Effect of Queen Conch (*Strombus gigas*) on Populations of *Enterococci* sp. accumulated in the Benthonic Surface of the Utila Island Shores, Bay Islands, Honduras

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ABSTRACT

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The queen conch (*Strobus gigas*) is an important comercial product in Honduras. By 1998 the surveys established a population of seven *S. gigas* ha^{-1} in contrast to surveys in the 70's showing populations over a thousand *s.gigas* ha^{-1}. Now a days, the *S. gigas* is considered an endangered species by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and the Honduran Government. The objective of the study is to analyse the capacity of the Queen Conch to filtrate *Enterococci* sp. from the benthonic interface of the Utila Island shores. A second objective of the study is to determine if there is any risk of cross contamination of *Enterococci* sp. during the conch sacrifice that can lead to potential health problems of the consumers. The experiment uses 16 *S. gigas* placed in four plots of 10 m^2 with four conch in each plot. Samples from water and the benthonic interface are taken for nine consecutive days, performing the sacrifice 10 days after the experiment started. All samples are run and quantify with the method of Quimioluminiscence (QL) Defined Substrate Technology (DST) of Enterolet® by IDEXX®, specific for *Enterococci* sp. Results show that there is a reduction of 78.5% of *Enterococci* sp. CFU (Colony-Forming Units) from the first to the eighth day of sampling from the benthonic interface in the plots containing conch, while there is an increase of 1.2% in the plots that contained no conch. At sacrifice, most contaminated viscera samples had above the 700 CFU of *Enterococci* sp. which can lead to the cross contamination of the meat.

Key Word: Bioremidiation, bacterial quantification, water quality indicator, quimioluminiscense, Defined Substatrate Technology, cross contamination.

INTRODUCTION

The Queen Conch (*Strobus gigas*) is the biggest Caribbean gastropod (Hensen 1994) from South Florida to Venezuela (CITES 2004). It is found mostly in marine grasses and the benthonic surface of the reef systems (Hensen 1994). It can live between 40 and 48 years, and it is ready for commercial means above 9 years old (Randall 1964).

In Honduras, the *S. gigas* is an important product for exportation and local consumption (FAO 2006). During the rise of commerce during 1991 to 2003, the country had a 27% increase in the species trading (CITES 2004), while reducing its local population 21.3% annually (Morales 2003). In 1998 surveys established a population of seven *S. gigas* ha^{-1} (Tewfik *et al.* 1998), in contrast, surveys from the 70's estimated populations over 1,000 *S. gigas* ha^{-1}. In 2003 the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) catalogued *S. gigas* as an endangered species in Honduras, prohibiting the exportation of Queen Conch from the country (CITES 2004). In 2006 the Agriculture and Cattle Agency (SAG) emitted a decree prohibiting the capture of Queen Conch (FAO 2006) justifying the non existent of management systems that can be proven sustainable for the prolongation of the species.

