EFFECTIVENESS OF MANAGEMENT REGULATIONS AND UPDATED ANALYSIS OF POPULATION HEALTH AND TRENDS OF QUEEN CONCH, *STROMBUS GIGAS,* IN PUERTO RICO

By

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Abstract

The queen conch resource continues to support a commercial fishery in Puerto Rico, despite a history of overfishing and low conch densities. The primary goal of this study was to generate density estimates for queen conch off western Puerto Rico using diver-based visual surveys and to assess trends and evaluate hypotheses of management interest using generalized linear mixed models. Density data were supplemented by size/age data. The spawning stock was also calculated and compared to a population (Abrir La Sierra) recently discovered at depths of 38-44m. Forty-six sites were surveyed and data on habitat, depth, estimated length, age class and reproduction were collected. Total density was 14.05/ha (adults = 7.32/ha; juveniles = 6.63/ha). Year (current and past surveys), depth and location were all significant factors influencing adult density. Lower densities of both juvenile and adult conch were observed in 1997 compared to 2006 and 2013. This result alone indicates some level of improvement in the population, though not recently. A location effect compared sites within the US EEZ (greater than 9NM from Puerto Rico) which is closed to fishing versus local waters which are open to fishing. Adult density was higher in shallower water; and regardless of depth or year, adult densities were higher in the EEZ than in local waters, though a greater proportional increase occurred in the EEZ. This suggests that though not statistically significant, the closure of the EEZ is having a positive effect on conch density. Length-frequency diagrams showed an increase in the proportion of adults within the 16-20 cm size class in 2006-2013 pooled relative to 1997. This suggests an effect of the 9inch minimum size limit implemented in 2004. In 1997, juveniles comprised 70% of the population, and no very old adults were found. In 2013, 50% of the population was juveniles, and adults were found in all the age classes, including very old adult. This suggests an overall decrease in fishing mortality. The spawning stock on the broad, shallow shelf was estimated at 172,705 individuals, significantly greater than the 29,092 individuals reported at Abrir La Sierra. Changes in survey methodology are recommended, including but not limited to shortening the transects, not utilizing scooters, standardizing area surveyed, stratifying between depth and habitats, and increasing sites in the EEZ area.

Resumen

En Puerto Rico, el recurso concha reina (carrucho) sigue apoyando la pesquería comercial a pesar de una historia de sobre pesca y la baja densidad poblacional de la concha. El objetivo principal de este estudio fue generar estimaciones de la densidad de concha reina en el area oeste de Puerto Rico utilizando modelos mixtos lineales generalizados para evaluar las tendencias y evaluar hipótesis de interés sobre la gestión de manejo. Los datos de densidad se complementaron con datos de talla / edad. La población reproductora también se calcula y se compara con una población recientemente descubierta en Abrir la Sierra a profundidades de 38-44m. Cuarenta y seis sitios fueron muestreados utilizando equipo autónomo de buceo y se recogieron datos sobre el hábitat, la profundidad, el tamaño estimado, clase, edad y la reproducción. Densidad total fue de 14,05 / ha (adultos = 7,32 / ha; los juveniles = 6,63 / ha). Se observaron densidades más bajas de ambos juveniles y adultos de caracol en la encuesta de 1997 en comparación con 2006 y 2013. Este resultado puede indicar algun nivel de mejora en la poblacion total, aunque no necesariamente. Año, la profundidad y la ubicación fueron factores significativos que afectaron a la densidad de adultos. Se compararon sitios como los EE.UU. ZEE (mayor que 9NM de Puerto Rico) versus aguas locales (cerca de la costa de la frontera 9NM). La densidad de adultos fue mayor en aguas menos profundas; independentemente de la profundidad o el año, las densidades de adultos fueron más altos en la zona económica exclusiva que en las aguas locales.Esto sugiere que aunque no estadisticamente significativo, el cerrar los ZZE resulta en un efecto positivo en la poblacion del carrucho. Diagramas de frecuencia de tamaño mostraron un aumento en el número de adultos dentro de la clase de tamaño de 16-20 cm en 2006-2013 agrupados en relación con 1997. Esto sugiere un efecto al limite de tamano minimo de 22.86cm implementado en el 2004. Los juveniles componen el 50% de la población en 2013 en comparacion a un 70% en 1997. Los adultos se encontraron en todas las clases de edad adulta, incluyendo adultos de mayor edad aunque estos no estaban presente en el 1997. Esto sugiere una baja en mortalidad por la pesca. La primera estimación de la población reproductora en la amplia plataforma,poco profunda era 172.705 individuos, significativamente superiores a los 29.092 individuos reportados en Abrir La Sierra. Algunos cambios recomendados en la motodologia incluyen, pero no se limitan a cortar el largo de los transectos, no utilizar sistemas de scooters submarinos, estandarizando el area muestrada, estratificando entre profundidades y habitats y aumentando el numero de sitios en el area de ZZE.

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Introduction

Queen conch, *Strombus* (= *Lobatus*) *gigas*, is a valuable resource both commercially and recreationally in the Caribbean. In Puerto Rico, scuba divers that target queen conch are among the most successful commercial fishermen on the island (Matos-Caraballo *et al.* 2012). After spiny lobster, queen conch contributes most to overall commercial landings (~11%). In 2007, a total of 65,300 kg (143,653 lb) meat weight was caught by commercial fishers. At an average price of $3.78 USD per pound (Matos-Caraballo *et al.* 2012), the commercial fishery is valued at around $543,000 USD.

Management of this commercially important species throughout the Caribbean is difficult due to a variety of factors. Key among these is that conch change the manner in which they grow. As juveniles, they increase in shell length, but at about the onset of maturity they cease growing in length and form a broad shell lip that thickens over time (Appeldoorn 1997; Tewfik *et al.* 1998). As a consequence, length and biomass of conch are largely fixed at the time of maturation (Appeldoorn 1988). Additionally, there is a wide variation in the size at maturity, with a strong environmental influence. Thus, length and biomass are not a function of age (Appeldoorn 1988). At present, there is no established way to age conch that could be used in standard growth models for assessment. In addition, conch require copulation for reproduction, and maintaining minimum densities is important, yet exact densities needed are difficult to assess (Stoner and Ray-Culp 2000, Appeldoorn *et al.* 2011a). In addition, genetic connectivity of individual stocks is generally not known. Yet conch are vulnerable to overfishing; they are slow moving with limited home ranges (e.g. Delgado and Glazer 2007), and during the extended reproductive season (Avila-Poveda and Baqueiro-Cárdenas 2009) they migrate to shallower depths and preferentially inhabit sandy bottoms where they are conspicuous and easy to catch (Randall 1964, Weil and Laughlin 1984, Coulston *et al.* 1987). In 1992, following the collapse of conch fisheries in a number of countries, conch were listed under Appendix II of CITES. This requires exporting countries certify through their local scientific authority that harvest and export are not negatively affecting the stock. This has helped by forcing exporting countries to collect non-detrimental findings to ensure export does not negatively affect the wild population (Theile 2001).

