Fine-Scale Population Structure of *Lobatus gigas* in Jamaica

Estructura Poblacional a Escala Detallada de *Lobatus gigas* en Jamaica

Structure de Population à Petite Échelle de *Lobatus gigas* en Jamaïque

KIMANI A. KITSON-WALTERS[1]*, ADAM S. CANDY[4], NATHAN K. TRUELOVE[3], MARCIA E. ROYE[1], MONA K. WEBBER[2], KARL A. AIKEN[2], and STEPHEN J. BOX[3]
[1]*Biotechnology Centre*
2 St. John's Close, University of the West Indies, Mona Campus, Jamaica.
kimash21@gmail.com
[2]*Department of Life Sciences*
4 Anguilla Close, University of the West Indies, Mona Campus, Jamaica
[3]*Smithsonian National Museum of Natural History*
Smithsonian Marine Station, Fort Pierce, Florida 34949 USA.
[4]*Environmental Fluid Mechanics Section — Faculty of Civil Engineering and Geosciences, Delft University of Technology, Delft, The Netherlands.*

ABSTRACT

Jamaica is one of the few remaining countries in the Caribbean region with an abundant population of *Lobatus gigas* (queen conch) able to sustain a lucrative fishery. Efforts to understand and maintain queen conch populations must involve an investigation into genetic connectivity. This connectivity facilitates population replenishment and continuity via the transport of veliger larvae by ocean currents. Due to the lack of knowledge in this regard to queen conch populations in Jamaica, the fine-scale population structure of *Lobatus gigas* populations in the country's Exclusive Economic Zone (EEZ) has been analysed by comparing the allele frequencies of nine microsatellite loci on a total of 459 individuals collected across twelve sites encompassing nearshore and offshore locations. Samples were grouped into five broad scale geographic clusters for statistical analysis. Our findings indicate that a weak but significant population structure exists (Global Fst = 0.004, $p = 0.01$) suggesting that mainland Jamaica acts as a weak divide between populations north and south of the island. Greater levels of connectivity are suggested between north coast populations and those present at the Formigas Bank, an offshore site northeast of the island. The island's primary conch fishing ground located offshore on Pedro Bank, receives limited gene flow from the other sampled populations and may be heavily dependent on local recruitment or receive recruits from sources external to Jamaica's EEZ. An analysis of surface ocean currents strongly supports these three findings and further that conch populations on Pedro Bank very likely receive recruits from sources distinct to those that supply nearshore populations. Further genetic studies into the recruitment patterns and sources for the community on Pedro Bank are therefore critical to ensure sustainable management of this commercially threatened population. Decades of intense fishing pressure has resulted in the establishment of the Allee effect on the island shelf, significantly hampering reproduction and consequently recruitment. If the question of recruitment on Pedro Bank is not addressed, further development of the Allee effect there and eventual population exhaustion are inevitable. These findings, their implications and recommendations for the management of the queen conch fishery in Jamaica are discussed.

KEYWORDS: Queen conch, Jamaica, genetics, *Lobatus gigas*, microsatellites, hydrodynamics, ocean currents

INTRODUCTION

The conservation of commercially and ecologically important species has become a multidisciplinary effort that has evolved far beyond just biological and environmental monitoring, to now including genetic methods. The field of conservation genetics has proven to be an informative tool in understanding the complex interactions of populations across spatial and temporal scales. Using this approach, we conduct the first examination of the fine-scale population structure of a commercially endangered species, the queen conch (*Lobatus gigas*), in one of the last remaining abundant populations within the species' geographic range (Aiken et al. 1999, Prada et al. 2008, Tewfik 1997). The queen conch supports an export-focused industrial scale fishery in Jamaica that is based on Pedro Bank, the island's primary fishing ground. The increase in demand for conch in the early 1990's led to unsustainable increases in annual exports with 7,500 tonnes exported between 1993 and 1999 (Aiken et al. 2006). Jamaican exports accounted for 46% of conch meat exported internationally during this time (Thiele 2001), with exporters making an average of US$ 8 million/year between 1997 - 2000.