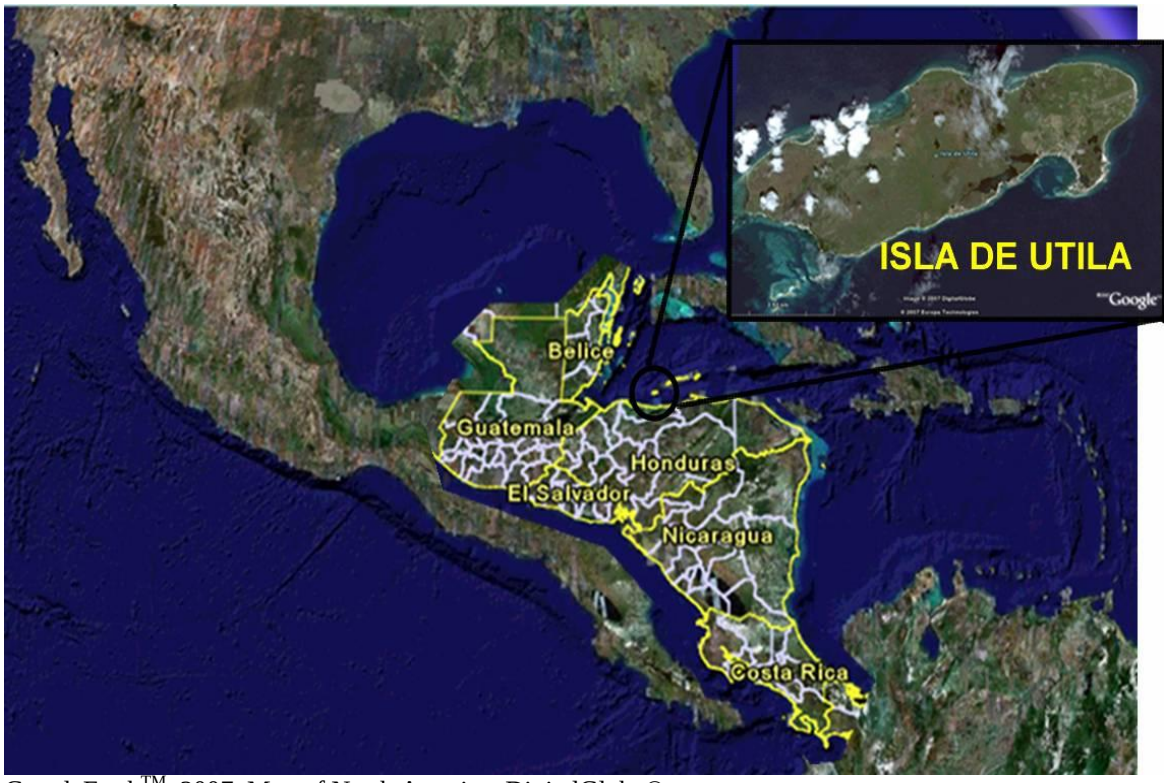
Essentially the *S. gigas* has a great value for marine ecology. It is crucial to regulate the population of microorganisms that cause trophic cascades on reefs and benthos surface as well as prevent mayor ecological changes by filtrating invasive organisms (Stoner *et al.* 1995). It feeds itself from detritus, epiphytes, microalge and microorganisms (Randall 1964; Hensen 1994). It is still uncertain if the *S. gigas* is effective to filtrate human pathogens accumulated on the benthonic surface from beach shores.

The genus *Enterococus* is conformed most by facultative, stable bacteria. Most of the genus is salinity tolerant (Fischetti *et al*. 2000), antibiotic resistant, and highly associated with human diseases from faecal source (Ryan y Ray 2004). For all these characteristic it is used as the official standard indicator for water quality in the public beaches around the World (Jin *et al*. 2004), reason for which it is also used in this experiment.

The objective of the Project is to study the capacity of the *S. gigas* to filtrate and clean the benthonic interface from the shores of Utila Island using the *Enterococcus* genus as the indicator of efficiency. The second objective is to determine if there is any risk of cross contamination that may imply risk for human health when the *S. gigas* is sacrificed.

MATERIALS AND METHODS

Location. The experiment was conducted in Utila Island, Bay Islands, Honduras; during January of 2007. Utila Island is peculiar for its rich marine and terrestrial ecosystems that have been perturbed and modify by men. In the island two spots were chosen to settle the experiment: 1) "Bay House"[1], selected for been a central spot, of high faecal contamination and low water flow; and 2) "Trade Winds[2]", isolated spot, of extremely low contamination and high water flow (Figure 2; Table 1).



GoogleEarth™. 2007. Map of North America. DigitalGlobe©.