The queen conch resource in Puerto Rico is managed jointly by the territorial and U.S. federal governments. From the shoreline out to 9 nautical miles (NM) (16.87 km), the regulations governing harvest are mandated by the territorial government. Outside 9 NM is the United States' Exclusive Economic Zone (EEZ), where the federal government oversees and imposes regulations regarding queen conch harvest through the Caribbean Fisheries Management Council. In 1997, the US Caribbean EEZ, with the exception of St. Croix (US Virgin Islands) was closed to conch fishing. Also at this time, a closed season was implemented in territorial waters (1 July to 31 September), later amended to 1 August to 31 October in 2012. In 2004, additional regulations in local waters included a 9-inch (22.86 cm) minimum shell length or 3/8-inch (9.5 mm) minimum lip thickness and a bag limit of 150 and 450/day per person and per boat, respectively.

Puerto Rico's conch fishery is currently overfished, but recovering from severe overfishing and loss of habitat in the 1980's. In the mid 1980's one boat trip could average 73 kg of meat, while the same trip in the early 2000s could only average 33 kg (Valle-Esquival 2002). Catch was based on juveniles (Appeldoorn 1991), and fishing mortality was greater than natural mortality (Appeldoorn 1987). A general trend of decreasing catch has been observed since the early 1980s (SEDAR 2007). To try and combat this decreasing trend, the Puerto Rico Department of Natural and Environmental Resources (DNER) through the Southeast Area Monitoring and Assessment Program - Caribbean (SEAMAP-C) has been conducting periodic visual surveys to collect data that will help with management. Prior to the standardized SEAMAP surveys, a survey was conducted in 1985-86, but was restricted to 81 sites on the SW corner of the island. Average total density was 8.11/ha (Torres Rosado 1987). The first SEAMAP survey was done in 1997, and covered both the east (29 sites) and west (60 sites) coasts. Average densities were 7.49/ha and 8.49/ha, respectively (Mateo 1997, Mateo *et al.* 1998). Sixty sites were again surveyed on the west coast in 2001, and density had increased to 14.42/ha (Appeldoorn 2002). The 2006 survey added 14 sites on the south coast to the sampling regime and resampled the west (46 sites) and east (40 sites) coasts (Jiménez 2007). Average densities were 17.74/ha, 22.4/ha and 46.58/ha, respectively (revised from Jiménez 2007). Direct comparison is complicated by temporal variation, as the four surveys were conducted at different times of the year, ranging from April to December. From 1997 to 2006, a trend of increasing density was noted, though direct statistical comparisons were not made. Improvements in the health of the population were additionally supported by analysis of length and age distributions, which showed a greater proportion of total adults, especially older adults (SEDAR 2007).

The low densities of conch observed repeatedly throughout the course of these surveys, combined with data from the Bahamas showing that reproductive rates dropped below a minimum density of 50/ha (Stoner and Ray-Culp 2000), suggest that the functional spawning stock of conch in Puerto Rico may be critically low. In 2012, a survey of commercially important species at three mesophotic reefs off the west coast of Puerto Rico (38-44 m) found large numbers of adult conch (672 individuals) at one of the sites (Abrir La Sierra) (Garcia-Sais *et al.* 2012). This site is along the insular slope off the western platform of Puerto Rico. Average density per habitat type was as follows: 3.3/ha for the wall habitat, 7.09/ha for the top habitat and 194.93/ha for the rhodolith habitat (see Garcia-Sais *et al.* 2012 for specific habitat classification details). 4.23ha were surveyed for both top and wall habitats, and 3.22ha of rhodolith habitat were surveyed. Approximately 25 transects were surveyed at each Abrir La Sierra site. A total population estimate for Abrir La Sierra is 29,092 individuals. Of these, 95% had shell lengths of 20-28 cm. The average lip thickness was 21mm, with 72% between 20 and 30mm indicating these were old adults. Conch were observed to be reproductively active, but the extent of this activity was not quantified (Garcia-Sais *et al.* 2012). This high density of reproductively active conch may be contributing larvae for settlement further inshore.

The purpose of this study was to re-survey the shallow water conch population off the west coast of Puerto Rico as part of the ongoing SEAMAP survey program. Although there is fishing on the east, south and west coasts, the broad shelf in the west is the primary fishing grounds. Additionally, the west coast has the longest time series of past surveys, dating back to 1987 (Torres Rosado 1987). The primary goal of the survey was to generate density estimates and size/age data that could be used to assess trends and current status. However, unlike past surveys, density data analyses employed generalized linear mixed models to generate more robust statistical comparisons to test several hypotheses of management interest, as follows:

Hypothesis 1 – Conch densities are increasing relative to previous studies, presumably in response to management measures limiting fishing effort and catch and

Hypothesis 2 - Mean density within the EEZ will be higher compared to local waters after being closed to fishing for 16 years.

The above density comparisons were augmented by analysis of changes in length/age frequency distributions. Additionally, spawning stock abundance on the platform was estimated for comparison to that observed by Garcia-Sais *et al*. (2012) in deep water at Abrir La Sierra to evaluate the significance of the latter.

Methods

Visual Surveys

All SEAMAP surveys for conch in Puerto Rico have utilized strata of expected areas of high and low conch density based on fisherman interviews (Mateo 1997, Mateo *et al.*1998). In 2006, interviews with fishermen identified areas of (i) past conch fishing grounds, (ii) present conch fishing grounds and (iii) areas known to have juveniles. These interviews covered the west, east and south coasts. The maps were digitized into a geographic information system database using ArcMap, and the pooled area was used as a boundary or frame of the survey area. All categories (past, present and juvenile) were given the same weight during the site selection, though many of the polygons overlapped. A total of 46 random sites (transect start coordinates) were chosen off the west coast, within the 27-m (90-ft) isobath (Figure 1). The depth limit was chosen for diver safety. The direction of the transect was a pre-selected random compass heading.

