Preliminary Report on Population Genetic Structuring among Queen Conch (*Strombus gigas*) from The Bahamas

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Introduction

Identifying genetically distinct populations is a critical link for guiding sustainable management of heavily exploited fishery species. Early genetic studies with queen conch (*Strombus gigas*) using electrophoretic methods indicated a high degree of gene flow among populations dispersed over the species' geographic distribution, with definitive separation observed only between populations in Bermuda and those in the Caribbean basin (Mitton et al. 1989). Two relatively recent developments indicate that a closer look at population connectivity is warranted. First, we know now that pelagic larvae are often retained within close proximity to the parental stocks by mesoscale and fine-scale ocean circulation in the Caribbean region allowing for localized self-recruitment patterns (Kool et al. 2010). Second, new, more sensitive genetic tools have been developed revealing previously undetected genetic structure within populations of Caribbean species (see Christie et al. 2010) that is relevant to fisheries management. Knowledge of genetic connectivity among stocks is critical in determining the appropriate geographic units for fisheries management including quotas, the design of marine protected areas, and international relations related to sources of recruitment for fishery stocks in a complex geopolitical environment such as the Caribbean Sea.

Queen conch are currently harvested in nearly all of the 30 nations and territories occupying the Greater Caribbean region. Declining stocks have resulted in the closure of some of the fisheries, queen conch trade is regulated under the Convention for International Trade in Endangered Species, and the species is currently being considered for Endangered Species status in the United States. This provides important impetus for exploring the molecular genetics of queen conch.

This study was conducted to evaluate whether polymorphic DNA microsatellite markers isolated in queen conch by Zamora-Bustillos et al. (2007) can be used to differentiate geographically distant populations of conch in The Bahamas. We concluded that if this preliminary screening revealed genetic distinction in populations separated by 500 kilometers then microsatellite markers can be used for stock identification over the broader Caribbean region and within large nations such as The Bahamas, Cuba, and Venezuela where important fisheries for queen conch are prosecuted.

Methods

During November 2013, 30 adult queen conch were collected in each of two locations for analysis of genetic structure: 1) near West End Grand Bahama Island, and 2) in the northern Jumentos Cays (Figure 1). As conch were extracted from their shells for market small pieces of mantle tissue (< 1 cm^2) were excised and placed on filter paper for drying. These dried materials were shipped to the Hatfield Marine Science Center for analysis.