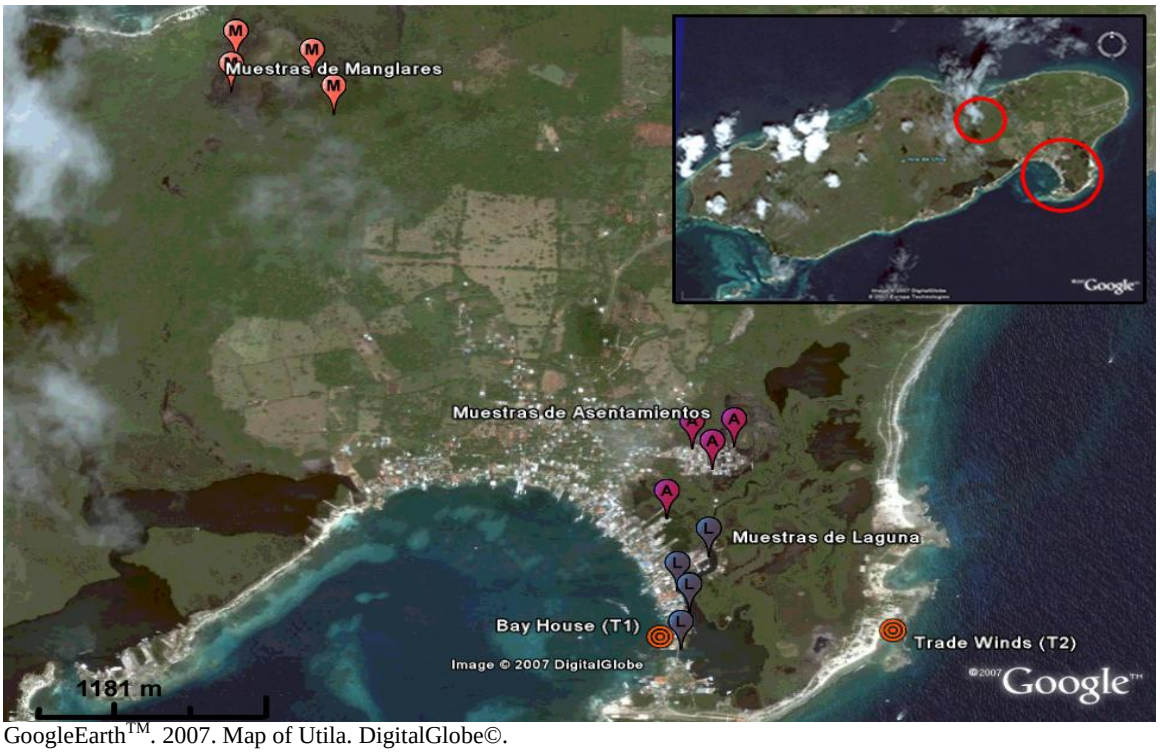
Figure 1. Utila position in Central America.

[1] Depth 1.60 m; wind direction coming from Northeast (NE); water stream ± 63 m h^{-1}; full light penetration.
[2] Depth 1.70 m; wind direction coming from NE; water stream ± 884 m h^{-1}; full light penetration.

Experiment Setting. Sixteen adult *S. gigas* were randomly captured in depths between 15 y 25 m below sea level. Four plots of 10 m^2 where delimited in each site, separated by 6 m from each other. In each site eight conch were placed in two plots (four in each plot), and two plots stood with no conch only effected by the environment. The conch were stringed to a concrete block tightened with fishing line of 1.78 m long. The two variables measured during the experiment were 1) contamination in water; and 2) contamination in the benthonic interface.

The treatments were labelled according to the site they were sat in. Treatment one (T1) was established in "Bay House" which was located in a centric house of the Utila Bay; particular for the great loads of contamination and presence of bacteria. Treatment two (T2) was established at "Trade Winds" which was a non-contaminated and isolated spot in Utila (Figure 2).

For additional information, random samples were taken in three different ecosystems non-related to the treatments. Since the water stream runs through these sites into the Utila Bay, taking samples of these places gave a broad view of the contamination stream flowing into the sea. The ecosystems sampled were: 1) The mangrove system; 2) The centric lagoon by which most of the population has settled; and 3) The marginal living ghettoes that have been built upon destroyed mangroves (Figure 8). Eight samples were taken in each site; four from the water and four from the benthonic interface.



GoogleEarth™. 2007. Map of Utila. DigitalGlobe©.

Figure 2. Sampling sites in Utila Island, Honduras.

Field Sampling. The water samples were taken with a 100 mL sterile flask, filling it completely 1 m beneath the water surface. The samples from the benthonic interface were

recollected burying a 50 mL flask 4 cm into the interface while letting 10 mL of water in. Four random recollections were done for each field sampling in order to have one representative lab sample of each treatment. Sampling was done every 24 hours for nine consecutive days.

Table 1. Description of the treatment used for the experiment with *S. gigas* in Utila Island, Honduras.

Tratamientos	Parcelas	Descripción
	T1	Contaminated with conch (1)
"Bay House"†	$T1_A$	Contaminated with conch (2)
(Contaminated)	C_{T1}	Contaminated / no conch (1)
	C_{T1A}	Contaminated / no conch (2)
	T2	Non contaminated with conch (1)
"Trade Winds"††	$T2_A$	Non contaminated with conch (2)
(Non Contaminated)	C_{T2}	Non contaminated / no conch (1)
	C_{T2A}	Non contaminated / no conch (2)

† N16°05'27.44", W86°53'30.28"

†† N16°05'39.05", W86°52'56.59"

Lab Samples and Quantification of *Enterococci* sp. For the water samples, 2.5 mL of water were taken from the each of the 4 subsamples recollected in the field and placed into a 100 mL IDEXX® sterile flask. The sample was then measured up with distilled water for a final dilution of 1:10. For the samples from the benthonic interface, subsamples were shaken for a minute so the bacteria could be suspended in water. After the solids precipitated for a few seconds, 2.5 mL were taken from the water substrate of each subsample. The procedure followed as described for the water samples.

