Chemistry and Preliminary Coliform Microbial Communities in Laguna Bacalar, Yucatan Peninsula, Mexico.

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Introduction

It may come as a surprise that there is very little scientific literature pertaining to Mexico's second largest lake—Laguna Bacalar. In the state of Quintana Roo, the 56 kilometer long lake is nestled in the jungles of the eastern Yucatan Peninsula (figure 1), its southernmost tip just over 13 km from the city of Chetumal. It is one of the world's most unique freshwater lakes in that it takes on the visual appearance of a marine system—the range of intense turquoise blues seen in its clear waters have earned it the nickname "Laguna de Siete Colores", or "Lake of Seven Colors" (figure 2). There are also several marine species that have adapted to survive Laguna Bacalar, including mangroves (figure 3), stromatolites, tarpon, bonefish, and skate (Lagunabacalarinstitute.com/ Research). The laguna also harbors the many cichlid species, small livebearers, and Cayman that inhabit other freshwater bodies in the Yucatan ("Poet3 Fauna" pdf, figure 4).

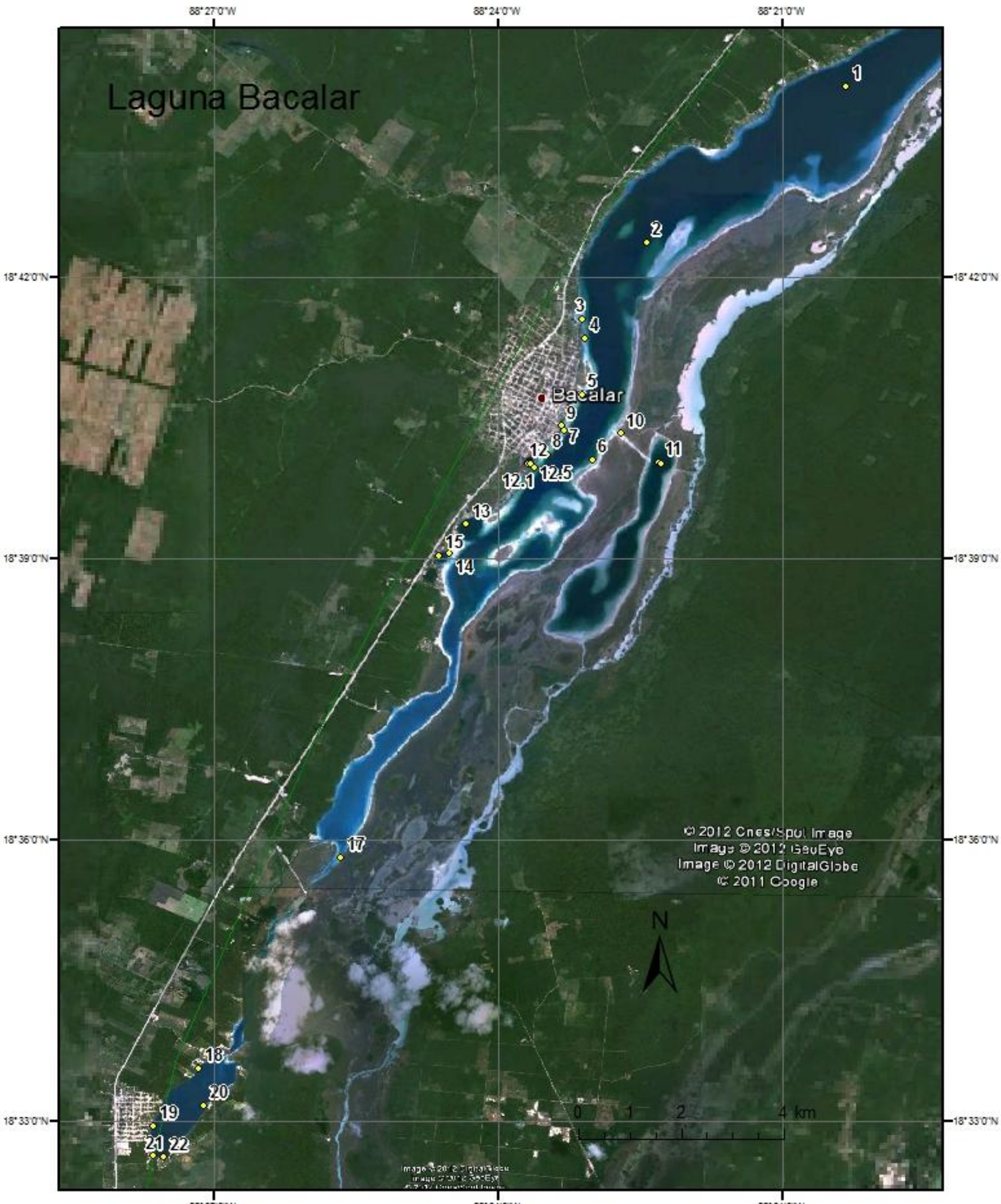


Figure 1: Overview of Laguna Bacalar indicating sample locations (yellow circles) with station numbers. Station 12, Black Cenote, was given points of 12.1 and 12.5 to indicate various locations within the cenote, but all are considered Station 12.

One major property of the Laguna Bacalar ecosystem that makes it unique is its geology. The cavernous subterranean realm of the Yucatan Peninsula is composed primarily of porous karst limestone, allowing for the existence of the world's largest freshwater lens system. A freshwater lens exists when seawater from a marine coastline infiltrates groundwater. The denser, saline seawater sits below less dense freshwater; the lens is the barrier where the two meet (Moore et al 1992). The Yucatan is dotted with entrances to this underground cave system—the cenotes. Effectively large freshwater wells, the ancient Maya believed the cenotes to be the gates to their underworld, and several exist in and around Laguna Bacalar (figure 5).

Figure 2 (above left)**:** Western shore aerial photo—illustrates the lake's "7 colors." (*www.lagunabacalar.com*)

Figure 3 (above)**:** Freshwater mangrove in southern Laguna Bacalar.

Figure 4 (left)**:** Cichlid species common in Laguna Bacalar (*http://www.belowwater.com/blog/mexico-laguna-bacalar-yucatan*)

The chemical properties of this body of water also make it relatively unique. Laguna Bacalar is considered a *marl* lake. The term marl refers to a mud consisting chiefly of calcium carbonate (the substance must contain at least 35% carbonate to be considered marl), and also including various amounts of clay and aragonite (Pettijohn 1957). The marl on the floor of Laguna Bacalar is very fine in particulate size, and the lake's usually clear, oligotrophic waters can easily become clouded by storms or heavy boat traffic.