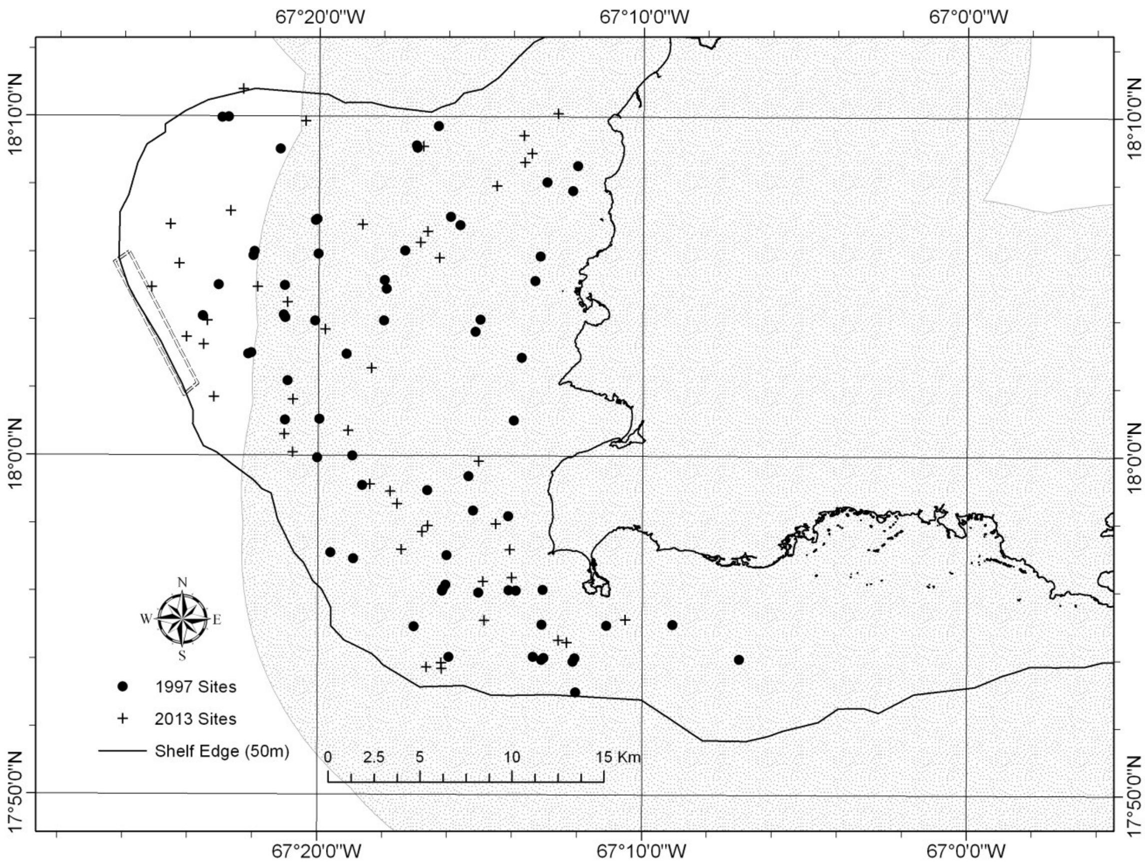

Figure 1: Location of random sample sites for the 1997 and 2013 conch visual surveys on the western platform of Puerto Rico. Double dashed box represents approximate location of the Abrir La Sierra site surveyed by Garcia-Sais *et al.* 2012. Grey stippled area represents waters under local jurisdiction.

Methods for this survey were kept identical to previous years' surveys under the SEAMAP protocol to facilitate comparison of results, with the exception of the timing of the surveys (see Discussion). The 2013 survey was done during the months of October and November. All divers participating were trained in the following: identification of *Strombus gigas,* use of underwater scooters including maintaining constant direction and speed as well as safety protocols, estimating length, identifying age classes using an established reference collection, completing practice transects and recording all applicable data.

At each of the sites, paired visual surveys were done on scuba with the help of underwater scooters to maximize distance traveled. Each diver surveyed a 4m wide transect of variable length depending on depth, current and available dive time, but for a maximum of 45 minutes. One diver trailed a safety buoy, which helped identify the end point of the transect and allowed the surface support vessel to track the divers; the other diver carried a compass set to a fixed random heading so the dive pair could follow a straight line. At the end of the transect, divers signaled to the boat by pulling on the buoy line; the boat then approached the buoy and marked the position using GPS. During the survey, habitat, depth, age class and estimated siphonal length (nearest 1cm using a reference object 20cm long) were recorded for each conch and the time at which they occurred, as well as observations of copulation or egg laying. Any observed changes along the transect of depth and habitat type were also recorded. Classifications of habitat included sand, gorgonians, *Thalassia, Syringodium,* algae, reef, hard bottom or any combination of these. Possible age classes were juvenile (J), newly mature adult (NMA), adult (A), old adult (OA) and very old adult (VOA) and are classified based on shell appearances and lip thickness (Table 1, Figure 2). Distance of each transect was calculated in ArcMap by measuring the straight line distance connecting the start and end positions.

Table 1: Definitions of adult queen conch age classes. Numbers in bold are measurements of lip thicknesses of reference specimens (Appeldoorn *et al.* 2003).

Newly Mature Adult	Flared lip starting to grow or very thin (lip generally <5 mm thick). Periostrocum tan and clean. Often the lip is thin enough to allow the perisotrocum to give color to the underside of the lip. (**4, 7**)
Adult	Flared lip is fully formed, with minimal to moderate erosion. Periostrocum tan but may be sand covered or with some algal growth, Lip underside generally white with pink interior. (**15, 15**)
Old Adult	Outer lip starting to erode (as viewed from bottom). Top of shell still well formed, but periostrocum is lost and spines have rounded, with moderate erosion and fouling on the outside shell. Lip underside may have platinum color, with darker pink interior. (**30, 33**)
Very Old Adult	Lip is very thick and flared portion may be completely eroded away, Outer shell is highly fouled and eroded, often resulting in a short total length. Viewed from the underside, the lip is squared off, the white portion is often completely eroded and the interior is a dark pink. (**42, 59**)

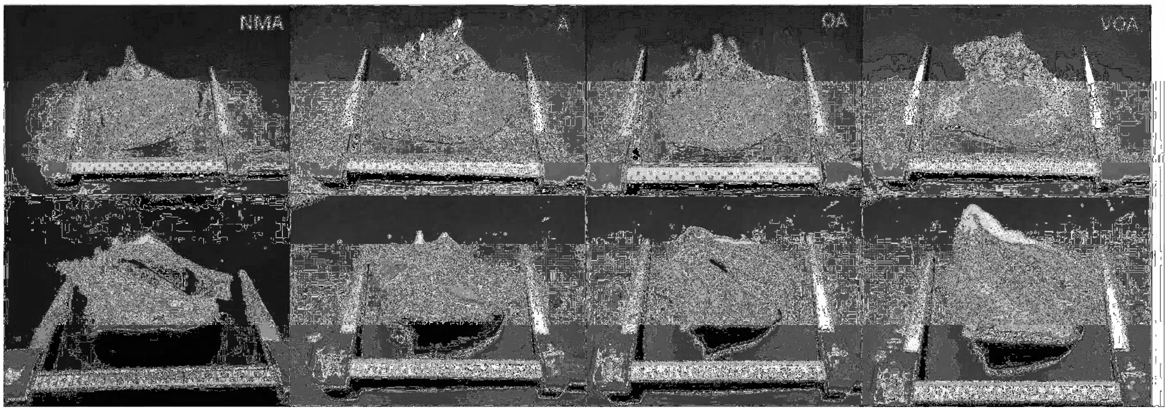

Figure 2: Photographic representation of each of the adult age classes. Abbreviations in the top right are consistent throughout the text. NMA-newly mature adult; A-adult; OA-old adult; VOA-very old adult. Letters inside the shells are arbitrary and numbers are shell length. Specimens are from Puerto Rico. Photographs by D. Sanabria.