Six *S. giga* were selected to be sacrificed for the viscera samples. Two conch were randomly chosen for each site and killed 10 days after the experiment started, and two were sacrificed one day after been caught in their natural habitat. A traditional sacrificed was performed; a fissure was made in the third growing line on the shell of the conch and a knife was introduced through the fissure to push the conch out. Using a sterilized scalpel, 3 subsamples of 2 × 2 × 1 cm were randomly cut off from each conch and blended together in low revolutions with 100 mL of distilled water for 30 seconds. Samples were not moved for 10 minutes for solids to precipitate. The procedure followed as described for the water samples.

A standard unit of Enterolet® Defined Substrate Technology® (DST) by IDEXX® was added to each flask that contained a diluted sample. It was shaken until completely diluted. Each sample was poured into a quantifiable trade by IDEXX® of 48 × 48 cells with ± 0.05 of accuracy.

Quimioluminiscence (QL) was used for the quantification of specific *Enterococci* spp. The counting was done under ultraviolet 350 nm (UV_{350}) light (IDEXX 2001). This method is based on the detection of photons emitted as a metabolic REDOX reaction to a highly concentrated glucose substrate (Philippe *et al.* 2005). Estimation was done using the Most Probable Number (NMP) curve standardized for bacteria quantification.

Experimental Design. The experiment used an analysis of variance ANOVA ($P \leq 0.05$) in factorial arrangement for repeated measures in time; a One Way ANOVA ($P \leq 0.05$) to compare between treatments and repetitions; and regression graphs elaborated to compare the tendencies of the treatments. All the Analysis were run in MINITAB® 15 (2006).

Results were given based on geometric means (Table 2) for bacteria quantification had no normal tendency and the effect of the accumulation of bacteria population over time (Spencer y Chan 1997; Philippe *et al.* 2005) had to be estimated.

RESULTS

Efect of *Strombus gigas* on the levels of *Enterococci* sp. The contamination mean from the benthonic interface of the contaminated site "Bay House" was 100 times greater ($P \leq 0.005$) than in the non-contaminated site "Trade Winds". This reflects the contamination pressure placed upon the highly populated centric spots in Utila.

Table 2. Means and results of the contaminated and non-contaminated treatments after the effect of *Strombus gigas* in the population of *Enterococci* sp. Utila, Honduras.