Prior to the industrialization of the fishery in the 1990s, queen conch exports were less than 50 tonnes per year (Aiken et al. 2006). In 1992, severe exploitation of the conch fishery in the Caribbean resulted in the species being listed in Appendix II of the Convention on International Trade in Endangered Species [CITES] (Thiele 2001). The convention requires permits for collection and export to be issued by the relevant authorities only if the authority is satisfied that any removal will not be detrimental to the survival of the affected population (Prada et al. 2008). Efforts to implement measures for sustainable use of the queen conch fishery in Jamaica have been limited to restricting the number of licensed conch fishers, setting of quotas and the designation of marine reserves (Aiken et al. 2006, Morris 2012). These measures along with stock assessments via abundance surveys are the only tools currently used by Jamaican authorities to manage a marine territory that is more than sixteen times the size of mainland Jamaica; the mainland is approximately 10,991 km^2 while the Exclusive Economic Zone (EEZ) is approximately 181,190 km^2 (Qu et al. 2001, U.S. Department of State 2004). In order to sustainably manage a fishable resource such as the queen conch, it is essential to understand the underlying biological processes that maintain the affected populations and ensure their survival. Information on these processes can be elucidated through genetic methods.

Early attempts to assess the population structure of the queen conch, *L. gigas*, in the Caribbean reported high levels of

genetic connectivity but explicitly stated the potential exists for greater levels of genetic differentiation among populations across the region (Mitton et al. 1989, Morales 2004). Collecting samples from nine (9) locations (Bermuda, Turks and Caicos Islands, St. Kitts, Nevis, St. Lucia, Bequia, the Grenadines, Barbados, and Belize) using eight allozyme loci, Mitton et al. (1989) identified increased gene flow between the Greater Antilles and the Eastern Caribbean as well as genetic differentiation at all spatial scales. For example, the geographically isolated countries of Bermuda and Barbados were genetically different, which is expected. However, two sites relatively close in proximity, north and south of St. Lucia (separated by approximately 44 km) were significantly different. Estimates of gene flow and the frequency of private alleles observed along with the large dispersal potential of *L. gigas* larvae led Mitton et al. (1989) to establish connectivity relationships throughout Caribbean populations. Despite the limitations associated with the use of allozymes (Hellberg et al. 2002, Sunucks 2000), a genetic study conducted by Morales (2004) comparing partial 16S rRNA mitochondrial DNA sequences determined similar results of connectivity and genetic differentiation. Studies conducted in the Mexican Caribbean (Pérez-Enriquez et al. 2011, Machkour-M'Rabet et al. 2017) and the Bahamas have detected a spatial genetic structure for *L. gigas* populations within their territorial waters. Pérez-Enriquez et al. (2011) amplified the cytochrome oxidase subunit I (COI) and cytochrome-b (cty-b) mitochondrial genes subsequently analyzing the variation between individuals of the species using restriction fragment length polymorphism (RFLP). This study identified a genetic cline along the southern Mexican Caribbean to north of the Yucatan Peninsula with a reduced gene flow observed between the two most distant sites representing an increase in genetic differences as geographic distance increases. Machkour-M'Rabet et al. (2017) providing an update to research done by Pérez-Enriquez et al. (2011) using Inter Simple Sequence Repeat (ISSR) molecular markers found similar results with the exception of the apparent genetic isolation of samples collected on Isla Cozumel. This isolation was not detected by Pérez-Enriquez et al. (2011). Biophysical models have also played a significant role in assessing connectivity of the species by accurately estimating the probability of larval exchange among locations (Paris et al. 2008; Pérez-Enriquez et al. 2011). This approach has been applied to other commercially important species such as the Caribbean spiny lobster, so as to enhance management efforts and increase fisheries yield (Chollett et al. 2016).