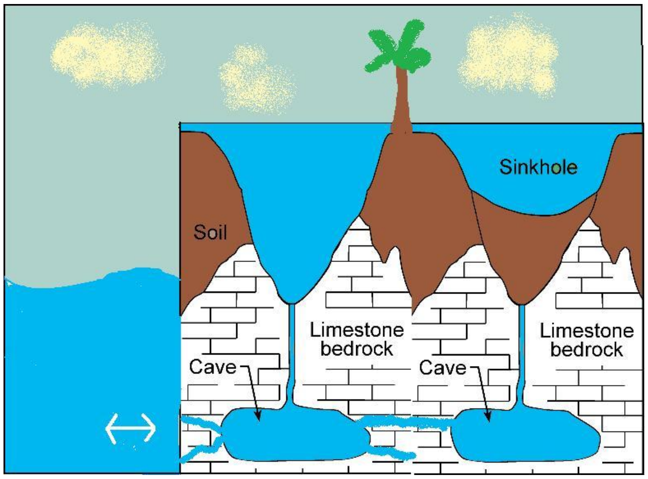


Figure 5: The formation of cenotes (sinkholes) into caves and resulting subterranean flow describes the groundwater system of Laguna Bacalar.

Little is known about the hydrology of Laguna Bacalar, and its bathymetry has never been systematically mapped. Its depths vary greatly from upwards of forty meters in the cenotes to just a meter or two in other areas. There is also great variation in its width, from over two kilometers wide in several spots in the north, down to just a few meters in the region known as "the rapids" (figure 1- area between sites 17 and 18*)*. In the gently flowing current of the rapids, one can find some of the most accessible and largest concentrations of possibly the *most* unique feature in the laguna—the stromatolites.

Figure 6: Stromatolites on the Western shore of Laguna Bacalar.

Figure 7: Image of a submerged stromatolite in the Rapids Region of Laguna Bacalar.

The Holocene giant microbialite structures known as clouted stromatolites (thrombolites) (figures 6 and 7) are representative of some of the oldest fossil forms of life on Earth. Structurally, they consist of layers of microbial organisms, with colonies of those more photo-dependant (i.e., Cyanobacteria) closest to the surface (Gischler et al 2008). They dominated in the Precambrian and date back to roughly 3.5 billion years in the fossil record (Awramik 1971) (their decline may have begun with the increase in grazing animals brought on by the Cambrian substrate revolution (Allwood et al 2009)). Living stromatolites, like those in Laguna Bacalar, which date back to the Holocene (Gischler et al 2008), are rare today and exist only in a handful of locations around the globe. Most commonly, they are found in hypersaline lagoons (as in the famous example at Shark Bay in Western Australia), thus making those found the freshwater of the laguna even rarer. Laguna Bacalar also harbors some of the largest living stromatolites on the planet (Gischler et al 2008).

The study of the Laguna Bacalar system is still in its early stages. The aim of our study is to provide a base of information in regard to the physical, chemical, biological, and ecological properties of this body of water. More specifically, our objectives and methods are outlined in the following sections. From its deep cavernous cenotes, to its unusual chemistry, to rare examples of primitive life on Earth in the stromatolites, to its stunning crystal clear and turquoise waters, it is undeniable that Laguna Bacalar is an extremely unique feature in need of both research and protection. It is our hope that this piece of literature contributes to a growing body of knowledge centered on the ecosystem of this world class lake.

Objectives

1) Obtain basic water column data by performing water surveys at both the surface and depth.   Parameters of interest include dissolved oxygen (DO), specific conductivity, temperature, and pH and secchi depth. These parameters were chosen based upon their ease of measurement using a conventional water quality sonde equipped with the appropriate sensors
2) Conduct bacterial sampling and analyze for various bacteria, including *E. coli*, Coliform, and other bacteria using standard water quality assays.
3) Estimate black-striped mussel (*Mytilopsis sallei)* population densities associated with the giant stromatolites.
4) Track navigational waypoints near the City of Bacalar, cenotes, deep basins within the lake and other areas of interest in Laguna Bacalar basins.

Methods

<u>Station locations:</u>

GPS was used in navigating throughout Laguna Bacalar in Quintana Roo, Mexico. The GPS device allowed us to mark the waypoints when collecting our data for water samples, black-striped mussel counts, and basic water column data. After the data were collected, the positions were plotted on Google Earth and in ArcGIS (figure 1).

<u>Multiparameter Sonde Data:</u>

To obtain critical data from the lake, our team used the YSI 6600 V2-4 Multi Parameter Sonde. We recorded the data using two different techniques, depth profiles and transects. To create depth profiles, we chose locations around the lake, for example specific targets such as the submarine cenotes and what we believe are natural springs in the lake. A profile consisted of specific measurements that the sonde took every two seconds from the surface to the bottom of the lake. Furthermore, to begin a profile the sonde first had to be calibrated and the sensors cleaned by the integrated wiper system. Next, while one person lowered the unit in to the lake the other could view the data on the hand held output device. The sonde was equipped with sensors for temperature, pressure (depth), pH, and optical measurements of dissolved oxygen, turbidity, chlorophyll and phycocyanin fluorescence. In addition, we used the sonde to obtain data by making transects in certain sections of the lake as well. Creating a transect was similar to creating a depth profile, however in creating a transect the sonde was held just under the surface of the water while the boat was moving at a speed just above idle. Additionally, one 24-hour diel deployment of the sonde was completed nearshore to Ecotucan (18.73156 N, 88.3518 W). At the end of each day the raw data was compiled in Excel (attached Appendix 1, Table 1) and plotted in Sigma Plot.

The oxygen sensor was calibrated at the beginning of each sampling day and pH was checked at the completion of sampling and the sensor was calibrated. The other sensors typically have negligible drift over a short sampling period, such as this. Turbidity, chlorophyll α and phycocyanin fluorescence are not reported here.

<u>Bacterial Assays:</u>

Water samples were collected at various depths (surface, 1, 5, 10, 20 and maximum water depth), depending on the sites, in a one liter Van Dorn style water bottle sampling device. The depths included the surface, one meter, five meters, ten meters, and twenty meters. Approximately ten milliliters of water was extracted using a plastic syringe, after rinsing 3 times, labeled, and stored in the dark until they were ready to be cultured. For culturing, one and/or five milliliter sub-samples were taken from the syringe, mixed with five milliliters of Coliscan EasyGel (from MicrologyLabs.com) agar and plated on petri dishes to check for coliforms, and *E.coli.* The bacterial samples were analyzed using the procedure outlined by *MicrologyLabs* (attached Appendix 1, Table 2). The blue/purple colonies are *E.coli*, while the pink/red colonies are other coliforms.