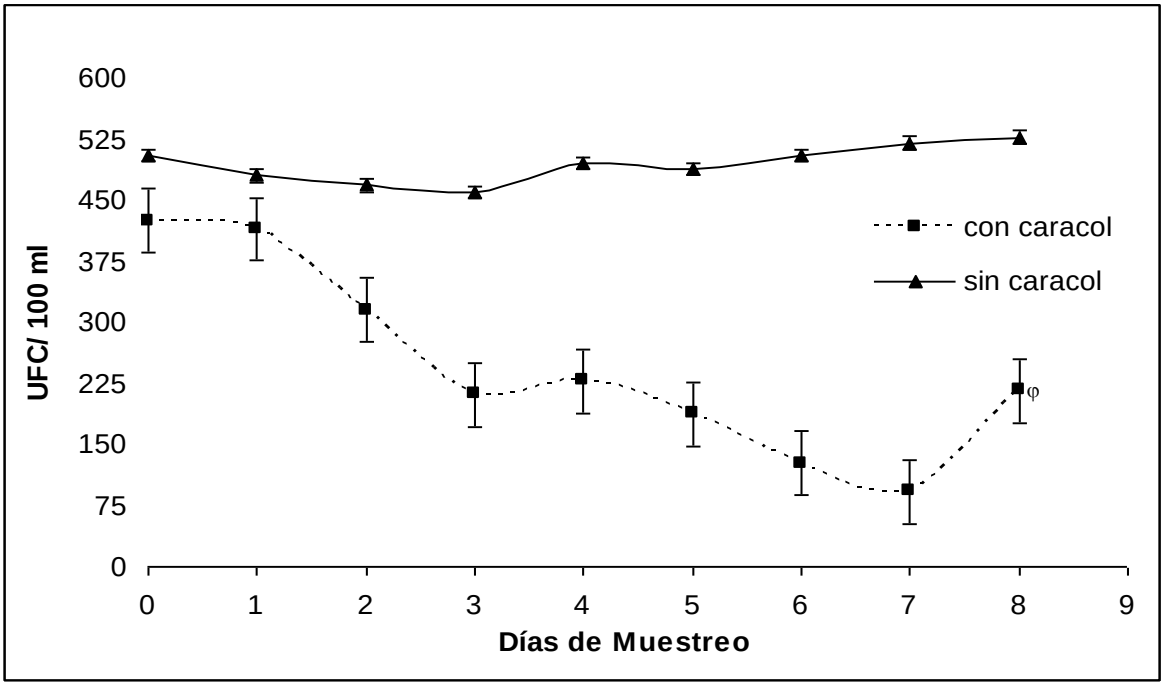
Site	Treatment	Initial Value	Final Value	Geometric Mean	CFU Variations over Time
		CFU[Ω]	CFU	CFU	%
Bay House	Interface with conch	471.3	100.1	>234.6	-78.5**
Contaminated	Interface / no conch	504.0	520.0	>493.5	1.2
	Water with conch	104.7	37.5	>50.4	-32.0[Φ]
	Water / no conch	111.3	81.2	>91.6	-27.0
Trade Winds	Interface with conch	0	0	>4.4	0
non contaminated	Interface / no conch	0	0	>3.3	0
	Water with conch	0	0	>1.1	0
	Water / no conch	0	0	>2.2	0

[Ω] Colony-forming Units [Φ] Non significant (P = 0.084) ** Highly significant (P<0.005)

A significant difference ($P<0.005$) was obtained by comparing treatment with *S. gigas* versus treatments with no *S. gigas* (control) in the contaminated benthonic interface of "Bay House" (Figure 3). In the first eight days of the experiment the plot with conch had a reduction of 78.5% in the population of *Enterococci* sp., compared to an increase of 1.2% in plot with no conch in the same period of time. Data demonstrates that the eight *S. gigas* were effective to reduce contamination of human pathogens (Figure 3). However, when a storm broke into Utila in the eighth and nineth day of the experiment, the benthonic interface was disrupted by the sudden change of the stream, causing the displacement of the microorganisms which lead to an increase of the population of *Enterococci* in the plots sampled.

The effect of *S. gigas* in the contaminated water of "Bay House" was not significant (P =0.084) (Figure 4) since a higher ecological balance is needed to achieve an impact upon populations of organisms in the water column. A closely significant statistical value in the experiment (P=0.084) suggests that a greater population of *S. gigas* placed in the site for a longer period of time could become significant for the overall marine ecosystem.

Results obtained from the non-contaminated site "Trade Winds" showed no difference between the first and last sampling day, resulting in 0 CFU accumulations (Figure 5). Generally all the plots, with or without conch, had no variation. Four samples showed low CFU counting (<50 CFU), non significant for the experiment, due to the randomness of the sampling and the generic quantification of the method.



φ Data affected by storm

Figure 3. Variation of *Enterococci* sp. population during the experiment with *Strombus gigas* in the contaminated benthonic interface from Bay House, Utila Island.