Our study determines the fine-scale population structure of *L. gigas* in the Jamaica's EEZ using nine microsatellite markers (Truelove et al. 2016, Zamora-Bustillos et al. 2007). This will provide insight into the population dynamics present to help guide conservation and sustainable management strategies by the relevant Jamaican authorities. These markers have been used in the analysis of other *L. gigas* populations throughout the Caribbean Basin in Mexico (Zamora-Bustillos et al. 2011) and unpublished work by Community Conch in the Bahamas. Based on the findings, implications and recommendations for management are discussed.

MATERIAL AND METHODS

Sample Collection

Mantle/muscle tissue was collected from 526 *L. gigas* individuals selected at random from 12 sites within Jamaica's EEZ (Figure 1). Tissue (≤ 100 mg) was diced, placed in tubes with Autogen M2 Buffer and DNA extracted using the AutoGenprep 965 automated high throughout system which utilizes the phenol/chloroform extraction method. Nine microsatellite loci (Truelove et al. 2016; Zamora-Bustillos et al. 2007) were divided into three multiplexes for PCR (Multiplex 1 = ConchPR11F and Sgig1; Multiplex 2 = ConchF17, ConchF29, ConchPR1F and Sgig2; Multiplex 3 = ConchF21, ConchF23 and Sgig6). The Type-it® Microsatellite PCR Kit (Qiagen) was used for the PCR with the following profile: 95°C for 5 min, 28 cycles of 95°C for 30 seconds, 57-60°C for 90 seconds (Multiplex 1 = 57°C; Multiplex 2 and 3 = 60°C) and 72°C for 30 seconds with a final extension at 60°C for 30 min. The final concentrations of the reagents were 1X PCR master mix, 2 µM 10X Primer mix and <200 ng DNA using a reaction volume of 10 µl. The 10X Primer mix for each multiplex was made with a concentration of 2 µM for each primer pair. Genotyping the amplified samples were conducted with an ABI 3730xl automatic DNA sequencer (Applied Biosystems) at the Smithsonian Institute's Laboratory of Analytical Biology. Alleles were manually scored using GeneMapper®v3.7 software (Applied Biosystems).

Data Quality Checks

Binning of the detected alleles was achieved using the R package MsatAllele version 1.05 (Alberto 2009). Due to the variability in the number of samples collected at each location, samples were grouped based on geographical location into five broad scale sites for statistical analysis. The number of alleles, number of private alleles, observed heterozygosity (H_o), expected heterozygosity (H_e) and the probability of departure from Hardy-Weinberg equilibrium (pHWE) were calculated at each locus for each group of sites using GenAlEx 6.502 (Peakall and Smouse 2012). Linkage disequilibrium among all loci at each site was determined using ten thousand permutations in Arlequin ver 3.5.2.2 (Excoffier and Lischer 2010). The false discovery rate was used to correct for Type I errors which occur during multiple comparisons without being overly conservative (Benjamini and Hochberg 1995). MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to detect any evidence of scoring error caused by stuttering or large allelic dropout as well as the presence of null alleles.

Population Structure

Pairwise genetic differences (Global Fst and Global Fst corrected for null alleles) were analysed using the genetic software FREENA and GenAlEx 6.502 (Chapuis and Estoup 2007; Peakall and Smouse 2012). Genetic distance between each group of sites was also determined to provide insight on population structure using the same software. GENODIVE version 2.0b23 (Meirmans and Van Tienderen 2004) was also used to conduct an analysis of

