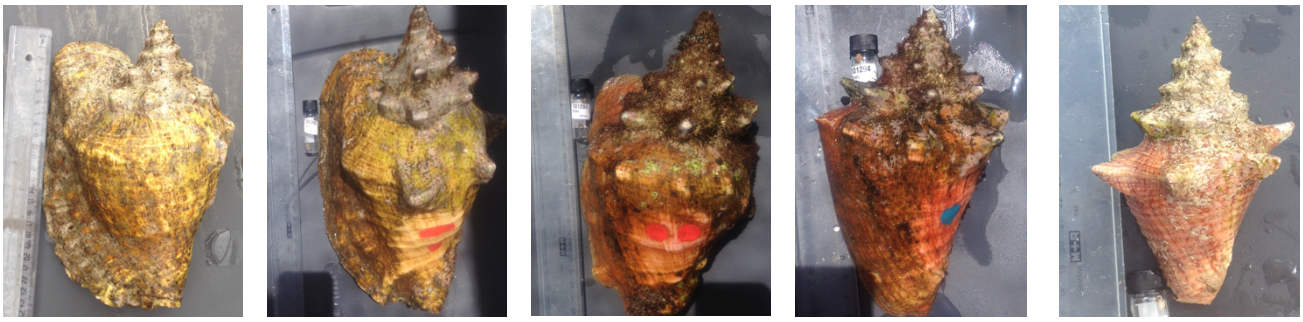

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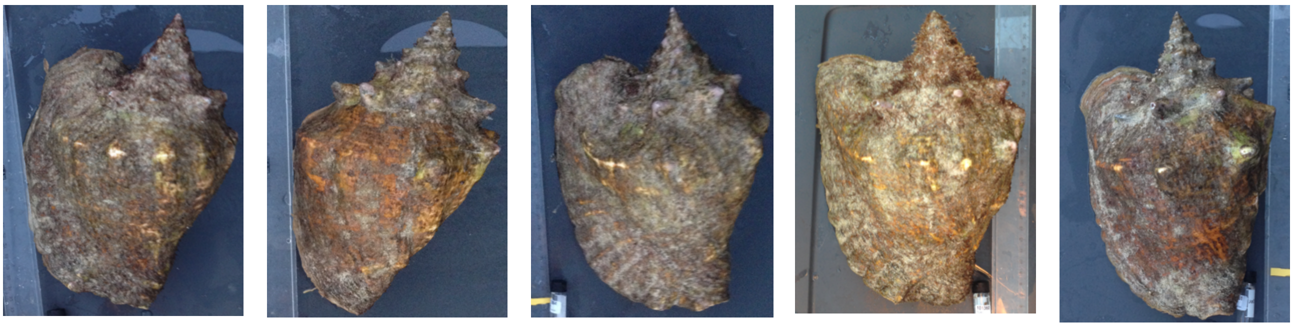