During the bacteriological sample incubation the temperature control on the incubator became inoperable. Therefore, the majority of all samples were incubated at outdoor temperature, which ranged from 20 to 30˚C. Samples were incubated at outside temperature for 48 hours, and were checked intermittently at 24 and 36 hours. While these temperatures still allowed growth, this occurred at varying temperatures which may have affected colonization. Additionally, no known, standard sample was plated for comparison with the field samples. Another source of error may have been inconsistent readings on the plates; multiple individuals made readings at various periods.

<u>Mussel Densities:</u>

Black-striped mussel densities were estimated via counting the number of individual mussels observed in a known area. Six individual snorkel divers each took five different counts randomly throughout Laguna Bacalar's rapids. Known areas were outlined using clear plastic Petri dish tops (area = 63.6 cm^2) and bottoms (area = 56.7 cm^2). After being dropped off at the beginning of the rapids each diver stopped at random locations counting the amount of individual black-striped mussels that fit in their petri dish, collecting five counts along the way. Thirty counts were thus obtained for estimating black striped mussel densities (attached Appendix 1, Table 3).

Results

Various analyses, discussed above, were performed on a variety of field stations (figure 1) including open waters, springs and cenotes (table 1). Two sonde casts were performed, on various days, at Black Cenote (Station 12) and Hotel Bacalar Cenote (Station 13). Almost all bacteriological samples were prepared in duplicate and read for bacteria after 12 and 36 hours. Secchi disk depth measurements were taken at every site that had adequate water depth.

Depth profiles were prepared for all sonde casts in open lake sites deeper than 5 meters and for all casts in cenotes (figures 8 and 9). Additionally, a contour plot was created to visualize the difference in chemistries between open water and cenotes, specifically Cenote Negro (figure 11. Throughout the whole study temperatures ranged from 24 to 28.5 degrees Celsius, with minimal variation over depth. The largest temperature differences were less than 2 degrees, even over 40+ meters of depth. Specific conductivity measurements generally ranged from 2.3 to 2.5 mS/cm, with few exceptions. Dissolved oxygen concentrations experienced greater fluctuations varying from 3.5 to 9.0 mg/L, although over depth the differences were all less than 2 mg/L. All stations were relatively neutral, with pH values ranging from 6.86 to 7.86. A 24-hour sonde deployment also provided information about daily fluctuations in dissolved oxygen concentrations (figure 10), among other parameters.

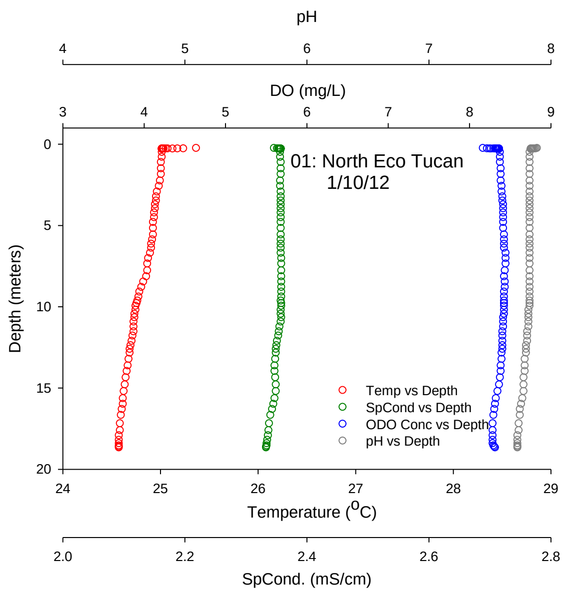
Two in-lake springs were also sampled for basic water chemistry parameters. The spring in the southern most point of Xul Ha (Station 22) differed from Xul Ha (Station 19) by having very low dissolved oxygen (1.17 mg/L) and reduced pH (6.65). The springs at Station 04 had very high specific conductivity (3.03 mS/cm). It is also likely that oxygen concentrations were extremely low here (<1 mg/L), however high turbidity prevented accurate measurements from being taken.

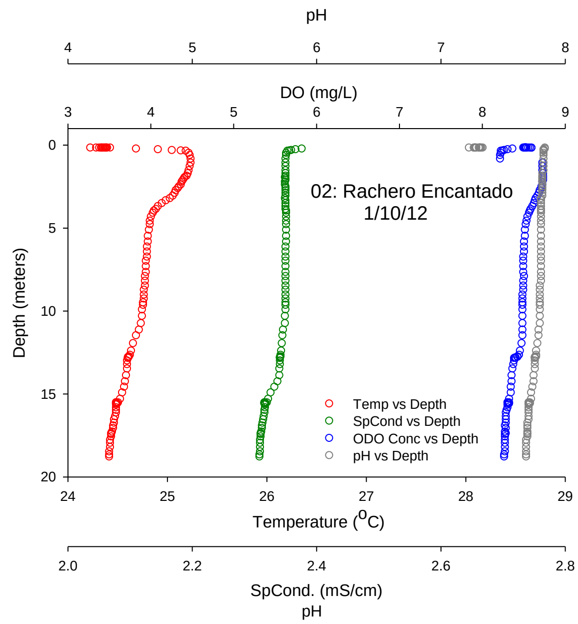
Bacteriological sample results are shown in figure 14. Only the 36 hour readings are presented, to maximize time for bacterial growth. *E. coli* levels ranged from 100 to 100,000 CFU/100 ml, with 30% of the samples containing *E. coli* above the EPA standard of 235 CFU/100 ml. Samples containing other forms of coliform and bacteria ranged from 100 to 18,500 CFU/100 ml.

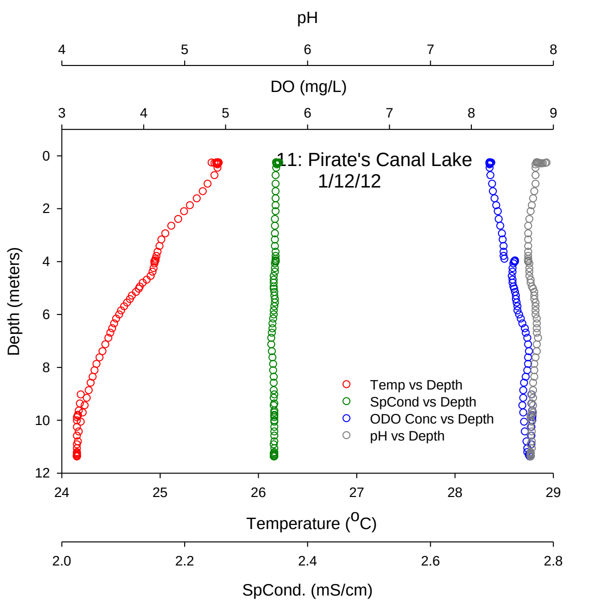
For full results, please refer to Appendix 1.