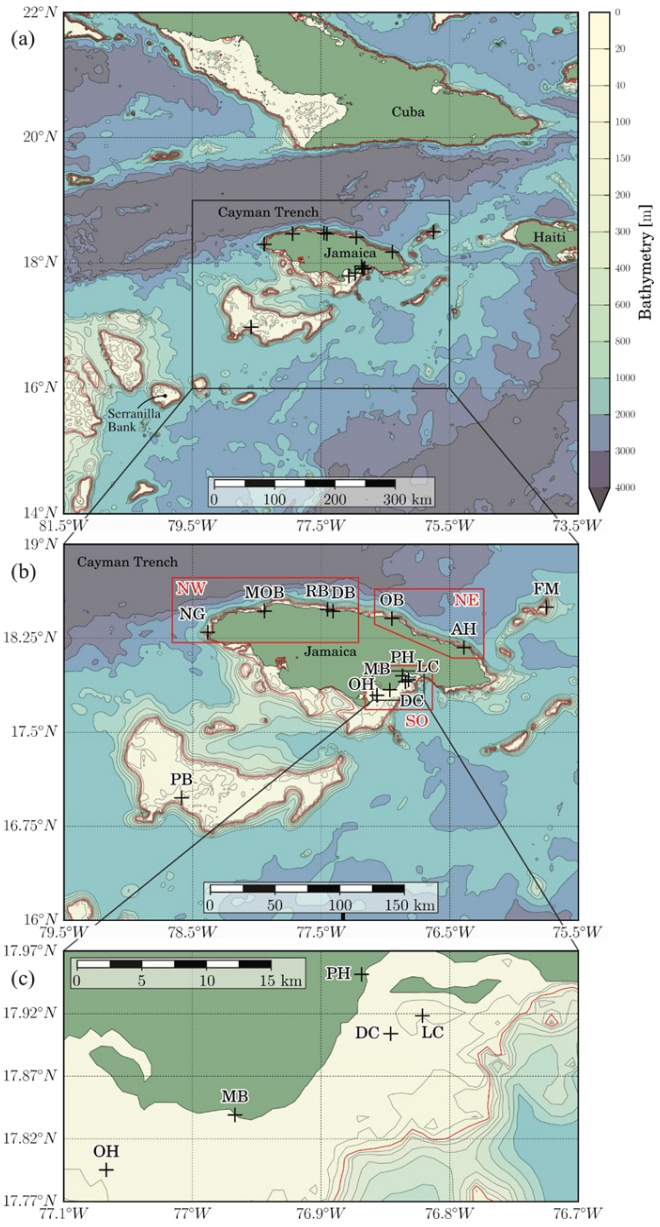

Figure 1. Map of sample site groupings together with sea bed ocean bathymetry of (a) the regional Caribbean Sea surrounding Jamaica, Pedro Bank and the sampled sites (b) the coastal waters and (c) the eastern side of the Southern Shelf of Jamaica. Bathymetry data derived from the updated 2014 version, 30 arc-second grid General Bathymetric Chart of the Oceans (GEBCO, 2014; Weatherall, 2015) and presented under an equidistant cylindrical projection (with distances approximately accurate to scale shown). To identify the location of submerged atolls, notably Pedro Bank and Formigas Bank, a red line marks the 100m depth contour. Sample sites are marked by black crosses and labeled, including: Negril (NG), Montego Bay (MOB), Rio Bueno (RB), Discovery Bay (DB), Oracabessa Bay (OB), San San (AH), Lime Cay (LC), Port Henderson (PH), Drunkenman's Cay (DC), Old Harbour Bay (OH),Manatee Bay (MB), Pedro Bank (PB) and Formigas Bank (FM). Grouped sample sites, based on their proximity to mainland Jamaica, are boxed in red in (b), and include Northwest (NW), Northeast (NE) and Southeast (SE). The location of SE sites are more accurately shown in (c) and in the context of sea bed bathymetry.

molecular variance (AMOVA) to detect the presence of population structure (Fst) at varying hierarchical levels using an infinite allele model (Weir and Cockerham 1984). Fifty thousand permutations were used to determine the level of significance. This was done at each loci and overall loci for each group of sites.