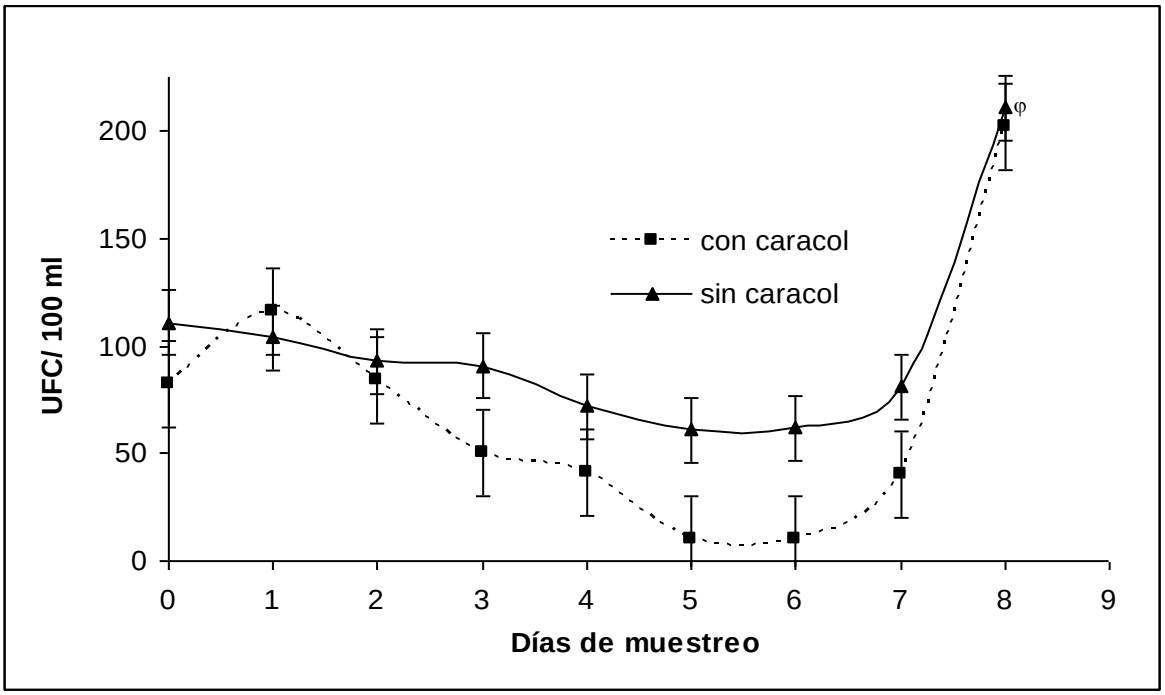


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Figure 4. Variaton of *Enterococci* sp. population during the experiment with *Strombus gigas* in the contaminated water from Bay House, Utila Island, Honduras.