Data Analysis

Total area surveyed was calculated by multiplying the length of the transect by 4m width and then doubling the area (two transects per site) and finally summing over all 46 sites (92 transects). Densities were calculated by dividing number of conch observed at each site by the area surveyed. Transects were pooled for one estimate per site. For analyses, each site was classified by the average depth and the dominant habitat type(s) found along the transect. Comparisons of both adult and juvenile densities between years (1997, 2006 and 2013) were made using a model that included year, depth and habitat, and for sites where more than one habitat was dominant, counts of conch were attributed to both habitat types. The data analysis was generated using SAS® software, Version 9.3 (SAS Institute, Cary NC). Analyses were conducted using the PROC GLIMMIX of SAS, based on a negative binomial distribution for the counts. This distribution was chosen over a Poisson because it is better equipped to handle overdispersion. The area of the transect was included in the model as an off-set term. No spatial correlation term was included in the model because the inclusion of the depth and habitat terms explained most of the variability. A separate model substituted a location term for habitat to compare the mean density of adults and juveniles in local waters versus in the EEZ. A Komologov-Smirinoff test was used to assess differences in length frequency distributions between 2006/2013 pooled versus 1997 and a Pearson's chi-squared test was used to test for differences in frequencies of individuals in each age class between 2013 and 1997. An ANOVA and Tukey post-hoc test were done to compare the average length in each age class. The spawning stock for the west coast was calculated using the pooled density for only the older adult age classes (adult, old adult and very old adult) multiplied by estimates of suitable habitat area, i.e., the area of overall polygon used for site selection. These spawning stock estimates were compared to the mesophotic population estimate at Abrir La Sierra (Garcia Sais *et al.* 2012) to determine the potential contribution of the mesophotic population relative to the shallow water stock assuming equal sex ratios and reproductive output per adult.

Results

Forty-six sites were sampled during the course of the 2013 survey (Table 2). Total area surveyed was 37.45 ha, with transect area averaging 0.814 ha and ranging from 0.3 ha at site 5 to 3.97 ha at site 11 (Appendix Table 1). Differences in the amount of area covered are based on a variety of factors including but not limited to depth and current. The total number of conch observed was 380: 194 juveniles and 186 adults (Appendix Table 2). This does not include site 6, where 1,399 juveniles less than ten cm shell length were observed. This site was not included in subsequent analyses due to statistical distortion effects and the lack of observation of this phenomenon in other surveys. Juvenile density ranged from 0 at multiple sites to 34.37/ha at site 37; adult density ranged from 0 at multiple sites to 44.72/ha at site 16 (Appendix Table 2). Total density ranged from 0 at multiple sites to 61.51/ha at site 37. Average total average density was 14.05/ha. This information for all survey years is presented in Table 2.

Table 2: Descriptive statistics for all queen conch visual surveys in Puerto Rico.

Year	Total Number of Sites	Sites in Local Water	Sites in the US EEZ	Total Area Surveyed (ha)	Transect Average (ha)	Total Juveniles	Total Adults	Total Conch
1987	81	81	0	40.81	0.2535	224	107	331
1997	67	58	9	51.32	0.3834	207	85	292
2001	60	54	6	23.58	0.3881	89	60	149
2006	46	38	8	25.2	0.5479	240	205	445
2013	46	37	8	37.45	0.814	194	186	380

Average lengths for each age class are as follows: J 14 cm, NMA 19 cm, A 22 cm, OA 22 cm and VOA 23 cm. Differences in mean length per age class were tested and confirmed through an ANOVA ($F=307.4$, $p<0.001$) and all pairs were significantly different at the $p=0.05$ level except OA-A ($p=0.46$) VOA-A ($p=0.98$) and VOA-A ($p=0.99$) (Table 3).

Table 3: Tukey multiple comparison of means at the 95% confidence level. Diff is average difference between lengths and the p values are adjusted for multiple comparisons.

	diff	Lower	upper	p adj
J-A	-7.924	-8.617	-7.232	0
NMA-A	-2.775	-3.882	-1.667	0
OA-A	0.8675	-0.573	2.3077	0.4683
VOA-A	0.4433	-1.637	2.5235	0.9777
NMA-J	5.1497	4.1265	6.1728	0
OA-J	8.792	7.4156	10.168	0
VOA-J	8.3678	6.3311	10.404	0
OA-NMA	3.6423	2.0172	5.2675	0
VOA-NMA	3.2181	1.0057	5.4304	0.0007
VOA-OA	-0.424	-2.821	1.972	0.9889

To address temporal differences in the mean juvenile, adult and total densities, counts were modelled as a function of year, depth and habitat. Table 4 lists the mean densities of past surveys, though not all were included in this analysis. Surveys for 1987 and 2001 were not included because of limited access to raw data. Table 5 summarizes the results of the analysis.

Table 4: Comparison of mean and range of densities for juveniles, adults and total (conch/ha) for all 5 visual surveys conducted off western Puerto Rico. Where separate juvenile and adult numbers were not reported, original data were analyzed to calculate these densities. Individual transect densities were not available for 1987.

	Total		Juvenile		Adult		
Year	Mean	Range	Mean	Range	Mean	Range	Source
1987	8.11	NA	5.48	NA	2.62	NA	Torres Rosado 1987
1997	8.49	0-247.2	6.24	0-175.07	2.24	0-30.9	Mateo 1997
2001	14.42	0-509.26	10.13	0-445.61	4.29	0-63.66	Appeldoorn 2002
2006	22.4	0-125	11.4	0-120	11	0-53.92	Re-analyzed from Jimenez 2007
2013	14.05	0-61.51	6.73	0-34.37	7.32	0-44.72	This report

Table 5: Model outputs from the analysis of conch counts as a function of year, depth and habitat type. 2013 is the reference year, so its estimate was set to 0. Underlined numbers are significant at the p=0.05 level. Numbers with * are significant at p=0.10 level. SE=standard error. Chi-square values (Chi^2) indicate the fit of each model. Numbers close to 1 indicate a strong fit of the model to the data. Total is adults + juveniles. Numbers in parentheses are the amount of transects with the habitat type present for all 3 years.

Chi^2 =	1.70		1.13		1.60	
Effect	Juvenile Estimate	SE	Adult Estimate	SE	Total Estimate	SE
Year						
1997	-0.6938*	0.3870	-1.0988	0.2943	-0.8406	0.2840
2006	0.4947	0.4689	0.3739	0.3226	0.4652	0.3334
2013	0		0		0	
Depth	-0.00779	0.009189	-0.00946	0.007189	-0.00907	0.006806
Habitat						
Hard Bottom (45)	-1.0300*	0.5466	-0.3161	0.3685	-0.5939*	0.3603
Seagrass (47)	0.4277	0.5229	0.8206	0.3954	0.7175*	0.3809
Reef (21)	-1.8225	0.6266	0.2125	0.4257	-0.6517	0.4121
Sand (45)	-0.6202*	0.3525	-0.2546	0.2886	-0.4675*	0.2624
Algae (50)	-0.3162	0.4375	0.4147	0.3030	0.08332	0.3033
Gorgonians (21)	0.4978	0.5052	0.3455	0.3831	0.2736	0.3704
Mud (9)	-1.3077*	0.6958	-2.0881*	1.1317	-1.2282	0.5679

Depth was not a significant controller of mean density for either juveniles or adult conch, while habitat strongly controlled juvenile mean density. Transects with reef present, (estimate = -1.8225, p=0.05) had significantly lower densities of juvenile conch, while transects with hard bottom (estimate = -1.0300), sand (estimate = -0.6202) and mud (estimate = -1.3077) were also inversely related to juvenile density but at a lower significance level (p=0.10). Higher mean densities of adults were found on transects with seagrass present (estimate = 0.8206, p=0.05). *Thalassia* composed 40% of the seagrass sites, 54% were a mix of *Thalassia* and *Syringodium* and the remaining 6% were *Syringodium* alone.