Discriminant Analysis of Principal Components

The discriminant analysis of principal components (DAPC, Jombart et al. 2010) was implemented with and without any *a priori* delineation using the *dapc()* function in the R package ADEGENT. When using no *a priori* delineation, K-means clustering was used to determine the lowest Bayesian information criterion using the *find.clusters()* function and retaining all principal components which helps to identify the number of unique genetic clusters present prior to the DAPC. On executing the *dapc ()* function in both analyses, the number of principal components that explained 90% of the variance was retained. Thereafter, a plot of the discriminant functions was constructed using the *scatter()* function. Only the first two discriminant functions were visualized using the *colorplot()* function for the *a priori* analysis. For the *a priori* DAPC analysis, the probability of each individual being assigned to the other grouped sites was illustrated using *assignplot()*. The DAPC also calculates the percentage of individuals that were successfully reassigned to their original groups.

Detection of Genetic Outliers and Migrants

A kinship analysis (GenoDive, Meirmans and Van Tienderen 2004) was conducted on all samples successfully amplified at seven or more loci by calculating the genetic distance between each individual using the method of Loiselle et al. (1995) which determines the relative probability of identity by descent. A principal coordinates analysis (PCoA) was carried out on the pairwise matrix of the kinship analysis and a plot visualized using *cmdscale ()*. The *s.skde2()* function in the ADEGENT R package was used to plot a density kernel on the PCoA plot around individuals that displayed a high level of relatedness. Those individuals outside the density kernel were identified as genetic outliers or migrants. Individuals outside the kernel but within the first grid in any direction along the x and y axes of multivariate space around the center of the PCoA plot were identified as genetic outliers. Those individuals outside the density kernel by two grids or more in any direction were identified as migrants (Truelove 2014).

Genetic Relatedness within Grouped Sites

The R package *related* (Pew et al. 2015) was used to analyse the relatedness within the grouped sites to determine if observed within-group relatedness was greater than expected. The *grouprel()* function calculated the relatedness within the groups using the method of Wang (2002) with fifty thousand iterations.

RESULTS

Data Quality and Summary Statistics

Lobatus gigas (526) from twelve locations in the Jamaican fishery were successfully genotyped using nine polymorphic microsatellite loci. Only 459 of the 526 individuals collected were successfully amplified at seven or more loci (87% amplification). These individuals were used for the subsequent analysis. A random subset of 39 and 37 samples were used from the Formigas Bank and Pedro Bank samples respectively to prevent any unbiased analysis. North-western Jamaica had the highest number of private alleles across all loci (n = 24) followed by north-eastern Jamaica (n = 15), Formigas Bank (n = 8), south-eastern Jamaica (n = 7) and the Pedro Bank (n = 4). The effective number of alleles did not vary substantially across sample sites with a range of 5.796 – 7.316 with Pedro Bank and Northeast Jamaica recording the lowest and highest values respectively. The mean number of alleles did not vary significantly either, ranging from 10.7 - 14. However, the greatest values were observed at north coast sites (North-eastern and north-western Jamaica) and the lowest at Pedro Bank (Table 1). There were 25 significant departures from Hardy-Weinburg equilibrium (HWE) of 45 comparisons with only the Conch23 locus exhibiting significant departure across all sites (Table S1). MICRO-CHECKER analysis determined that departures from HWE were not due to scoring error or allele dropout but could be as a result of null alleles. However, FREENA determined that the potential bias of null alleles to departure from HWE was negligible (Global Fst = 0.0045; Global Fst corrected for null alleles = 0.0047). All microsatellites were therefore used in the analysis of population structure.

Population Structure

Pairwise Fst values ranged from 0.008 to 0.016 (Table 2). Pedro Bank and north-western Jamaica reported the highest pairwise value and are the only two sites that are significantly differentiated (Fst = 0.016, p = 0.001). However, Fst values were greater when north coast sites (north-eastern and north-western Jamaica) were compared to southern sites than among sites in each grouping. An AMOVA determined that significant differences were present among individuals (Fis = 0.143, $p < 0.001$) and among populations (Fst = 0.004, p = 0.01). When sites were grouped based on proximity to mainland Jamaica (island shelf sites vs. offshore bank sites), a marginal significant difference was identified among populations within these groups (Fsc = 0.004, p = 0.055).