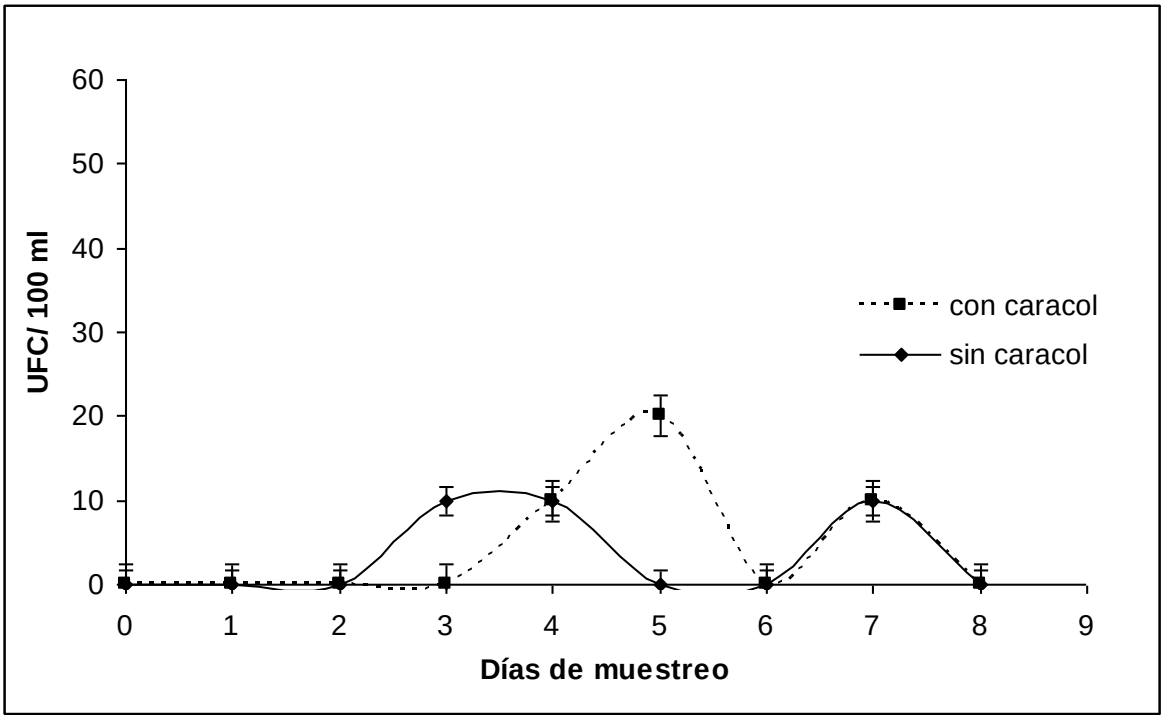
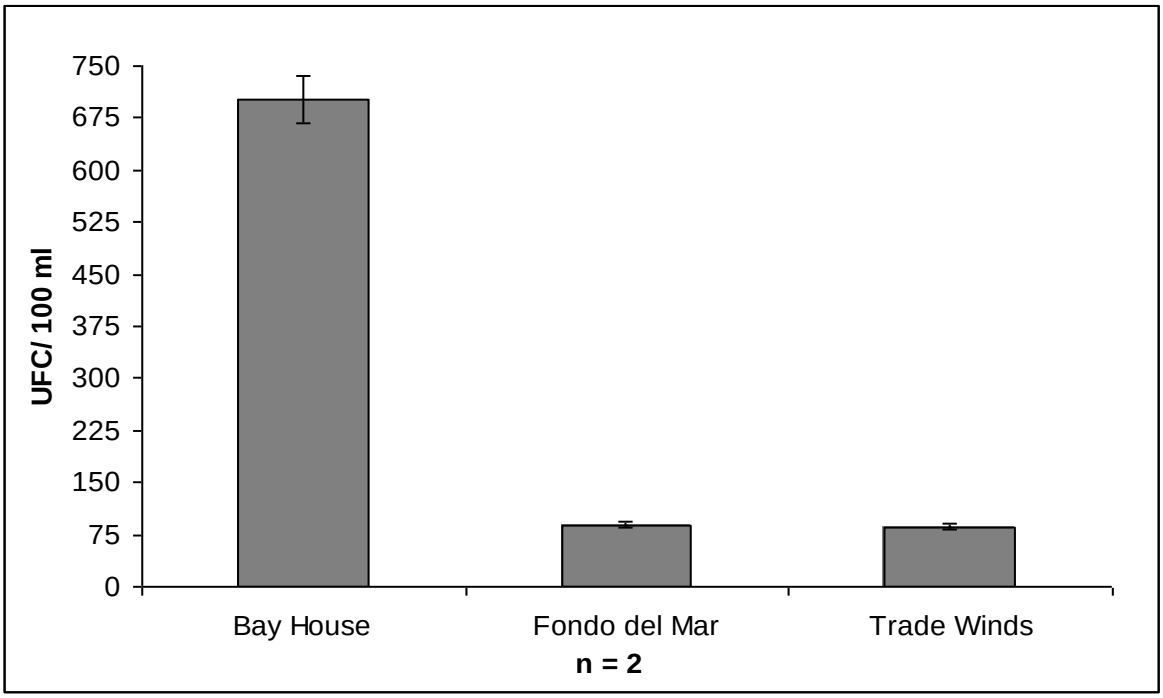


Figure 5. Variaton of *Enterococci* sp. population during the experiment with *Strombus gigas* in the non-contaminated benthonic interface from Trade Winds, Utila Island.

Quantification of *Enterococci* spp. in *Strombus gigas* viscera. For ethical reasons only six *S. gigas* were sacrificed for the experiment so no statistical analysis was run. However, results suggest a relation between the accumulation of *Enterococci* and the sites where the conch were recollected. Conch coming from a non-contaminated site ("Trade Winds") had 88 CFU quantified (Figure 6), similar to the viscera from the conch subjected to no-treatment (control) coming from a deep natural habitat. Quantification of *Enterococci* sp. in the viscera coming from a contaminated site ("Bay House") was over 700 CFU (Figure 6). It is suggested that high loads of faecal contamination in Utila Bay resulted in an accumulation of *Enterococci* CFU in the conch viscera.