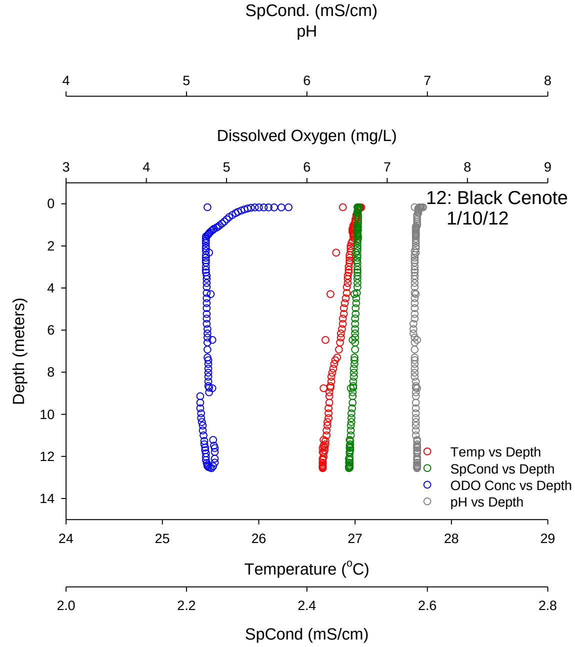
Table 1: Twenty-five stations and 4 transects were the sites of various water analyses including sonde profiles, bacteriological samples, plankton tows and mussel counts. Station numbers were only given to those stations that were sampled while underway. The locations of the transects are only at the beginning of each course took.

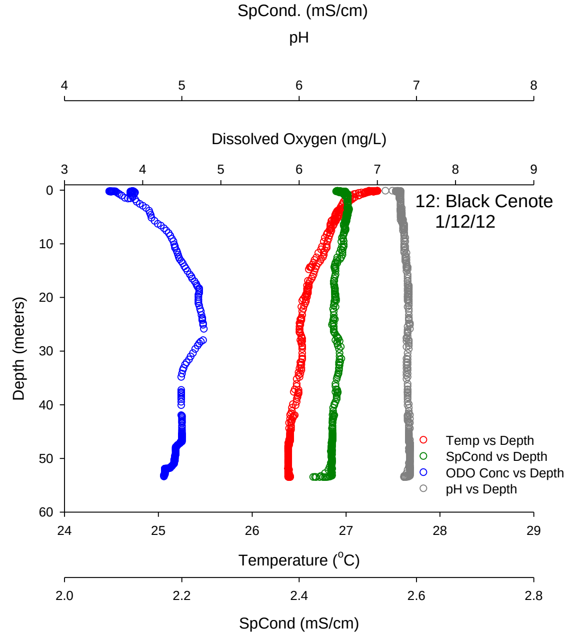
Station	Station Number	Latitude	Longitutde	Secchi Disc	Sonde	Bacteriological Sample	Plankton Tow	Mussel Count
North Eco Tucan	1	18.73388333	-88.33878333	X	x	X		
Ranchero Encantado	2	18.70618333	-88.3739	X	x	X		
North Bacalar Cove	3	18.69248333	-88.38516667		x	X		
Jobs Springs	4	18.6891	-88.38476667		x			
Public	5	18.67916667	-88.3853		x	X		
City Surface 1	6	18.677009	-88.386791			X		
City Surface 2	7	18.67273333	-88.38838333			X		
City Surface 3	8	18.66845	-88.39143333			X		
Pirate's Canal	9	18.6722	-88.37853333		x			
POLLY'S HOUSE	10	18.67353333	-88.38881667		x	X		
Croc Bay	11	18.6671	-88.3718	X	x		X	
Black Cenote	12	18.66685	-88.39483333		x	X		
Hotel Bacalar Cenote	13	18.656	-88.40563333		x	X		
South China House	14	18.65098333	-88.40868333	X	x	X		
Parallel Cenote	15	18.650316	-88.410164	X	x	X		
Hotel Cenote-Deep	16	18.65471667	-88.40525		x	X		
Xul Ha Deep	19	18.54913333	-88.45635	X	x	X		
Shoreline White Film	20	18.55273333	-88.45176667	X	x	X		
Xul Ha	21	18.5436	-88.45888333		x	X	X	
South Dock	22	18.54376667	-88.46076667			X		
Palmer Springs		18.462776	-88.520552		x			
Xu Hal Transect Transect		18.5593	-88.45275		x			
Xu Hal Transect 2 Transect		18.54598333	-88.45678333		x			
Into Black Cenote Transect		18.65435	-88.40525		X			
Out of Cenote Negro Transect		18.66691667	-88.39433333		X			
Shrimp Pier		18.685027	-88.385395			X		
Rapids		18.592619	-88.432761					X

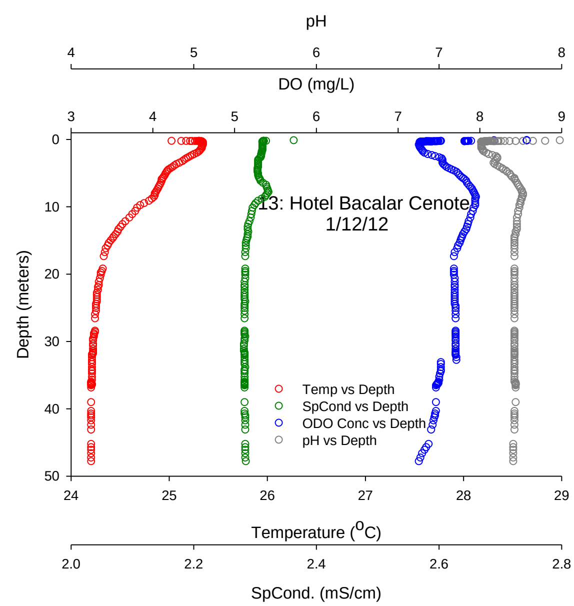

pH
DO (mg/L)
01: North Eco Tucan
1/10/12
Depth (meters)
Temp vs Depth
SpCond vs Depth
ODO Conc vs Depth
pH vs Depth
Temperature (°C)
SpCond. (mS/cm)