There was a lower mean density of total conch in 1997 (estimate = -0.8406) compared to 2006 or 2013 (Table 5, Table 6). There was also a lower mean density of adult conch in 1997 (estimate = -1.0988) compared to 2006 and 2013 (Table 5, Table 6). The same trend was observed for juveniles, but at a lower significance level (estimate = -0.6938, p=0.10) (Table5, Table 6).

In 1997, conch fishing grounds in Puerto Rico within the US EEZ were permanently closed to conch fishing. To test for population differences as a function of location and management regime, (closed EEZ versus open local waters) density was modelled as a function of year (1997 or 2013=reference), depth and location (local or EEZ=reference). A separate model that included a year*location interaction term showed that this term was not significant, tested at the p=0.05 level (estimate = -0.1097), so the simpler model without the interaction term was used. For adults, the terms year, depth and location were all significant at p=0.05 (Table 6). The analysis shows that on average, in 1997 adult density across the shelf was less than in 2013. Regardless of year or location, adult density was higher in shallow water; and regardless of depth or year, adult densities are higher in the EEZ than in local waters. Adult density increase disproportionally in the local waters (by a factor of 2.6) from 1997 to 2013 compared to the EEZ waters (factor of 6.3) for the same years.

Table 6: Summary of the effects estimates from the analysis of juvenile and adult queen conch density as a function of year, depth and location. Local refers to local waters or 0-9NM from Puerto Rico; EEZ refers to 9-200NM from Puerto Rico. 2013 and EEZ were the reference points, so the estimates were set at 0. Chi^2 is the Pearson's chi-squared value divided by the degrees of freedom and indicates the fit of the model. Numbers close to 1 indicate a good fit of the model. SE=standard error. Underlined numbers are those significant at the p=0.05 level. * indicate those effects significant at p=0.10.

Chi^2=	1.18		1.54	
Effect	Adult Estimate	SE	Juvenile Estimate	SE
<u>Year</u>				
1997	<u>-1.1099</u>	0.2718	-0.3186	0.3376
2013	0		0	
<u>Depth</u>	<u>-0.02542</u>	0.006679	<u>-0.02773</u>	0.006391
<u>Location</u>				
Local	<u>-1.4388</u>	0.4293	-0.7810*	0.4787
EEZ	0		0	

Modelling of the mean density of juveniles, including the same terms as in the adult analysis, showed different results. Again, a model including the year*location term interaction term was not significant (estimate = 0.7828) tested at p=0.05. Only the depth term was significant at p=0.05 (estimate = -0.02773). The location term was significant (higher juvenile density in the EEZ) at p=0.10 level (estimate = -0.7810) (Table 6), and the year effect was not significant at either level.

Population changes were also assessed using age and length-frequency analyses. The Kolmogorov-Smirnov test was used to test for differences in the length-frequency distributions between 1997 and 2006/2013 pooled. The Pearson's chi-squared test was used to test for differences in age-class structure, between the 1997 and 2013 surveys. The length frequency distributions (Figure 3)

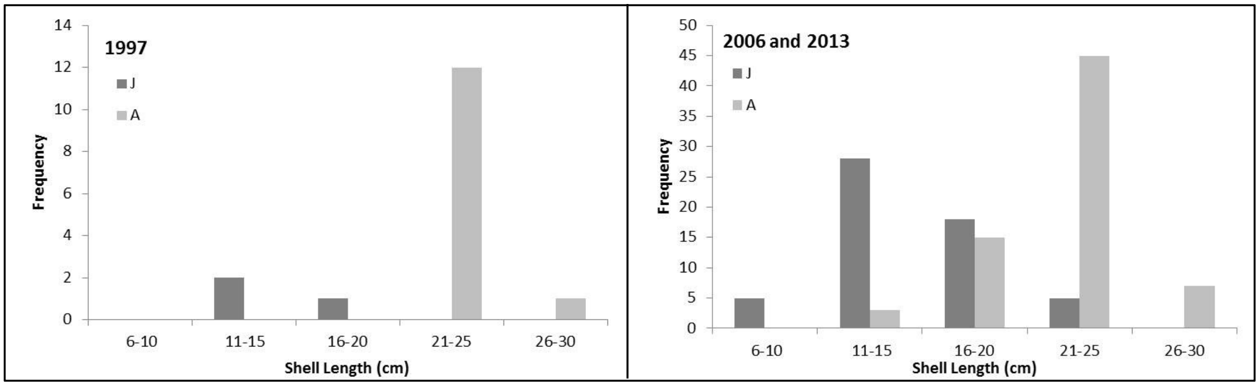

Figure 3: Length-frequency distributions for juveniles (dark) and adults (light) between 1997 and 2006/2013 pooled.

show a statistically significant proportional increase in the number of adults less than 20-cm, specifically in the 16-20-cm range in the later years (D=0.2854, p<0.001).

The age class structure of Queen Conch in 2013 is markedly different from that of 1997 (x^2=50.0427, p<0.001) (Figure 4). One of the most obvious differences is the absence of VOA in 1997. Additionally, in 2013, approximately 50% of the population were juveniles, whereas in 1997, 70% of the Queen Conch were juveniles.

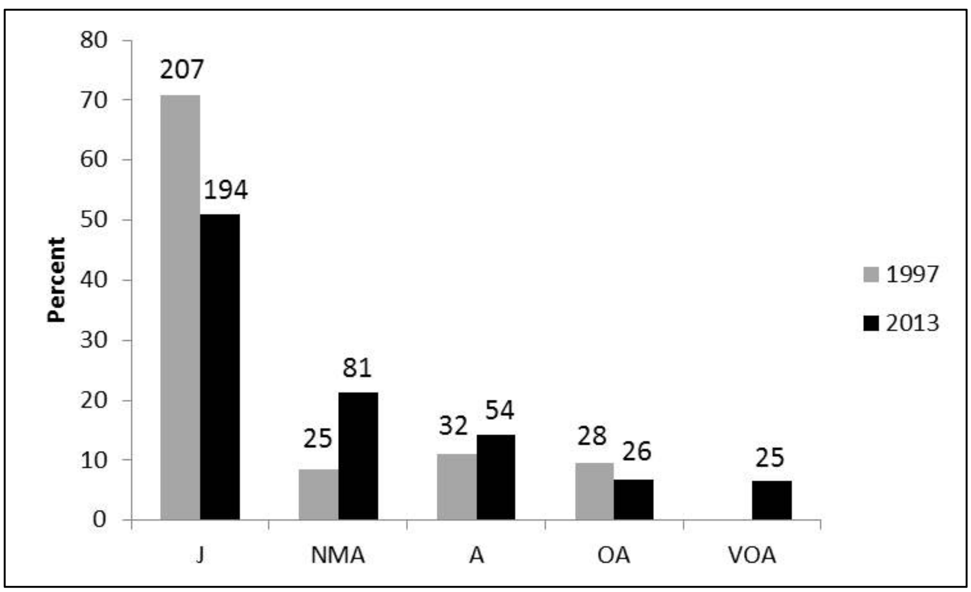

Figure 4: Age class structure of queen conch off the west coast of Puerto Rico for 1997 (grey) and 2013 (black). Numbers above the bars are frequencies.