K-means clustering analysis without any *a priori* delineation identified three genetically unique clusters. No sample site was exclusively assigned to one cluster, that is individuals from all sites were well admixed across clusters (mean membership probability in Table 1). Mainland sites (north-eastern Jamaica NE, north-western NW and south-eastern Jamaica SO) accounted for 72%, 52% and 46% of clusters 1 - 3 respectively.

Conducting a DAPC using site location as a criterion revealed reduced levels of genetic differentiation among sites suggested by the considerable partial overlap of the 95% ellipses for each site (Figure 2). A weak genetic differentiation was identified between sample sites north and south of the island, with the greatest differentiation occurring between Pedro Bank (PB) and north-western Jamaica (NW), which supports the pairwise Fst results (Figure 3). The Formigas Bank (FM), north-eastern Jamaica (NE) and south-eastern Jamaica (SO) sites are essentially indistinguishable genetically.

Membership probability of all individuals were analysed to determine successful reassignment to their original group. No group of sites had successful reassignment ≥ 90%. Pedro Bank (PB) site had the highest (89%) with only a few individuals being assigned to Formigas Bank (FM) and south-eastern Jamaica (SO). Individuals from FM (44% successful reassignment) had a high probability of being assigned to all other sites suggesting that Formigas Bank's (FM) upstream position has a strong genetic influence on them. Similar results were observed for the south-eastern Jamaica (SO) sites (43% successful reassignment) with individuals being reassigned to all sites (mostly Formigas Bank, FM and north-eastern Jamaica, NE) except north-western Jamaica (NW). Individuals from NW sites had the second highest successful reassignment (68%), those not successfully reassigned were assigned to Formigas Bank (FM) and north-eastern Jamaica (NE). NE had the lowest successful reassignment of 38% with the majority being assigned to north-western Jamaica (NW) and Formigas Bank (FM) with very few individuals being assigned to Pedro Bank. This analysis suggests that most sites are well admixed except for NW and Pedro Bank (PB) sites that are more dissimilar from the other sites as well as each other.

Genetic Outlier/Migrant Detection

Only 13% of the individuals sampled were determined to be outliers or migrants with the majority being from the Pedro and Formigas Banks (55% and 27% respectively,

Table 1. Genetic variability among *Lobatus gigas* samples at nine microsatellite loci

Population	Mean # of Alleles	Effective # of Alleles	Private Alleles	Mean Heterozygosity		Mean Membership Probability		
				Observed H_o	Expected H_e	Cluster 1	Cluster 2	Cluster 3
FM	11.7	7.089	8	0.692	0.743	0.15	0.50	0.34
NE	14.0	7.316	15	0.621	0.776	0.21	0.49	0.30
NW	13.9	6.623	24	0.667	0.796	0.38	0.45	0.17
SO	11.0	6.599	7	0.671	0.766	0.26	0.48	0.26
PB	10.1	5.796	4	0.649	0.714	0.11	0.52	0.37

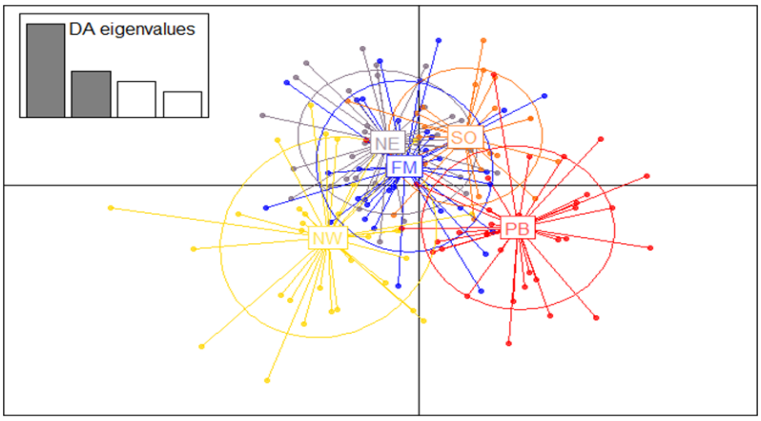

Figure 2. Discriminant analysis of principal components results using the geographic sites criteria, for Pedro Bank (PB), Formigas Bank (FM), Northeast (NE), Northwest (NW) and Southeast (SO) sites.