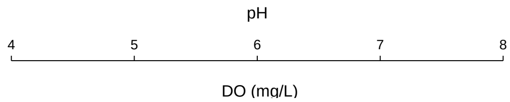

pH
DO (mg/L)
02: Rachero Encantado
1/10/12
Depth (meters)
Temp vs Depth
SpCond vs Depth
ODO Conc vs Depth
pH vs Depth
Temperature (°C)
SpCond. (mS/cm)


pH
DO (mg/L)
11: Pirate's Canal Lake
1/12/12
Depth (meters)
Temp vs Depth
SpCond vs Depth
ODO Conc vs Depth
pH vs Depth
Temperature (°C)
SpCond. (mS/cm)


pH
Dissolved Oxygen (mg/L)
12: Black Cenote
1/10/12
Depth (meters)
Temp vs Depth
SpCond vs Depth
ODO Conc vs Depth
pH vs Depth
Temperature (°C)
SpCond (mS/cm)


pH
Dissolved Oxygen (mg/L)
12: Black Cenote
1/12/12
Depth (meters)
Temp vs Depth
SpCond vs Depth
ODO Conc vs Depth
pH vs Depth
Temperature (°C)
SpCond (mS/cm)


pH
DO (mg/L)
13: Hotel Bacalar Cenote
1/12/12
Depth (meters)
Temp vs Depth
SpCond vs Depth
ODO Conc vs Depth
pH vs Depth
Temperature (°C)
SpCond. (mS/cm)



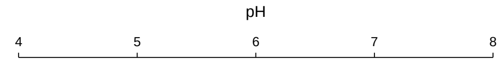
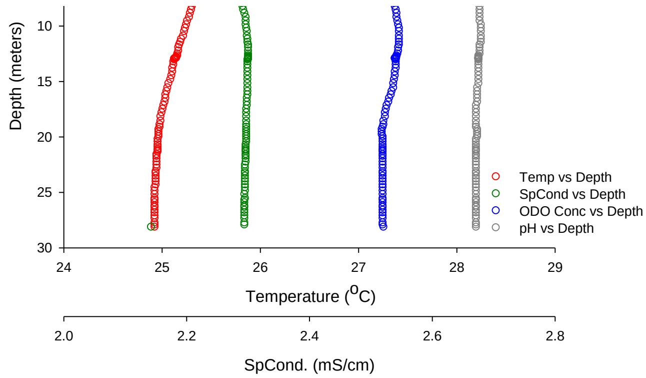
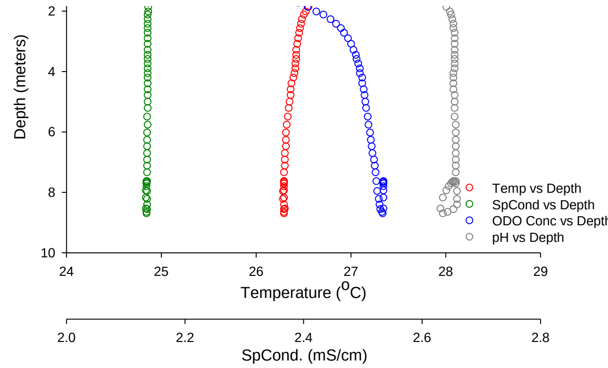


Figure 8: (clockwise from top left) Sonde profiles of the following sites: Station 1- north of EcoTucan,; station 2- Ranchero Encantado; Station 12- Black Cenote; Station 13- Hotel Bacalar Cenote; Station "12.5"- the deepest location in Black Cenote; and Station 11- Pirate's Canal Lake.





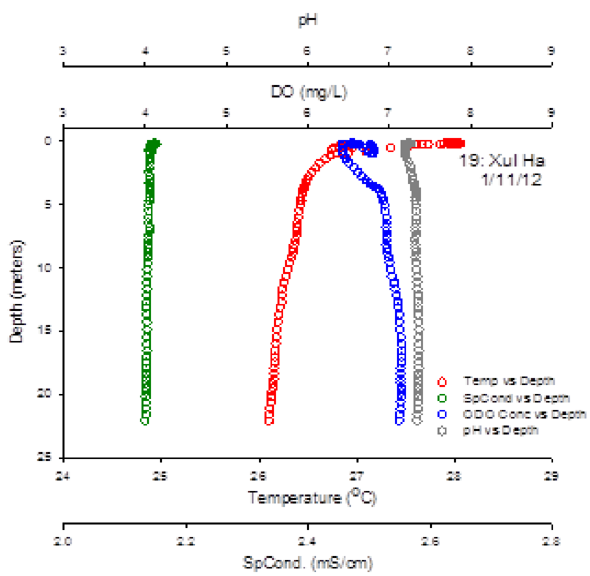
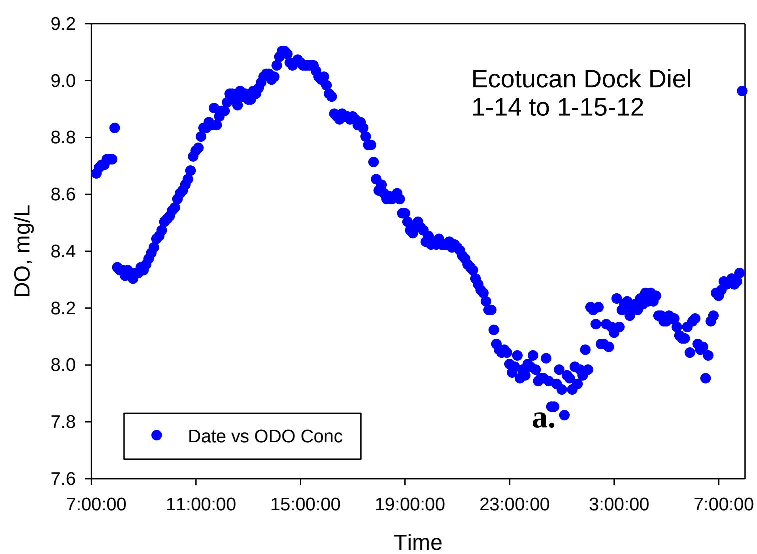


Figure 9 (Clockwise from top left): Depth profiles from Station 12- Black Cenote, Station 13- Hotel Bacalar Cenote, Station 18- Xul Ha at its deepest found location, Station 19- Xul Ha during a transect of the lake and from Station 15- Cenote Parallel.

Figure 10 (below): A 24-hour sonde deployment from the Ecotucan dock measured the daily fluctuation in dissolved oxygen concentrations. In this nearshore site the DO

was carried out within the rapids section of the lake which divides the large basin of Laguna Bacalar from Xul Ha. Between Station 17 and just north of Station 18 twenty-nine sites were counted. The average population density for the rapids region was 2,890 mussels/m^2, with a standard deviation of 650 mussels/m^2.