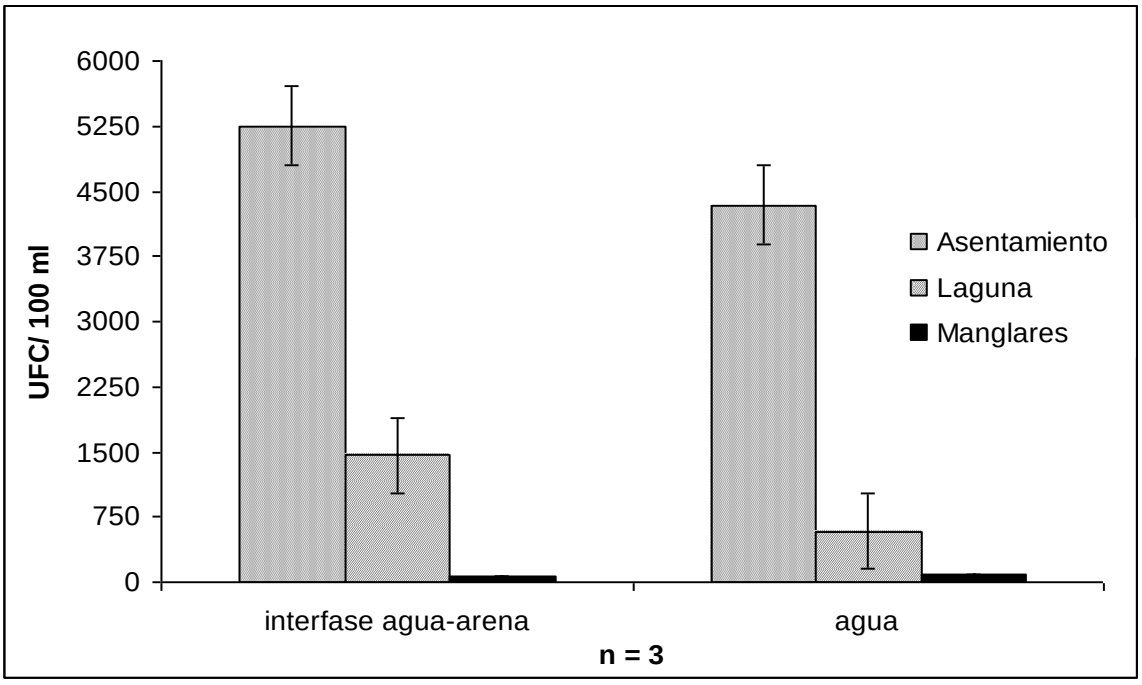
Attempts to quantify *Enterococci* sp. with QL in the dilutions made from the *S. gigas* tissue were inaccurate since white muscle particlesin the substrate had a reflection effect under UV light. It is not discarded that traditional forms of sacrifice may induce potential cross contamination from the viscera to the conch tissue.



n = number of conch to used to determine the mean.

Figure 6. Levels of *Enterococci* sp. in the viscera of conch from the contaminated treatment ("Bay House"), non-contaminated treatment ("Trade Winds") and the Control.

Source of Contamination. The biggest loads of contamination come from two main sources: 1) Buildings and establishments that pour their faecal and organic waste directly into the sea; and 2) The fresh water system that coveys and evacuates the contamination from the whole island. Within the water system, poor marginal ghettoes that have been built upon destroyed mangroves represent focal points of contamination with 4,338 *Enterococci* sp. CFU (Figure 6 and 7). As a contrast the quantification in the non perturbed mangroves was of 83 FCU (figura 7).



n = Number of simples recollected for each ecosystem.

Figure 7. Levels of *Enterococci* sp. in 3 ecosystems of the water system in Utila Island, Honduras.

Figure 8. Destruction of the mangroves to built living facilities in Utila Island, Honduras

CONCLUSIONS

- Quimioluminiscence is not useful to evaluate samples containing refracting particles (such as those from the meat of *S. gigas*) since the quantification under UV light becomes inaccurate.

- The *S. gigas* is capable of reducing populations of *Enterococci* sp. in a limited area of the benthonic interface. A higher ecological balance is needed to achieve an impact upon populations of organisms in the water column.

- The *S. gigas* did not have a significant effect on the bacteria content of the water samples.

- It was proven that the *S. gigas* carries the bacteria *Enterococci* sp. in the visceras which can lead to the contamination of the meat.

- The data suggests that there is a positive relation between the amount of pathogens found in the conch viscera and the contamination of the environment from which it was collected.

RECOMMENDATION

- Establish the experiment in an aquarium with controlled conditions. Complement it with the bacterial analysis of the digestive system so conclusions can be made about the *S. gigas's* capacity of degrading *Enterococci* sp.

- Alert about the consumption of *S. gigas* since it has been establish that is an important agent of contamination reduction as well as a potential hazard to health because of pathogenic bacteria accumulation.

- Publish documents about the problems of contamination and illegal fishery in Utila so the population of the Island can be aware about the danger of human and marine pathogens accumulating in centric spots of the island.

- Establishing education program to raise a culture of knowledge about the importance of species that can control contamination and prevent disease outbreaks in the island.

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