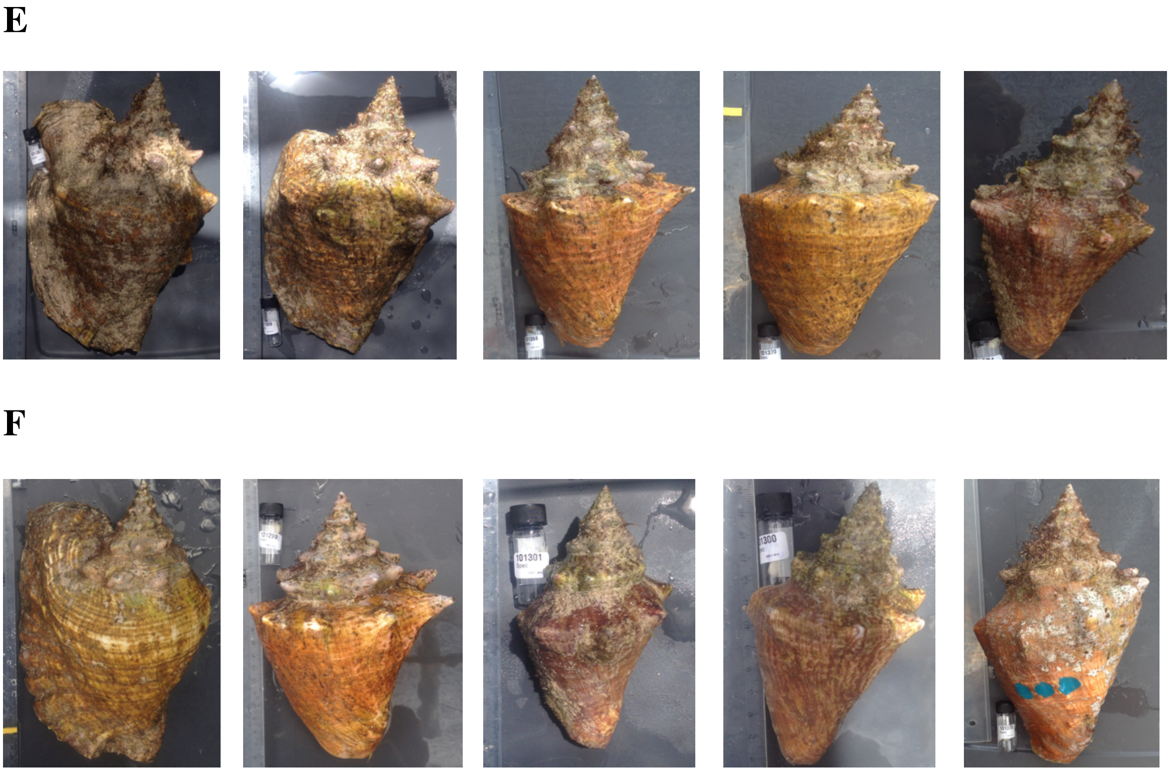
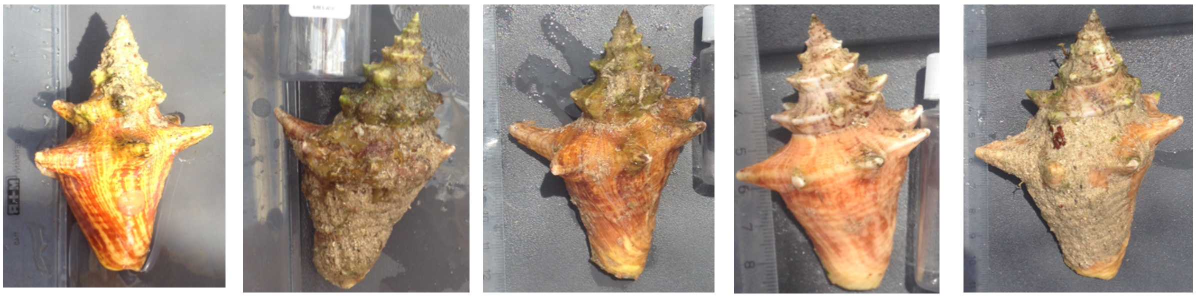


Figure 10. Pictures of the specimens collected in the facilities of the Sea Aquarium of Curaçao: A Animal encounter basin, B East basin, C Sea lion basin, D Therapy 1 basin, E Therapy 2 basin and F West basin.