The potential importance of the spawning population at mesophotic depths relative to that on the shelf was assessed by comparing the number of full adults on the shelf to the estimates of Garcia-Sais *et al.* (2012). The calculated density of spawners (i.e., the older age classes of adult, old adult and very old adult) on the shelf for the 2013 survey was 4.105/ha (90% confidence interval of ±1.61/ha). Over the 42,074ha that were identified as conch strata, (i.e., past and present fishing areas as well as juvenile areas; area of polygon used for site selection) there is an estimated spawning stock of 104,763-240,241 individuals (mean = 172,705). Therefore, while the mesophotic conch population at Abrir La Sierra is of high density (195/ha on the rhodolith reef), the available habitat is small (only 321ha), and its number of individuals (29,092) only constitutes 14% of the mean spawning population off the west coast.

Discussion

The high number of juveniles seen at site 6 is the emergence of the Age 1 year class. Due to the varying nature of the annual timing of the previous visual surveys (Table 7), and the lack of appearance of this phenomenon reported for those surveys, the site was excluded from statistical analysis. This site is in deep water (mean transect depth of 22m =72.5ft) with a mixed habitat of sand and algae. All conch observed were less than 10cm in shell length and represented the 2012 year class emerging from a first year of burial (Stoner *et al.* 1988, Appeldoorn 1990).

Table 7: Months of the year conch visual surveys were conducted for Puerto Rico. During the 1987 study, fieldwork was conducted each month of the year.

Year	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1997	x						x	x	X
2001	x	x			x	x	x	x	
2006				x	x	x			
2013							x	x	

Mean densities for temporal comparisons were modelled using the effects of year, depth and habitat. Depth and habitat are known controllers of both adult and juvenile distributions. Juveniles prefer shallow, seagrass areas with currents (Stoner and Waite 1991, Stoner *et al.* 1996, Stoner 2003). Adults on the other hand, are tolerant of a wider range of environmental conditions. Sand/algal flats provide nutrition, but adults also utilize hard bottom habitats (Torres Rosado 1987, Acosta 2001, Stoner and Davis 2010) and are commonly found up to 25m depth (Stoner and Schwarte 1994). Our findings are consistent with these documented habitat preferences. Juveniles were less dense in hard bottom, reef, sand and mud habitats. In our survey, adults were found in higher density in seagrass habitats, and in lower density in mud habitats. A large portion of the adults observed were in the NMA category (44.1%), and a majority of those, (37.8%) were found in seagrass habitats. NMA adults may not be reproductively active, and therefore not yet relocated to the sand/algae habitats of the older adults.

Characteristics of an increasingly healthy conch population would include higher adult density, especially in relation to spawning (see below), an increase in proportion of older adults, and evidence of sustained recruitment. Thus, the increase in mean adult density between 1997 to 2013 is a positive sign, as are the changes observed in length/age frequency distributions (Figures 3 and 4). Over the past 16 years, management regulations have been put in place to help the population recover from overfishing. In 1997, the EEZ was closed to fishing, and in 2004 minimum size limits, minimum lip thickness, seasonal closures and bag limits were all established. All of these regulations are aimed at lowering fishing mortality. Mean density of juveniles increased in the same time period, but not at the same significance level ($p=0.10$). Decreasing fishing pressure, which most juveniles are not subject to, does not affect the population the same way it does adults. These regulations cannot address natural limiting factors such as recruitment, which ultimately control juvenile density.

The density of conch in local waters was compared to that in the EEZ to investigate the effect of the EEZ closure in 1997. Model output showed depth was a significant factor affecting both juvenile and adult density; density of both decreased as depth increased. This is consistent with the shallow water preferences of juveniles. The decrease in mean density of adults with increasing depth may be due to their habitat preferences. No site over 17m deep was seagrass, the habitat type that had significantly higher adult densities. In contrast, 71% of the sites less than 17m were seagrass habitats, and habitat is a stronger controller of distribution than depth alone. The most interesting result of this analysis was the significance of the location effect, and subsequent lack of significance of the year*location term. Adults were found in higher density within the EEZ, but this effect was observed across years (i.e. higher adult densities were found in the EEZ in both 1997 and the recent surveys). It is interesting to note that the adult density in local waters increased only by a factor of 2.6 from 1997 to 2013 (from 2.26/ha to 5.98/ha) while the adult density in the EEZ increased by a factor of 6.3 (from 2.15/ha to 13.50/ha). Though the limitations of the sampling design prevent the separation of the effect of the reserve in the EEZ from the increase in density overall, the closure of the EEZ does seem to be having a positive effect.

One of the most striking differences between 1997 and recent surveys (2006-2013 pooled) was the proportional increase in smaller adults (16-20 cm size class). It is possible that the minimum size restrictions put in place in 2004 are responsible for this effect. This size class is below the 9-inch (22.86mm) minimum shell length needed to legally harvest. Conch that would mature within this size range (thus fixing adult shell length) would not be eligible for legal harvest until their shell lip-thickness reached 3/8-inch (9.5 mm) at which point they would be classified as normal adults. Accounting only for the required growth in shell lip-thickness, this provides a minimum of at least a half year of extra protection from fishing mortality (Appeldoorn 1988) beyond the additional protections afforded by the minimum shell length.

Additionally, a greater proportion of the current population is composed of adults and these adults are distributed across all age classes in a manner consistent with a significant decrease in overall total mortality. Unfortunately, because these adult age classes cannot be readily converted to age, an exact estimate of mortality is not possible. Nevertheless, having a higher percentage of adults, and presence of the oldest adult age class, means that there has been a marked increase in the spawning stock. While the average density of the spawning stock is low (see below), a recent field study on the western platform found maximum rates of egg laying and copulation to be 16% and 12%, respectively (Appeldoorn *et al.* 2011b), suggesting that conch occur locally in sufficiently high density to maintain reproductive activity.

In contrast to previous survey reports, the inclusion of a robust statistical analysis here helped to clarify trends in the recovery of the conch population. Based on a trend of increasing densities (Table 4) and the presence of more and older adults, previous reports have argued that, although the population was overfished, it was improving (SEDAR 2007). But in the present survey, total density did not continue the upward trend, and the statistical analysis came to a different conclusion. Though total density is higher since 1997, no significant differences were found in total density since 2006, and in fact, the total density was less in 2013. It may be that the protections offered by the current regulations have reached their maximum impact relative to the present level of fishing pressure, or that the current sampling design and effort are insufficient for addressing temporal density changes in the face of strong habitat and depth effects.