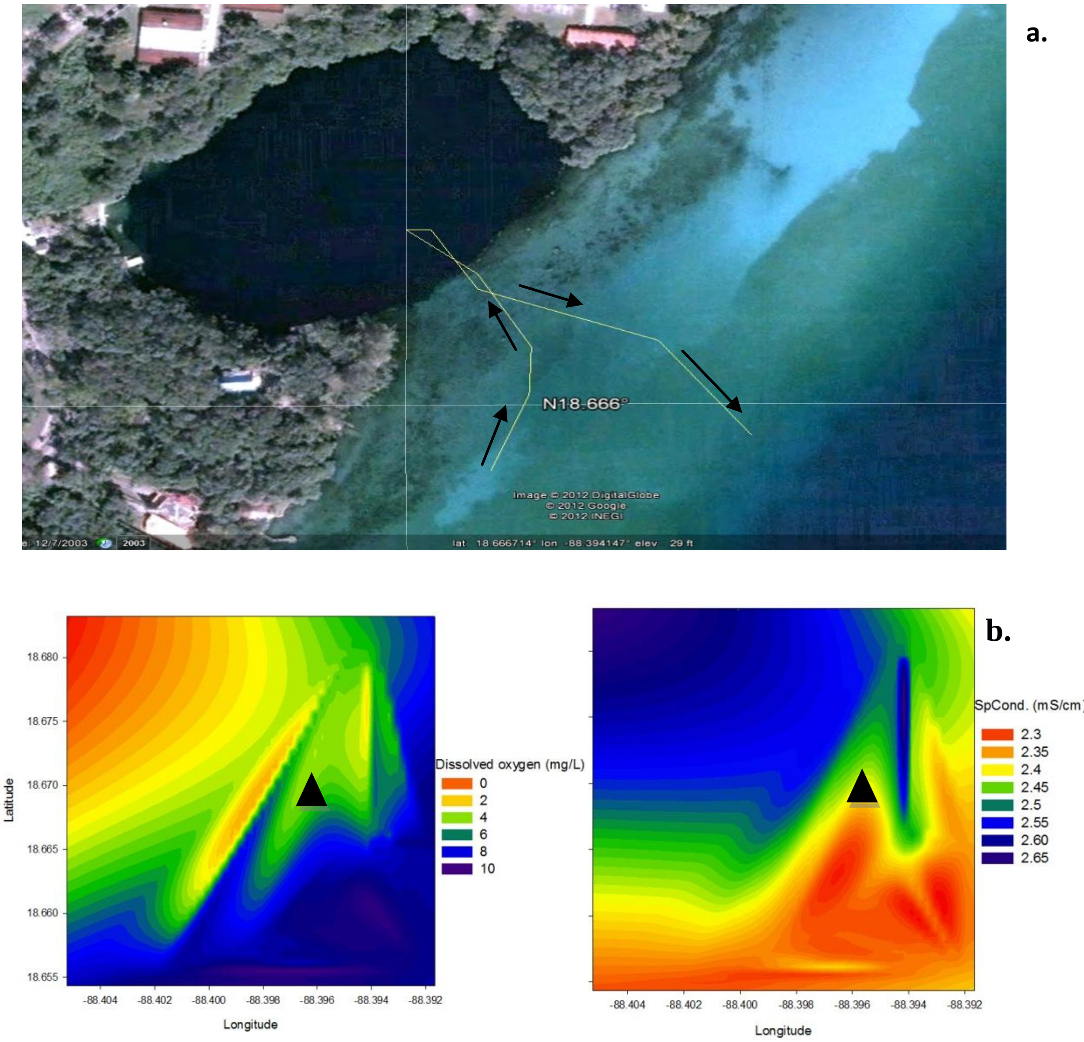


Figure 14: a) The path taken into and out of Black Cenote, with the yellow line indicating course and black arrows indicating direction. The location of entrance into Black Cenote was at 18.666714 N and 88.394147 W. b) Contour plots of dissolved oxygen and specific conductivity. The point of entrance is marked with the black triangle.

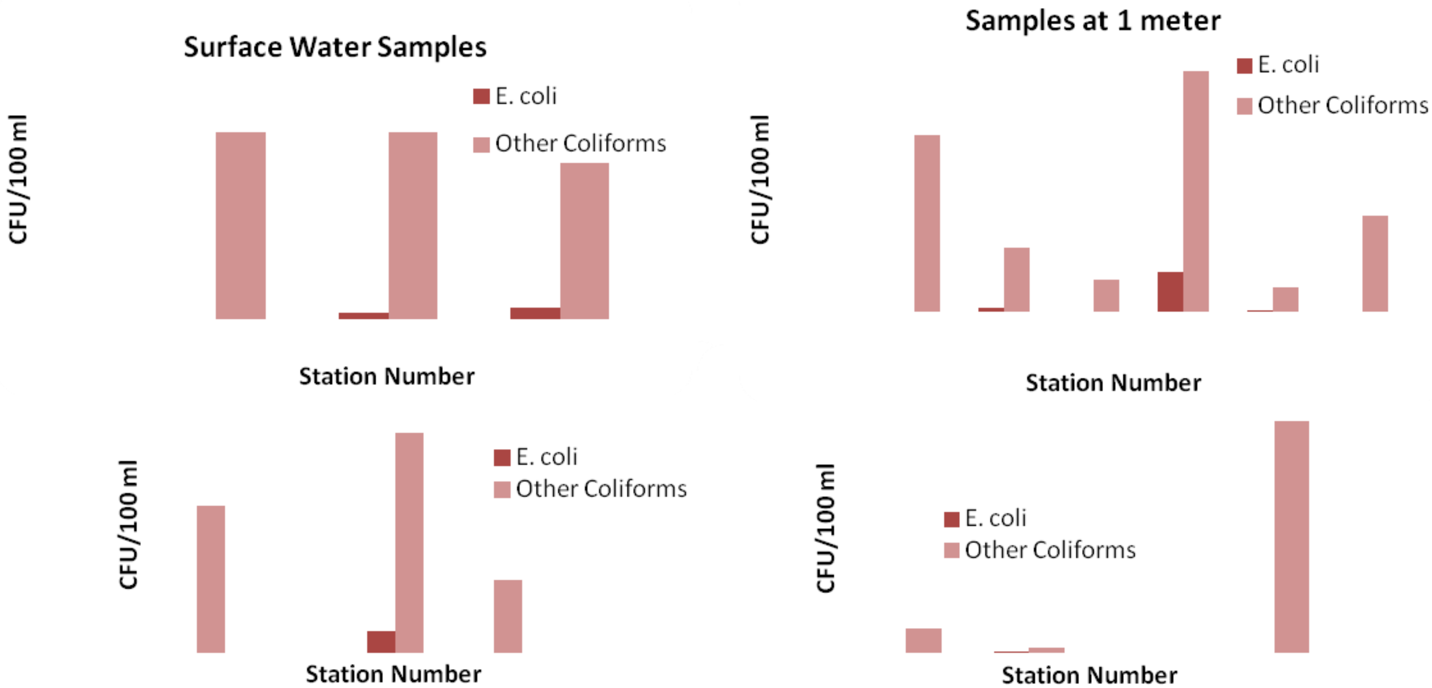


Figure 15: The *E. coli* and other coliform counts after 36 hours for various sample depths and locations around Laguna Bacalar.