A



B

C

D

E

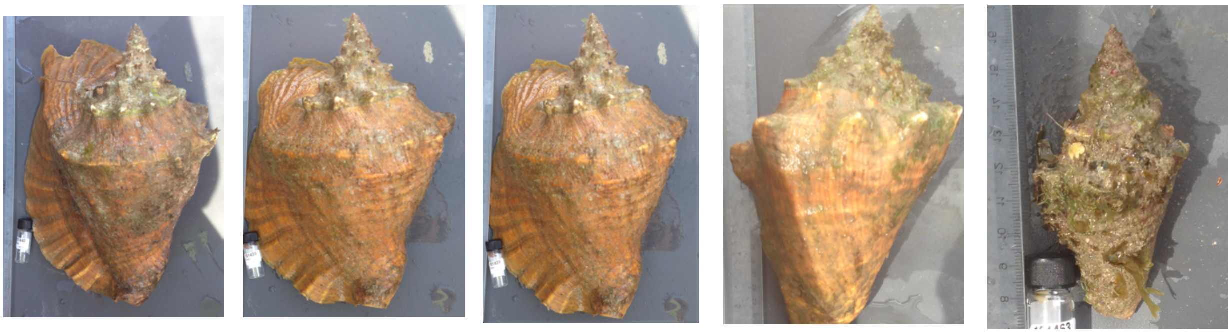


Figure 11. Pictures of the specimens collected in the wild sites sampled: A CARMABI Research Station, B East Point, C Klein Curaçao, D Piscadera and E Playa Grandi.

Appendix C- Additional tables

Table 5. Summary of $\delta^{15}N$ and $\delta^{13}C$ values obtained in the stable isotope analysis for the 60 conchs analysed in this study.

Location	Sample	$\delta^{15}N$	N (%)	$\delta^{13}C$	TOC (%)	Av. $\delta^{15}N$	St dev. $\delta^{15}N$	Av. $\delta^{13}C$	St dev. $\delta^{13}C$
Animal enc.	101464	6.19	13.61	-12.76	41.00	6.56	0.53	-13.53	0.64
Animal enc.	101465	6.76	11.47	-13.96	35.75				
Animal enc.	101466	6.42	12.95	-13.42	41.13				
Animal enc.	101467	6.06	11.38	-13.13	34.80				
Animal enc.	101468	7.38	12.03	-14.37	39.02				
CARMABI	101232	9.38	11.41	-13.18	35.52	8.44	1.35	-14.21	0.82
CARMABI	101235	8.01	10.69	-15.29	34.21				
CARMABI	101265	10.30	10.62	-13.70	35.31				
CARMABI	101266	7.16	11.33	-14.68	35.99				
CARMABI	101267	7.36	10.71	-14.23	35.20				
Dolphin canal	101228	5.64	9.99	-15.24	32.20	5.95	0.44	-15.55	0.43
Dolphin canal	101229	6.26	10.97	-15.85	33.89				
East Point	101233	1.56	11.71	-11.59	37.27	1.64	0.11	-11.30	0.41
East Point	101234	1.72	11.50	-11.01	36.23				
East Point	101256	1.93	8.54	-13.16	28.06				
East pool	101226	5.50	10.11	-14.54	32.94	5.51	0.47	-13.47	0.67
East pool	101227	5.78	9.44	-13.43	30.83				
East pool	101230	4.55	10.29	-12.33	31.95				
East pool	101225	5.65	7.26	-14.15	24.04				
East pool	101231	5.03	7.53	-13.09	25.78				
East pool	101302	5.58	9.62	-13.92	33.72				
East pool	101303	6.22	11.15	-12.85	32.88				
East pool	101304	5.94	7.76	-13.97	27.50				
East pool	101305	5.43	8.49	-13.28	30.65				
East pool	101306	5.46	9.29	-13.15	28.94				
Klein Curaçao	101499	2.47	11.64	-11.16	35.46	2.42	0.27	-11.88	0.51
Klein Curaçao	101500	2.81	10.42	-12.61	34.90				
Klein Curaçao	101501	2.13	11.74	-11.95	37.04				
Klein Curaçao	101502	2.22	10.39	-11.85	34.18				
Klein Curaçao	101503	2.49	10.95	-11.82	35.97				
Piscadera	101372	10.41	8.82	-13.86	28.26	10.54	0.40	-13.84	0.74
Piscadera	101373	9.97	9.99	-15.06	34.43				
Piscadera	101374	11.01	8.02	-13.42	28.66				
Piscadera	101375	10.52	11.24	-13.73	37.14				
Piscadera	101257	10.79	11.60	-13.14	37.02				
Playa Grandi	101439	2.48	10.17	-12.53	32.68	3.35	0.95	-13.60	1.48
Playa Grandi	101440	2.62	12.34	-12.04	36.61				