Results suggest that changes in methodology are needed to make stronger conclusions about temporal trends and the effectiveness of management regulations. Incorporation of the generalized linear model approach into future sampling should have several positive effects. First, it will give survey results a more powerful base of interpretation. More importantly, however, the statistical model can be used to structure the design of future surveys so as to greatly reduce sample variance and increase sample efficiency (e.g., Smith *et al.* 2011). For example, the statistical models confirmed what has been known about the importance of depth and habitat in the distribution of queen conch. Because of this, current sampling methods should be altered to more directly account for these factors. It would be much more effective to control for these variables when selecting sites. This can be accomplished by one of two ways: (1) choose sites that cover a variety of depths and habitat types, taking great care in the initial selection, and then re-surveying the same sites year after year or (2) selecting new sites each year, but ensuring the sites are stratified over a variety of depths and habitats. Stratifying the site selection by habitat would make the habitat analysis more clear, but this can only be done using a detailed habitat map of the entire west and southwest platform of which there currently is none. However, high resolution bathymetry (including back-scatter) is available for this region, as well as the technology for using this to develop detailed habitat maps (Costa *et al.* 2009, Pittman *et al.* 2009). With detailed habitat information, it may be beneficial to change the format of SEAMAP sampling to utilize a greater number of small, fixed area samples rather than the fewer but longer, underwater scooter-based transects currently used. As a consequence, each transect would then have only one specific habitat and depth. This would reduce the noise in the data generated by trying to account for these variables in the post-hoc analysis. A prime example of the effect of this is the average depth of each of the surveys (Figure 5). The average depth across all sites in 2006 is shallower than 1997, 2001 and 2013. This raises the question as to whether the higher density seen in the 2006 survey represents a true improvement in the population (changes in adult age distribution notwithstanding), given that statistical analysis showed shallower depth is related to higher densities. Changes in mean depth of sites probably resulted from the change in sample allocation across the shelf due to the incorporation of new strata in 2006 and the lack of sample allocation outside those strata.

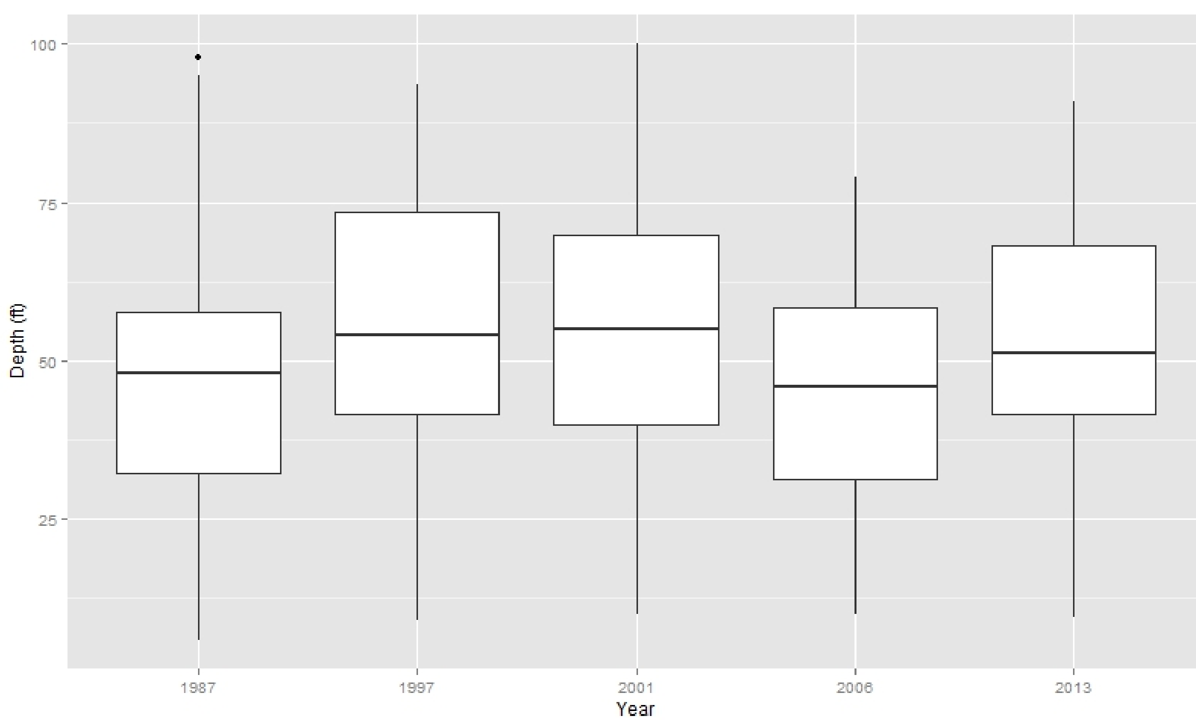

Figure 5: Box plots comparing average depth (ft) among queen conch visual surveys.

The power of the test to determine the effectiveness of the EEZ closure is also very limited because it was a post-hoc analysis. A small, and unequal number of sites were chosen in the EEZ area, which forced 2006 and 2013 data to be pooled, limiting the conclusions that can be drawn from the analysis. To increase power, the survey would have to be specifically designed to address this question, adding a much greater number of sampling sites within the EEZ, despite its relatively small area, while keeping the same number of sites in the local areas. This would still allow for characterization of the overall population, while testing the closure hypothesis.

The analysis of the spawning population on the shelf versus Abrir La Sierra suggests that the deeper population constitutes only 14% of the total population of spawning adults. The significance of this is not clear, however, because conch reproductive output is dependent upon a number of factors. One of the most important is adult density (Stoner and Ray-Culp 2000). Conch reproduce through copulation, and given their limited ability to move, maintaining high density is critical to ensuring reproduction. The density reported at Abrir la Sierra (195/ha) is well above the minimum density of 50 conch/ha reported by Stoner and Ray-Culp (2000) needed to avoid Allee effects, and approaches the density where mating frequency plateaus (Stoner *et al.* 2012). These values vary geographically, as probability of mating reaches 100% in the Exumas, Bahamas at 110/ha, but reaches only 90% probability at 350/ha in Andros and 570/ha in the Berry Islands (Stoner *et al.* 2012). The density at ALS also exceeds 140/ha, what is considered the population density needed to achieve maximum sustainable yield elsewhere in the Caribbean (SEDAR 2007, Appeldoorn *et al.* 2011a). In contrast, the highest individual density estimate observed for mature adult conch on the shelf was only 24.4/ha over a whole transect. Thus, the slope population at Abrir la Sierra may be contributing disproportionally more than its abundance alone would indicate. However, while Garcia *et al.* 2012 reported observing egg deposition at Abrir la Sierra, the rate was not quantified, so no comparisons can be made to those observed in shallower depths. Lastly, dispersal of conch larvae may be significantly different for eggs hatched in deeper waters on the shelf margin, than for those hatched on top of the platform, but comparative studies are not available.

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Appendix Table 1: Longitude and latitude coordinates (decimal degrees) for start and end positions for the 2013 paired visual transects. Depth (m) is the average between start and end depth. Habitat types are as follows: 1 – hard bottom/rubble; 2- seagrass; 3-reef; 4-sand; 5-algae; 6-gorgonians; 7-mud. Area surveyed at each site is in hectares. Station numbers are not sequential because initially selected sites deeper than 27m were not sampled.