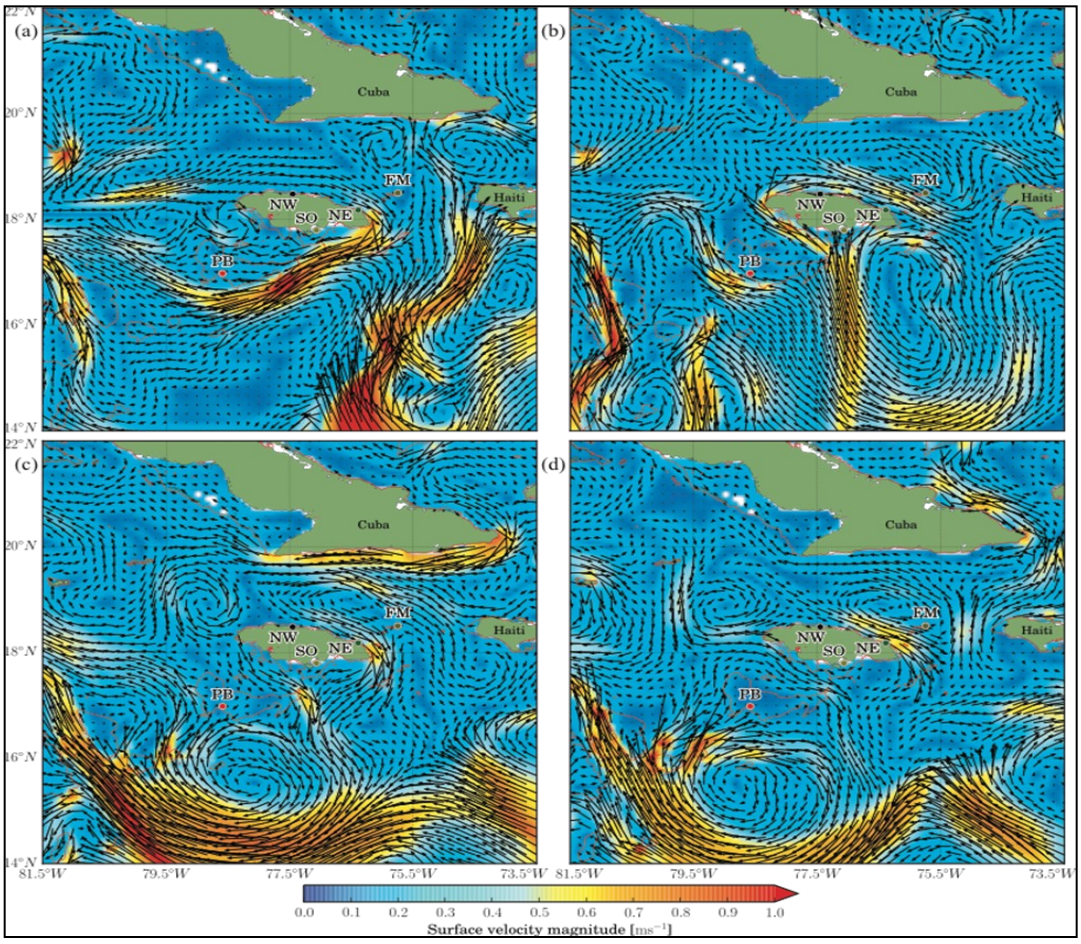

Figure 3. Typical daily mean surface currents of the regional Caribbean Sea surrounding Jamaica, Pedro Bank and the sampled sites, for (a) winter (Jan–Mar) (b) spring (Apr–Jun) (c) summer (Jul–Sep) and (d) autumn (Oct–Dec) in the year 2016. Velocity data from the Mercator operational 1/12º global ocean physics and analysis data set (Mercator Ocean, 2017). Bathymetry and coastlines determined from GEBCO (2014); Weatherall (2015), as above. To identify the location of submerged atolls, notably Pedro Bank and Formigas Bank, a red line marks the 100m depth contour. Circle markers present a visualization of the DAPC results using the geographic sites criteria. Similar colours indicate that individuals from these sites are more similar and differences in colour indicate dissimilarity. The locations follow the same labeling and include: Pedro Bank (PB), Formigas Bank (FM), Northeast sites (NE), Northwest sites (NW) and Southeast sites (SE).