Playa Grandi	101461	4.86	11.86	-15.85	37.72				
Playa Grandi	101462	3.52	12.61	-13.74	40.36				
Playa Grandi	101463	3.31	11.25	-13.83	38.88				
Sea lion	101293	10.40	10.22	-10.57	30.69	8.92	1.28	-12.09	1.41
Sea lion	101294	8.14	9.69	-12.32	32.96				
Sea lion	101295	8.23	9.98	-13.37	31.42				
Sea lion	101376	4.80	11.11	-14.60	36.96				
Sea lion	101377	5.11	10.07	-14.80	34.33				
Therapy 1	101360	6.16	12.00	-14.09	38.34	5.99	0.16	-13.87	0.51
Therapy 1	101361	5.90	11.50	-13.90	35.58				
Therapy 1	101362	5.87	8.27	-12.99	24.95				
Therapy 1	101363	5.96	8.74	-14.05	27.59				
Therapy 1	101364	6.04	8.66	-14.31	26.81				
Therapy 2	101367	6.05	9.61	-14.32	31.30	5.54	0.35	-13.71	0.80
Therapy 2	101368	5.13	8.77	-13.48	27.68				
Therapy 2	101369	5.69	10.49	-13.15	31.34				
Therapy 2	101370	5.40	10.38	-12.84	31.59				
Therapy 2	101371	5.45	12.52	-14.74	40.49				
West Pool	101296	5.93	11.80	-14.47	37.87	5.20	0.47	-14.08	0.35
West Pool	101299	4.99	11.12	-13.84	37.04				
West Pool	101300	5.00	9.46	-14.00	33.21				
West Pool	101301	4.71	10.19	-14.42	32.99				
West Pool	101378	5.38	12.92	-13.67	39.77				

Table 6. Summary of $\delta^{15}N$ and $\delta^{13}C$ values obtained in the stable isotope analysis for the 3 species of fish, the faeces of the sea lions and the suspended particulate organic matter (SPOM) of the 6 basins of the Sea Aquarium.

Description	Mean $\delta^{15}N$	St dev. $\delta^{15}N$	Mean $\delta^{13}C$	St dev. $\delta^{13}C$
Herring	12.18	0.18	-22.87	0.60
Mackerel	12.05	0.14	-19.65	0.03
Capelin	12.08	0.12	-19.97	0.10
Faeces	14.16	0.50	-21.93	0.25
SPOM Animal enc.	10.27	1.60	-21.19	2.14
SPOM East basin	10.45	0.40	-22.34	0.23
SPOM Sea lion	6.83	1.18	-18.81	2.39
SPOM Therapy1	11.68	1.62	-21.73	1.01
SPOM Therapy2	8.16	0.50	-21.74	0.29
SPOM West basin	7.74	0.48	-24.65	0.84

Table 7. Summary of δ^{15}N and δ^{13}C values obtained in the stable isotope analysis for all the algae collected in the wild and in the Sea Aquarium. Every alga was identified and the means and standard deviations of δ1^{5}N and δ^{13}C were calculated. The difference of δ^{15}N and δ^{13}C of every alga to the values of the conchs were also calculated. The asterisk shows the algae that are more likely to be in the diet of the Queen conch in every site.