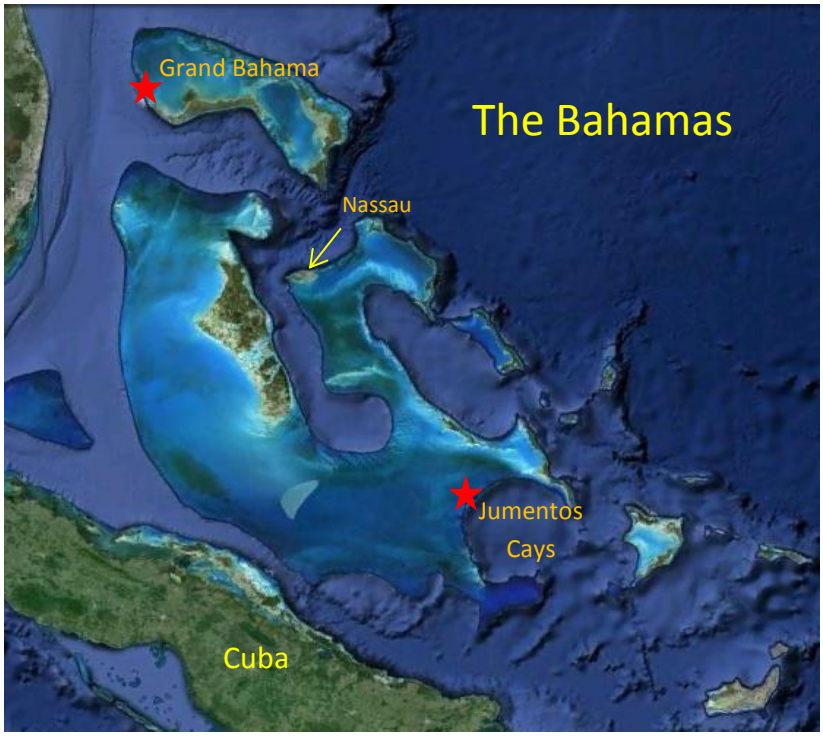

Figure 1. Map of The Bahamas showing the two locations where queen conch samples were collected for genetic analysis. Location of the capital city Nassau is also shown, where much of the queen conch is landed. Distance between the two collecting sites is approximately 500 kilometers.

All of the 60 conch where characterized using genetic markers known as microsatellites. DNA extraction of mantle tissue samples followed a silica-based method utilizing multichannel pipettes, PALL glass fiber filtration plates, buffer, centrifuge and transfer protocols described in Ivanova et al. (2006). Samples that failed to amplify were re-extracted using ethanol precipitation. Eight microsatellites (*Sgig-1-8*) were amplified using the polymerase chain reaction following methods described in Zamora-Bustillos et al. (2007). Fluorescently labelled products were electrophoresed using a 3700xl Applied Biosystems® DNA Analyzer and scored

using GENEMAPPER software. Preliminary analysis of allele frequency, population relationship (FCA) and genetic distinction (Fst) were assessed using tests available in the computer applications named GENETIX (Belkhir et al 1996-2004) and GENEPOP (Raymond & Rousset 1995; Rousset 2008).

Results and Discussion

Within population sample findings were not typical of normal random breeding populations, but we note that a sample size of 30 is too small to provide reliable allele frequency estimates, especially for about half of the loci which had more than 20 alleles (Table 1). *Sgig-1 & 5* had strikingly less observed heterozygosity (Ho) than expected (He) in both population samples, and overall significant Hardy-Weinberg-Castle (HWC) findings were large owing to heterozygosity deficiencies (all but *Sgig-2 & 7* were significant).

Table 1. Basic within population findings for Queen Conch using eight microsatellites (Sgig-1-8*)*.

	W Grand Behamas					Jumentos				
	# alleles	private alelles	He	Ho	HWC	# alleles	private alelles	He	Ho	HWC
Sgig-1	6	0	0.7878	0.4828	0.0011	6	0	0.7972	0.6000	0.0261
Sgig-2	4	1	0.6594	0.5667	0.252	4	1	0.5928	0.6000	0.1804
Sgig-3	13	4	0.8856	0.8000	0.0079	9	1	0.8228	0.5333	0
Sgig-4	20	4	0.9183	0.8000	0.0036	18	3	0.9178	0.5667	0
Sgig-5	20	7	0.9256	0.5333	0	17	4	0.9031	0.3793	0
Sgig-6	5	1	0.6628	0.4000	0.0001	6	2	0.7044	0.7333	0.0548
Sgig-7	5	1	0.7139	0.6333	0.1363	6	2	0.8022	0.9667	0.0078
Sgig-8	16	2	0.8835	0.6207	0	19	5	0.9156	0.8276	0.0133

Because of sampling shortcomings all results presented here are strictly preliminary as a means of indicating potential. Significantly larger sample sizes per population sample are required (approx. 160, see Banks et al. 2000) and probably twice the number of microsatellites should be observed before strong inference on population relationship can be drawn. Note also that the eight microsatellites used here vary substantially in their average number of alleles; *Sgig-1, 2, 6 & 7* all had less than 10 alleles, whereas *Sgig-3, 4, 5 & 8* had between 14 and 24 alleles (many more alleles than previously published by Zamora-Bustillos et al. 2007). Given sample numbers it is not reasonable to perform many tests for population relationship, distinction and assignment rates because preliminary results are certain to change once more conch are sampled and more microsatellites are observed. However, we did calculate Wright's fixation

index (Fst), a broadly applied assessment of within- and among-population sample genetic relationship often used to assess genetic distances among population samples:

> For all eight loci, Fst = 0.0088 and permutation tests indicate that this distance is significant (p = 0.026).
>
> For the four less polymorphic loci (*Sgig-1, 2, 6 & 7*), Fst = 0.0000 and permutation tests are not significant (p = 0.436).
>
> For the four more polymorphic loci (*Sgig-3, 4, 5 & 8*), Fst = 0.0157 and permutation tests indicate that this distance is significant (p = 0.002).