Station	Long Start	Lat Start	Long End	Lat End	Habitat	Depth	Area
1	-67.27755	18.11020	-67.28854	18.11044	4,5	18.5	0.9304
2	-67.20974	17.90930	-67.20416	17.90814	4,1	14.7	0.4832
3	-67.34033	18.16436	-67.32837	18.16621	6	20.1	1.024
4	-67.29630	17.98237	-67.28369	17.98374	4,5	20.4	1.0752
5	-67.30613	18.04306	-67.30534	18.04636	4,5	24.3	0.3
6	-67.31088	18.11362	-67.30772	18.11775	4,5	22	0.4528
7	-67.27985	17.96264	-67.27284	17.95759	2,5	14.6	0.7424
8	-67.28112	18.10493	-67.28001	18.09773	4,5	10.2	0.6432
9	-67.27983	18.15210	-67.28094	18.16126	3	7.6	0.8
10	-67.22765	18.14433	-67.22371	18.14843	2,4	7.3	0.4928
11	-67.24194	18.13265	-67.28827	18.13470	2	3	3.9272
12	-67.37877	18.12012	-67.37615	18.10837	4,6	23.9	1.0632
13	-67.29057	17.95380	-67.29922	17.95381	4,5	21.3	0.7336
14	-67.39237	18.05451	-67.38200	18.06071	4,3	20.8	1.0344
15	-67.20541	17.90834	-67.20291	17.90261	4,5	15.5	0.5496
16	-67.24787	17.91916	-67.24876	17.92464	2,5	12.8	0.492
17	-67.38714	18.02860	-67.38414	18.03045	4,5	15.6	0.3016
18	-67.23367	17.94018	-67.24352	17.94197	2	11.5	0.8496
19	-67.35084	18.01042	-67.35601	18.01454	3,4	18.5	0.5696
20	-67.34944	18.07535	-67.34183	18.08228	4,1	23.7	0.8904
21	-67.30673	17.98602	-67.29678	17.98479	3	19.2	0.8488
22	-67.41924	18.08263	-67.41341	18.08047	3	16.1	0.528
23	-67.17542	17.91961	-67.17207	17.91564	6,5	12.6	0.4504
24	-67.22394	18.14887	-67.22942	18.15018	2	8	0.3816
25	-67.36474	18.08293	-67.36566	18.07399	4	27.7	0.7784
27	-67.24203	17.96659	-67.24892	17.96795	4,5	8.2	0.596
28	-67.40976	18.11349	-67.40544	18.12057	1	24.2	0.7264

Appendix Table 1 continued

Station	Long Start	Lat Start	Long End	Lat End	Habitat	Depth	Area
29	-67.27746	17.89609	-67.28080	17.92260	1,6	21.1	2.3648
30	-67.24846	17.93828	-67.25859	17.93848	2,5	13.2	0.8592
31	-67.23480	17.95396	-67.23985	17.94881	2,5	10.6	0.5952
32	-67.27705	17.96561	-67.27782	17.96941	2,4	15.2	0.3424
33	-67.26975	17.89825	-67.26881	17.90867	6,4	16.7	0.9256
34	-67.31804	18.01221	-67.32545	18.01010	1	15.5	0.6552
35	-67.40501	18.09425	-67.40598	18.08797	6,3	18.5	0.5928
36	-67.21063	18.16835	-67.20764	18.17109	2,5	6	0.3976
37	-67.40116	18.05823	-67.39548	18.06139	1,6	14.9	0.5528
38	-67.29276	17.97640	-67.28926	17.96766	4,5	22.5	0.828
39	-67.39057	18.06617	-67.39052	18.07480	4,5	23	0.7648
40	-67.27128	18.09733	-67.27348	18.09109	2,6	5.9	0.5824
42	-67.26957	17.89536	-67.27339	17.90212	1,6	18.7	0.68
43	-67.34658	18.00154	-67.35453	18.00716	4,3	16	0.8384
44	-67.25080	17.99741	-67.23418	17.99046	2	13.5	1.5744
45	-67.34643	18.02754	-67.35651	18.02880	3,4	21.9	0.8608
46	-67.22816	18.15748	-67.22880	18.15103	2	12.6	0.5728
48	-67.27627	17.98872	-67.26698	17.99150	5,4,2	15.3	0.824
50	-67.17708	17.96208	-67.18580	17.95488	2	2.8	0.9752

Appendix Table 2: Counts and calculated densities for each of 46 stations sampled for the 2013 queen conch survey off western Puerto Rico. Densities are conch/ha.

	Count						Density		
Station	J	NMA	A	OA	VOA	Total	J	A	Total
1	14	0	1	0	0	15	15.05	1.07	16.12
2	0	0	2	0	0	2	0.00	4.14	4.14
3	13	2	1	0	0	16	12.70	2.93	15.63
4	2	1	2	1	1	7	1.86	4.65	6.51
5	4	0	0	0	0	4	13.33	0.00	13.33
6	1399	0	0	0	0	1399	3089.66	0.00	3089.66
7	8	0	1	0	0	9	10.78	1.35	12.12
8	4	1	0	0	0	5	6.22	1.55	7.77
9	0	3	1	0	1	5	0.00	6.25	6.25
10	5	5	1	0	0	11	10.15	12.18	22.32
11	4	1	0	0	0	5	1.02	0.25	1.27
12	4	4	1	1	0	10	3.76	5.64	9.41
13	7	0	1	0	0	8	9.54	1.36	10.91
14	2	2	0	0	0	4	1.93	1.93	3.87
15	1	0	1	0	0	2	1.82	1.82	3.64
16	4	10	8	1	3	26	8.13	44.72	52.85
17	1	0	2	2	5	10	3.32	29.84	33.16
18	4	0	0	0	0	4	4.71	0.00	4.71
19	0	0	3	1	0	4	0.00	7.02	7.02
20	0	0	0	0	0	0	0.00	0.00	0.00
21	0	0	0	0	0	0	0.00	0.00	0.00
22	4	2	5	0	0	11	7.58	13.26	20.83
23	10	1	3	3	3	20	22.20	22.20	44.40
24	0	1	0	0	1	2	0.00	5.24	5.24
25	0	0	0	0	0	0	0.00	0.00	0.00
27	0	6	0	2	0	8	0.00	13.42	13.42
28	4	0	1	0	0	5	5.51	1.38	6.88
29	2	0	0	2	3	7	0.85	2.11	2.96
30	8	2	0	2	1	13	9.31	5.82	15.13
31	6	3	1	1	0	11	10.08	8.40	18.48
32	4	1	0	1	0	6	11.68	5.84	17.52
33	11	0	1	0	0	12	11.88	1.08	12.96
34	0	0	1	0	0	1	0.00	1.53	1.53
35	14	8	3	0	3	28	23.62	23.62	47.23
36	7	7	0	3	1	18	17.61	27.67	45.27
37	19	6	8	1	0	34	34.37	27.13	61.51
38	2	1	1	0	1	5	2.42	3.62	6.04
39	0	1	0	2	1	4	0.00	5.23	5.23
40	4	2	0	0	0	6	6.87	3.43	10.30

Appendix Table 2 continued

	Count						Density		
Station	J	NMA	A	OA	VOA	Total	J	A	Total
42	2	1	2	3	0	8	2.94	8.82	11.76
43	0	0	0	0	1	1	0.00	1.19	1.19
44	3	0	0	0	0	3	1.91	0.00	1.91
45	0	0	2	0	0	2	0.00	2.32	2.32
46	17	10	1	0	0	28	29.68	19.20	48.88
48	0	0	0	0	0	0	0.00	0.00	0.00
50	0	0	0	0	0	0	0.00	0.00	0.00
Total	194	81	54	26	25	380			
Average							6.73	7.32	14.05