Discussion and Conclusion

The basic water chemistry information gathered with the sonde indicates homogeneity of parameters throughout major parts of Laguna Bacalar, with the exception of springs and some cenotes. The sonde measurements taken at the spring locations are indicative of a geothermally heated groundwater source -low oxygen, high specific conductivity and raised temperatures. Black Cenote also appears to be heavily influenced by a source of groundwater, as presented in figure 14. Dissolved oxygen is quickly reduced by 6 ppm, while specific conductivity increases 0.3 mS/cm. The studies from Xul Ha are indicative of groundwater conditions, consistent with results from Black Cenote- declined oxygen and warm temperatures. Additionally, warm groundwater rises, preventing formation of any stratified gradient via promoted mixing which was observed at all sampled sites. The 24-hour sonde deployment indicates that Laguna Bacalar experiences a cycle of diel cycle of dissolved oxygen, with concentrations differing more than 1 ppm between day and night. This could play a major role for organisms in the clear open waters of the lake since DO tends to vary little with depth and the differences are presumable due to photosynthesis. Additionally, photosynthetic fauna are apparently present even though chlorophyll concentrations were negligible.

The *E. coli* concentrations appeared to be highest closest to the city of Bacalar and near areas of development (e.g. Hotel Laguna Bacalar). The sample containing the highest amounts of *E. coli* was taken from a dock within the city limits of Bacalar. Almost all other samples with *E. coli* greater than 235 CFU/100 ml were taken from nearshore sites within view of the city; the exception being the sample taken directly in a pool of white film in Xul Ha. For recreational waters the US EPA limits are 400 total coliform CFU/100 ml and 235 *E. coli* CFU/100 ml (McLellan, pers. comm.), which nearly all of the samples in this study exceed.

The black-striped mussel is an invasive species that has established itself in the waters of Laguna Bacalar at densities of ~2900 mussels/m^2 on stromatolites. In the Laurentian Great Lakes, the *Dreissena* mussel rapidly spread and became well established within only two to three decades (Nalepa et al. 2009). This can likely be contributed to lack of competing species and the availability of food source in the water column. In Laguna Bacalar there are no species that consume the mussel, but rather compete with it (i.e. giant stromatolite) (Kaster, 2012). It is likely that low suspended material in the water column and oligotrophic status of the lake also helps keep mussels at low densities.

Future studies will be needed to further characterize the current condition and set standards to maintain Laguna Bacalar's high integrity. Laguna Bacalar is a unique system that should be preserved in quality and enjoyed, responsibly, by the world.

Sources:

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”POET3 Fauna” pdf

”POET 2_Flora” pdf

http://www.lagunabacalarinstitute.com/Research.html.

Appendix 1

Table 1. Water Quality Data (Sonde)

	Station Name	Station Number	Date	Time	Lat	Long	Secchi depth m	Depth m	Temp C	Sp Cond mS/cm	Cond mS/cm	DO mg/L	% sat %	pH
Open Lake														
	Xul Ha Deep	19	1/11/2012	12:33:33	18.54913	-88.45635	8.5	1.136	26.73	2.14	2.21	6.44	80.9	7.2
					18.54913	-88.45635	8.5	5.052	26.42	2.139	2.197	6.94	86.7	7.32
				12:35	18.54913	-88.45635	8.5	20.159	26.12	2.134	2.18	7.15	88.8	7.35
				12:35	18.54913	-88.45635	8.5	22.063	26.1	2.133	2.178	7.12	88.4	7.34
	North Bacalar Cove	3	1/10/2012	13:19	18.69248	18.69248		0.544	25.73	2.397	2.431	8.2	101.1	7.66
	Ranchero Encantado	2	1/10/2012	13:40	18.70618	-88.3739	6.1	1.091	25.24	2.351	2.362	8.73	106.8	7.83
					18.70618	-88.3739	6.1	5.132	24.82	2.352	2.344	8.52	103.5	7.81
					18.70618	-88.3739	6.1	18.681	24.42	2.309	2.284	8.27	99.7	7.69
	North Eco Tucan	1	1/10/2012	14:21	18.73388	-88.33878	8	1.132	25.01	2.358	2.358	8.38	102.2	7.83
					18.73388	-88.33878	8	5.194	24.93	2.358	2.358	8.42	102.5	7.83
					18.73388	-88.33878	8	18.227	24.58	2.336	2.317	8.29	100.2	7.73
	Public	5	1/10/2012	15:49	18.67917	-88.3853		0.787	26.32	2.353	2.412	8.74	109.1	7.83
	South China House	14	1/11/2012	8:50	18.65098	-88.40868		1.004	25.38	2.303	2.32	6.02		7.24
	Xu Hal Transect Profile	18	1/11/2012	11:06	18.55492	-88.46033	7	0.996	26.96	2.139	2.219	5.44	68.6	7.05
					18.55492	-88.46033	7	5.02	26.36	2.138	2.193	6.79	84.7	7.28
					18.55492	-88.46033	7	7.979	26.29	2.137	2.19	6.94	86.6	7.3
	Shoreline White Film	20	1/11/2012	12:48	18.55273	-88.45177		0.313	27.95	2.145	2.266	6.81	87.5	7.28
	Croc Bay	11	1/12/2012	10:28	18.6671	-88.3718	7	1.082	25.49	2.349	2.371	8.26	101.5	7.86
					18.6671	-88.3718	7	5.053	24.79	2.346	2.337	8.53	103.5	7.84
					18.6671	-88.3718	7	11.394	24.16	2.346	2.309	8.73	104.7	7.82

Table 2. Bacterial Assays

Bacteriological Analysis (F. coliform)