Overall association between the two collection sites was assessed through Factorial Correspondence of Analysis (FCA). This technique is suitable for categorical data, which allows investigation of correspondence between rows (i.e., individuals) and columns (i.e., alleles) in a two-way table. It enables graphical visualization of individuals in multidimensional space, with no *a priori* assumptions about grouping, using each allele as an independent variable. Axes are generated from combinations of alleles that explain portions of the total observed ‘‘inertia’’ of the table. Thus, those alleles exhibiting the strongest nonrandom association within groups of individuals will contribute most to the axes. The overall population relationship as depicted by FCA for these two populations from The Bahamas (Figure 2) indicates that the genetic relationships are more similar within population samples (same color) than between the populations. Minimal to no overlap of space occupied by the two population samples combined with the significant Fst observations detailed above indicate potential for application of similar genetics tools for population distinction and traceability among queen conch even within The Bahamas region.

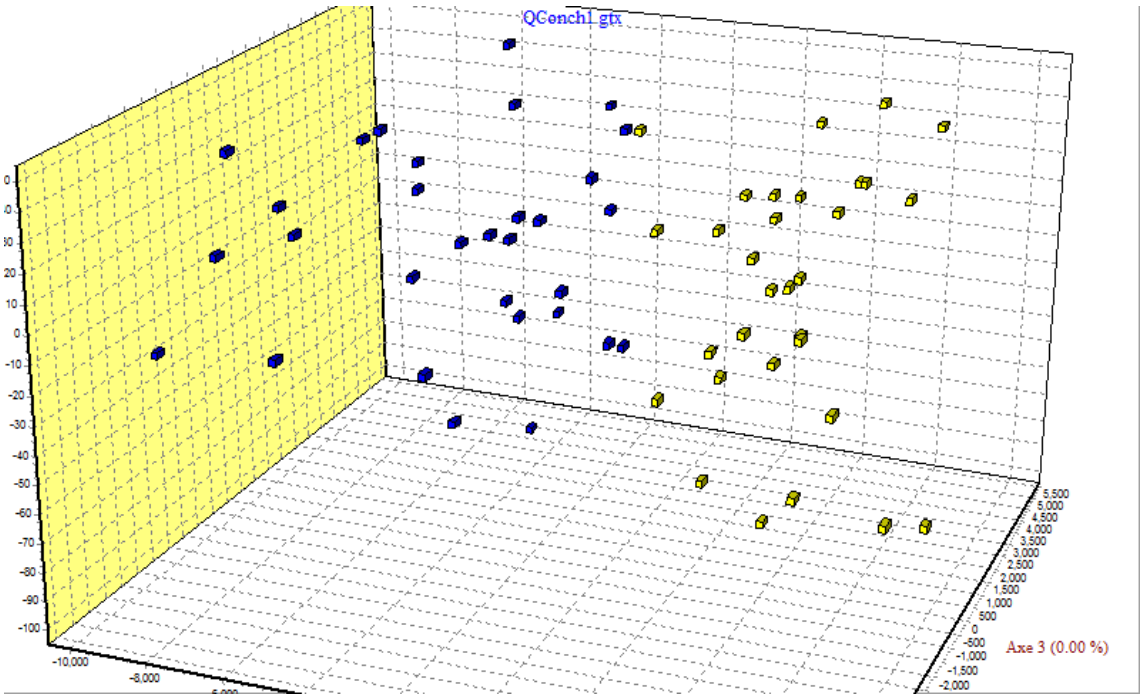

Figure 2. Factorial Correspondence Analysis for queen conch samples from two sites in The Bahamas (Grand Bahama (yellow) and Jumentos Cays (blue)).

Acknowledgements

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