	Description	Type algae	Mean δ^{15}N	St dev δ^{15}N	Distance δ^{15} N to conch	Mean δ^{13}C	St dev δ^{13}C	Distanc δ^{13}C to conch
Sea Aquarium								
	Animal enc Algae 1	*Cladophora*	5.86	1.21	- 0.70	-12.82	0.27	-0.69
	Animal enc Algae 2	*Hypnea*	7.29	0.17	+0.73	-10.81	1.93	-2.72
	Animal enc Algae 3	*Cladophora*	5.45	0.49	-1.11	-14.12	0.34	+0.89
*	Animal enc Algae 4	*Turf*	4.57	0.78	-1.99	-15.41	2.79	+1.88
*	East basin Algae 1	*Ceramium byssoideum*	3.17	0.35	-2.34	-14.89	1.13	+1.42
	East basin Algae 2	*Cladophora*	6.00	0.12	+0.49	-12.17	1.65	-1.3
	East basin Algae 3	*Cladophora*	5.19	0.62	-0.32	-12.31	1.95	-1.16
	Sea lion algae1	*Syringodium*	8.59	0.18	-0.33	-14.06	0.48	+2.02
	Sea lion algae2	*Turf*	8.58	0.18	-0.34	-12.74	0.64	+0.7
*	Sea lion algae3	*Ceramium byssoideum*	8.00	0.37	-0.92	-14.65	1.08	+2.61
	Sea lion algae4	*Dictyota*	7.62	1.28	-1.2	-15.11	1.41	+3.07
	Therapy 1 Algae1	*Dictyota*	5.56	0.41	-0.43	-12.98	1.73	-0.89
	Therapy 1 Algae2	*Dictyota*	5.44	0.39	-0.55	-14.46	1.15	+0.59
*	Therapy 1 Algae3	*Cladophora*	4.16	0.46	-1.82	-15.46	1.45	-1.59
	Therapy 1 Algae4	*Turf*	5.13	0.38	-0.86	-10.42	2.49	-3.45
	Therapy 1 Algae5	*Ceramium byssoideum*	5.05	0.33	-0.94	-14.96	1.19	+1.09
	Therapy2 Algae1	*Cladophora*	4.21	1.28	-1.33	-11.32	1.55	-2.39
	Therapy2 Algae2	*Hypnea*	2.76	1.28	-2.78	-9.23	1.55	-4.48
	Therapy2 Algae3	*Dictyota*	3.36	0.29	-2.18	-8.06	0.97	-5.68
*	Therapy2 Algae4	*Lyngbya*	2.16	0.45	-3.38	-13.08	1.31	-0.63
	Therapy2 Algae5	*Ceramium byssoideum*	3.28	0.41	-2.26	-15.95	0.61	+2.24
	West basin Algae1	*Halimeda*	2.12	0.30	-2.99	-12.35	8.29	-1.73
	West basin Algae2	*Hypnea*	4.43	0.46	-0.77	-11.02	1.11	-3.06
	West basin Algae3	*Cladophora*	4.74	0.26	-0.46	-13.40	1.57	-0.68
	West basin Algae4	*Cladophora*	4.30	0.55	-0.9	-12.04	1.65	-2.04
*	West basin Algae5	*Turf*	1.84	0.84	-3.36	-13.84	1.29	-0.24
Wild								
*	Carmabi algae1	*Turf*	5.61	0.37	-2.83	-14.52	1.08	+0.31
	Carmabi algae2	*Dictyota*	11.12	0.45	+2.68	-14.72	0.88	+0.51
	klein Cur algae1	*Thalassia*	2.82	1.00	+0.4	-12.03	2.56	+0.15
	klein Cur algae2	*Turf*	3.93	1.21	+1.51	-13.86	0.89	+1.98
*	klein Cur algae3	*Lyngbya*	0.29	1.00	-2.13	-12.03	2.56	+0.15
*	Piscadera Algae1	*Turf*	6.52	0.41	-4.02	-14.05	1.42	-0.18
	Piscadera Algae2	*Dictyota*	12.12	0.45	+1.58	-18.72	0.88	+4.88
*	P Grandi Algae1	*Turf*	3.04	0.39	-0.4	-11.46	1.51	-2.05
	P Grandi Algae2	*Thalassia*	3.18	0.10	0.24	-16.38	1.05	+2.87
	P Grandi Algae3	*Champia parvula*	3.63	0.22	+0.21	-17.26	0.98	+3.75
	P Grandi Algae4	*Cladophora*	2.76	0.26	-0.66	-19.45	0.71	+5.94
	P Grandi Algae5	*Halophila*	3.29	0.36	-013	-15.11	0.31	+1.6

Table 8: Comparison of $\delta^{13}C$ values of Queen conchs of the different sampled sites. The result is significant at p < 0.05. The treatments used in this analysis are: samples from Sea Aquarium, samples from pristine places in the wild and samples from human-influenced places in the wild.