Station #	Station Name	Date	12 Hrs	36Hrs	Time	Lat	Long	Depth m	Volume mL	Incubation Time (hr)	E. coli	Other Coliform	Other Bacteria	E. coli CFU/100 ml	Other Coli. CFU/100 ml	Other Bacteria CFU/100 ml
1	North Ecotucan	1/11/2012	X		7:45AM	18.73388	-88.33878	5	1	24	0	few	2	0		200
1	North Ecotucan	1/11/2012	X		7:45AM	18.73388	-88.33878	5	1	24	0	0	few	0	0	
1	North Ecotucan	1/13/2012		X	1:24PM	18.73388	-88.33878	5	5	48	0	25	5	0	2500	500
1	North Ecotucan	1/13/2012		X	1:24PM	18.73388	-88.33878	5	5	48	0	15	0	0	1500	0
2	Ranchero Encantado #1	1/11/2012	X		7:45AM	18.70618	-88.3739	1	1	24	0	1	0	0	100	0
2	Ranchero Encantado #1	1/13/2012		X	1:24PM	18.70618	-88.3739	1	5	48	0	100	0	0	10000	0
2	Ranchero Encantado	1/11/2012	X		7:45AM			5	1	24	0	few	0	0		0
2	Ranchero Encantado	1/13/2012		X	1:24PM			5	1	48	0	0	200	0	0	20000
2	Ranchero Encantado	1/13/2012		X	1:24PM				5	48	0	120	75	0	12000	7500
2	Ranchero Encantado	1/11/2012	X		7:45AM			5	1	24	0	0	0	0	0	0
3	North Bacalar Cove	1/11/2012	X		7:45AM	18.69248	18.69248	1	1	24	0	1	0	0	100	0
3	North Bacalar Cove	1/13/2012		X	1:24PM	18.69248	18.69248	1	1	48	0	30	0	0	3000	0
3	North Bacalar Cove	1/13/2012		X	1:24PM	18.69248	18.69248	1	5	48	5	50	0	500	5000	0
3	North Bacalar Cove	1/11/2012	X		7:45AM	18.69248	18.69248	1	5	24	0	90	few	0	9000	
5	Public Beach	1/11/2012	X		7:45AM	18.67917	-88.3853	1	5	24	0	2	1	0	200	100
5	Public Beach	1/11/2012	X		7:45AM	18.67917	-88.3853	1	1	24	0	few	0	0		0
5	Public Beach	1/13/2012		X	1:24PM	18.67917	-88.3853	1	1	48	0	20	0	0	2000	0
5	Public Beach	1/13/2012		X	1:24PM	18.67917	-88.3853	surface	5	48	0	100	30	0	10000	3000
6	City Surface #1	1/11/2012	X		7:45AM	18.66757	-88.38337	1	1	24	few	1 giant	110			11000
6	City Surface #1	1/11/2012	X		7:45AM	18.66757	-88.38337	1	5	24	1	160	160	100	16000	16000
7	City Surface #2	1/13/2012		X	1:24PM	18.67273	-88.38838	surface	1	48	4	100	0	400	10000	0
8	City Surface #3	1/13/2012		X	1:24PM	18.66845	-88.39143	1	5	48	25	150	60	2500	15000	6000
10	Pirates Canal	1/11/2012	X		7:45AM	18.6722	-88.37853	1	1	24	0	few	0	0		0
10	Pirates Canal	1/13/2012		X	1:24PM	18.6722	-88.37853	1	5	48	0	20	0	0	2000	0
10	Pirates Canal	1/13/2012		X	1:24PM	18.6722	-88.37853	1	5	48	1	10	0	100	1000	0
12	Black Cenote	1/11/2012	X		7:45AM	18.66685	-88.39483	5	1	24	0	2	2	0	200	200
12	Black Cenote	1/11/2012	X		7:45AM	18.66685	-88.39483	20	1	24	0	4	few	0	400	
12	Black Cenote	1/13/2012		X	1:24PM	18.66685	-88.39483	5	5	48	3	30	0	300	3000	0
12	Black Cenote	1/13/2012		X	1:24PM	18.66685	-88.39483	20	5	48	0	20	8	0	2000	800
13	Hotel Bacalar	1/11/2012	X		7:45AM	18.656	-88.40563	5	1	24	0	0	0	0	0	0
13	Hotel Bacalar	1/13/2012		X	1:24PM	18.656	-88.40563	5	5	48	0	10	0	0	1000	0
13	Hotel Bacalar Cenote	1/11/2012	X		7:45AM	18.656	-88.40563	10	1	24	0	1	few	0	100	
13	Hotel Bacalar Cenote	1/13/2012		X	1:24PM	18.656	-88.40563	10	5	48	0	10	5	0	1000	500
13	Hotel Bacalar Cenote	1/13/2012		X	1:24PM	18.656	-88.40563	10	5	48	3	lots	0	300		0
13	Hotel Bacalar Cenote	1/11/2012	X		7:45AM	18.656	-88.40563	1	1	24	0	0	3	0	0	300
14	South China House	1/13/2012		X	1:24PM	18.65098	-88.40868			24				0	0	0
15	Cenote Parallel	1/13/2012		X	1:24PM	18.55045	-88.41038	20	5	48	0	lots	0	0		0
15	Cenote Parallel	1/13/2012		X	1:24PM	18.55045	-88.41038	20	1		0	lots	few	0		
16	Deep Spot #1	1/13/2012		X	1:24PM			20	1		0	185	few	0	18500	
19	Deep Spot #2	1/13/2012		X	1:24PM			5	1	48	0	lots	0	0		0
20	White Film Surface	1/13/2012		X	1:24PM	18.55273	-88.45178	surface	5	48	0	150	100	0	15000	10000
20	White Film Surface	1/13/2012		X	1:24PM	18.55273	-88.45178		1		0	0	lots	0	0	
20	White Film Surface	1/13/2012		X	1:24PM			surface	5		20	100	100	2000	10000	10000
22	South Dock	1/13/2012		X	1:24PM	18.54377	-88.46077	1	1	24	0	lots	0	0		0
	Beach #1	1/13/2012		X	1:24PM				1	48	3	30	0	300	3000	0
	Beach #2	1/13/2012		X	1:24PM				1	48	20	175	0	2000	17500	0
	Shrimp Pier	1/13/2012		X	1:24PM			Surface	5	24	1,000			100000	0	0
														0	0	0
21	Jobs Prop	1/13/2012		X	1:24PM	18.6891	-88.38477		1	24	0	60	60	0	6000	6000

Table 3. Mussel Densities

Mussel Counts	Number	Average	Stdev	Area cm2	Mussels per m2
Bottom	4			56.716	705
	12				2116
	18				3174
	10				1763
	33	15.4	11.0		5818
Bottom	13				2292
	31				5466
	14				2468
	21	19.75	8.3		3703
Bottom	20				3526
	4				705
	13				2292
	31				5466
	7	15	10.8		1234
mean bot		16.5	9.46	57%	2909
				63.585	
Top	15				2359
	16				2516
	8				1258
	11				1730
	11	12.2	3.27		1730
Top	16				2516
	21				3303
	15				2359
	15				2359
	18	17	2.55		2831
Top	25				3932
	30				4718
	45				7077
	8				1258
	17	25	13.95		2674
mean top		18.07	9.51	53%	2841
	MEAN ALL				**2874**
	STDEV				**1574**
	%				**55%**