Summary ANOVA					
Source	**SS**	**df**	**MS**	**F ratio**	**p-value**
Between treatments	149160.199	2	74580.099	F=0.365	0.705
Within treatments	1633492.615	8	204186.577		
Total	1782652.815	10			

Table 9: Comparison of $\delta^{15}N$ values of Queen conchs of the different sampled sites. The result is significant at p < 0.05. The treatments used in this analysis are: samples from Sea Aquarium, samples from pristine places in the wild and samples from human-influenced places in the wild.

Summary ANOVA					
Source	**SS**	**df**	**MS**	**F ratio**	**p-value**
Between treatments	71.23	2	35.61	F=51.67	0.000027
Within treatments	5.51	8	0.69		
Total	76.75	10			

Table 10. Summary of the Tukey test results to analyse between which treatments there is a significant difference. The result is significant at p < 0.05. The treatments used in this analysis are: samples from Sea Aquarium, samples from human-influenced places in the wild (wild 1) and samples from pristine places in the wild (wild 2).

Summary Tukey test			
Treatment pairs	**Turkey HS Q statistics**	**Turkey HSD p-value**	**Turkey HSD interface**
Sea aquarium vs. wild (1)	4.85	0.0217	*p < 0.05
Sea aquarium vs. wild (2)	5.59	0.0103	*p < 0.05
Wild (1) vs. wild (2)	8.67	0.0010	**p < 0.01

Table 11. Description of all the conchs collected during this study. For every conch the length and the lip thickness (when present) was measured, and the sex was identified. Information about the date of sampling and recollection was also noted.

Sample	Location	Date collection	Date sampling	Sex	Length (cm)	Lip	Lip thickness (cm)
101464	Animal enc.	25/10/16	25/10/16	Female	23.4	Yes	0.6
101465	Animal enc.	25/10/16	25/10/16	Female	21.8	Yes	0.6
101466	Animal enc.	25/10/16	25/10/16	Male	24.7	Yes	0.8
101467	Animal enc.	25/10/16	25/10/16	Female	23.3	Yes	0.6
101468	Animal enc.	25/10/16	25/10/16	Male	26.2	Yes	0.4
101232	CARMABI	12/9/16	16/9/16	Unknown	9.6	No	Absent
101235	CARMABI	19/9/16	20/9/16	Unknown	8.6	No	Absent
101265	CARMABI	20/9/16	22/9/16	Unknown	11.5	No	Absent
101266	CARMABI	20/9/16	22/9/16	Unknown	9.7	No	Absent
101267	CARMABI	20/9/16	22/9/16	Unknown	7.5	No	Absent
101228	Dolphin canal	13/9/16	14/9/16	Female	13.2	No	Absent
101229	Dolphin canal	13/9/16	14/9/16	Female	25.3	No	Absent
101233	East Point	14/9/16	16/9/16	Unknown	8.8	No	Absent
101234	East Point	14/9/16	16/9/16	Unknown	9.4	No	Absent
101256	East Point	2/11/16	3/11/16	Female	14.3	No	Absent
101226	East basin	14/9/16	14/9/16	Male	22.9	Yes	1.2
101227	East basin	14/9/16	14/9/16	Female	23.7	Yes	0.6
101230	East basin	14/9/16	14/9/16	Female	19.3	No	Absent
101225	East basin	14/9/16	14/9/16	Male	21.3	No	Absent
101231	East basin	14/9/16	16/9/16	Female	23.7	No	Absent
101302	East basin	27/9/16	27/9/16	Female	19.2	No	Absent
101303	East basin	27/9/16	27/9/16	Male	23.1	Yes	1
101304	East basin	27/9/16	27/9/16	Female	14.4	No	Absent
101305	East basin	27/9/16	27/9/16	Female	14.8	No	Absent
101306	East basin	27/9/16	27/9/16	Female	22.1	No	Absent
101499	Klein Curaçao	12/11/16	14/11/16	Female	13.3	No	Absent
101500	Klein Curaçao	12/11/16	14/11/16	Female	20.1	No	Absent
101501	Klein Curaçao	12/11/16	14/11/16	Female	15.3	No	Absent
101502	Klein Curaçao	12/11/16	15/11/16	Female	13.1	No	Absent
101503	Klein Curaçao	12/11/16	15/11/16	Male	20.2	No	Absent
101372	Piscadera	4/10/16	7/10/16	Unknown	11.3	No	Absent
101373	Piscadera	4/10/16	7/10/16	Unknown	9.9	No	Absent
101374	Piscadera	4/10/16	7/10/16	Unknown	8.9	No	Absent
101375	Piscadera	4/10/16	7/10/16	Unknown	9.9	No	Absent
101257	Piscadera	1/11/16	3/11/16	Unknown	9.7	No	Absent
101439	Playa Grandi	14/10/16	15/10/16	Male	26.1	Yes	0.3
101440	Playa Grandi	14/10/16	15/10/16	Female	28.9	Yes	0.5
101461	Playa Grandi	21/10/16	21/10/16	Female	27.4	Yes	1
101462	Playa Grandi	21/10/16	21/10/16	Female	22.5	No	Absent
101463	Playa Grandi	21/10/16	21/10/16	Unknown	8.9	No	Absent

101293	Sea lion	24/9/16	24/9/16	Male	23.1	No	Absent
101294	Sea lion	24/9/16	24/9/16	Male	15.5	No	Absent
101295	Sea lion	24/9/16	24/9/16	Male	24.6	Yes	0.6
101376	Sea lion	11/10/16	11/10/16	Female	12.3	No	Absent
101377	Sea lion	11/10/16	11/10/16	Female	26.4	Yes	0.5
101360	Therapy 1	5/10/16	6/10/16	Female	24.3	Yes	0.6
101361	Therapy 1	5/10/16	6/10/16	Female	24.2	Yes	0.4
101362	Therapy 1	5/10/16	6/10/16	Male	25.4	Yes	0.4
101363	Therapy 1	5/10/16	6/10/16	Female	23.8	No	Absent
101364	Therapy 1	5/10/16	6/10/16	Female	25	Yes	0.5
101367	Therapy 2	6/10/16	7/10/16	Female	24.5	Yes	0.4
101368	Therapy 2	6/10/16	7/10/16	Female	20.4	No	Absent
101369	Therapy 2	6/10/16	7/10/16	Male	23.3	Yes	0.2
101370	Therapy 2	6/10/16	7/10/16	Female	20.4	No	Absent
101371	Therapy 2	6/10/16	7/10/16	Female	14.4	No	Absent
101296	West basin	27/9/16	27/9/16	Female	26.6	Yes	0.6
101299	West basin	27/9/16	27/9/16	Female	20	No	Absent
101300	West basin	27/9/16	27/9/16	Female	12.8	No	Absent
101301	West basin	27/9/16	27/9/16	Female	12.4	No	Absent
101378	West basin	11/10/16	11/10/16	Female	23.3	No	Absent

Appendix D- Additional graphs

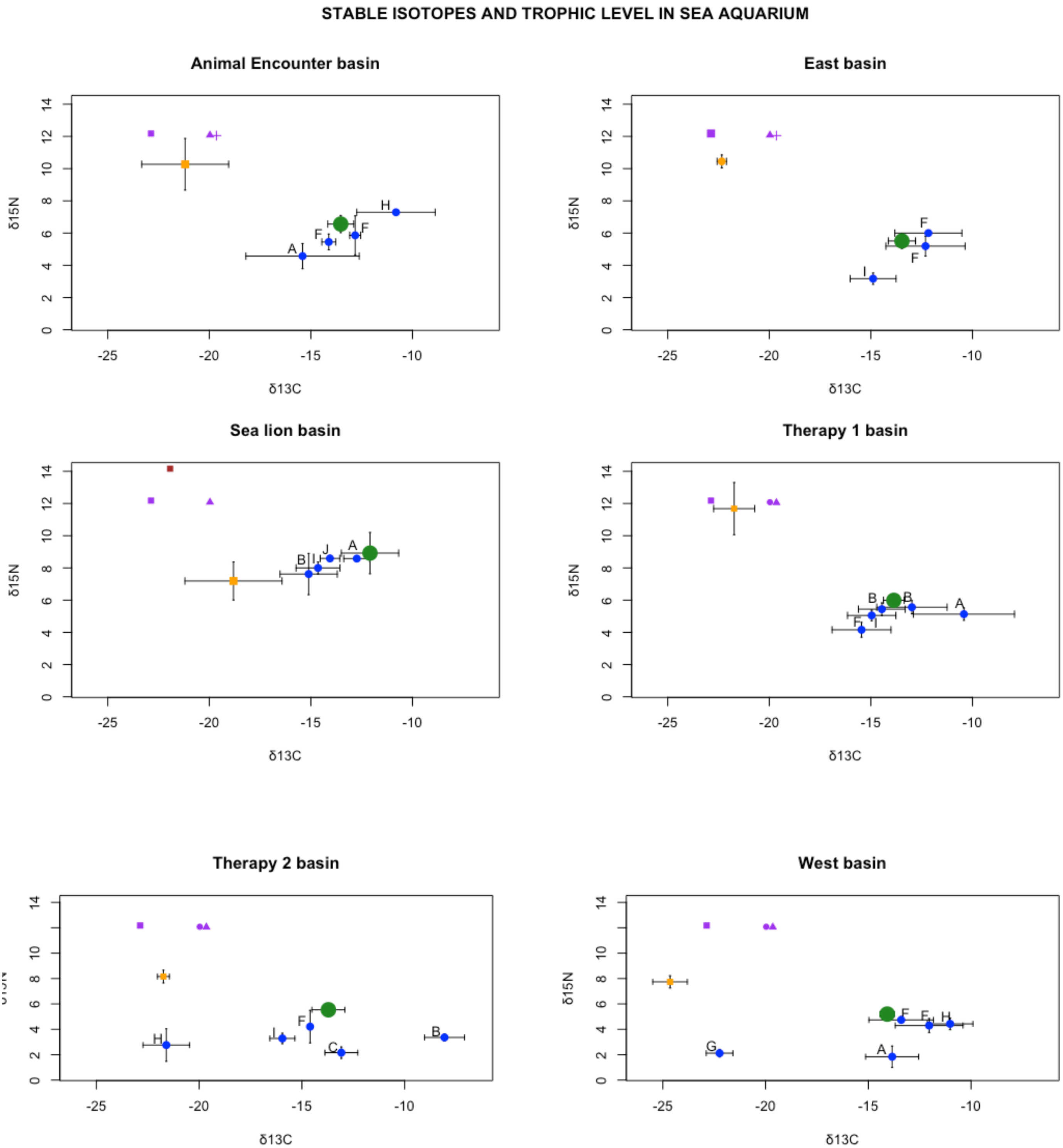


Figure 9. Bivariate plots of means and standard deviations of $\delta^{13}C$ vs. $\delta^{15}N$ values from tissue samples the Queen conchs (green dot), faeces (red square), SPOM (yellow square), fish (purple shapes) and the different types of algae (blue dots) collected in the different basins of the Sea Aquarium. The different algae are assigned with letters: A for Turf algae; B for *Dictyota*; C for *Lyngbya*; D for *Thalassia*; E for *Champia parvua*; F for *Cladophor*a; G for *Halophila;* H for *Hypnea*; I for *Ceramium byssoideum;* and J for *Syringodium*.