Table 2. Pairwise comparisons of genetic differentiation (Fst) of *Lobatus gigas* in the Jamaican fishery. p-values are above the diagonal (bold values are significant) and pair-wise Fst values below the diagonal

Sites[1]	FM	NE	NW	SO	PB
FM	---	0.411	0.067	0.230	0.138
NE	0.008	---	0.147	0.262	0.100
NW	0.010	0.011	---	0.097	**0.001**
SO	0.011	0.012	0.014	---	0.444
PB	0.009	0.010	0.016	0.010	--

both offshore sites). The results revealed 11% of the identified individuals were migrants. The number of outliers more than doubled that of migrants at all sites except north-eastern Jamaica (NE) which had no outliers and one migrant. NE was also the only nearshore site where migrants were detected, only outliers were detected at the other nearshore sites.

Genetic Relatedness within Grouped Sites

Our results revealed that only the Pedro Bank (PB) had an observed within relatedness that was greater than expected ($p < 0.0008$). Within relatedness for all other sites fell within the expected range determined by simulations.

HYDRODYNAMIC OCEAN CONDITIONS IN THE REGION OF JAMAICA

Population diversity, connectivities and isolation are strongly controlled by sea currents (Machkour-M'Rabet et al. 2017). It is a challenge to accurately monitor and model the relatively small-scale coastal island environments of the Caribbean Antilles. Local flows are influenced significantly by large-scale hydrodynamic changes in the basin and interacting processes, such as eddies, entering from the Atlantic Ocean. In the wider Caribbean Sea basin, surface circulation is typically dominated by the Caribbean Current, a generalized westward flow (Andrade and Barton 2000). This supports mesoscale eddies with meanders in flow over distances of 100 – 500 km and large anticyclonic gyres regularly migrate westward, with sizes ~200 km in diameter observed. For the coastal waters of the small islands typical in the region, these large fluctuations relative to the strength of mean currents result in highly variable, episodic events, such that it is difficult to identify stable, mean flow regimes.

Andrade and Barton (2000) infer sea surface flow velocities from satellite data and Alvera-Azcárate et al. (2008) use output from the Hybrid Coordinate Ocean Model (HYCOM). Both are good sources for studies on the basin scale, but their spatial resolutions are limited in applications to the small coastal scales of the Caribbean islands.

Ocean Bathymetry in the Region of Jamaica

Bathymetry has a significant, leading-order effect on ocean circulation (Alvera-Azcárate et al. 2008) and particularly in the Caribbean where there is a high density of steep slopes and channels. Sea bed ocean bathymetry of the regional Caribbean Sea surrounding Jamaica, Pedro Bank and the sampled sites are shown in Figure 1a, with a focus on the coastal waters in Figure 2b and the eastern side of the Southern Shelf (the only section of the southern shelf sampled) in Figure 1c. Bathymetry data is derived from the updated 2014 version, 30 arc-second (~1 km) grid General Bathymetric Chart of the Oceans (GEBCO 2014, Weatherall et al. 2015) and presented under an equidistant cylindrical projection (with distances approximately accurate to scale shown). This dataset consists of blended sources and the updated version is notably more accurate in coastal regions.

Ocean Surface Currents in the Region of Jamaica

L. gigas larvae tend to migrate at the sea surface (Paris et al. 2008), so we restrict attention to ocean surface flows, which at regional and coastal scales are considered in Figures 3 and 4, respectively. Here we examine data from the Mercator Ocean (2017) project which assimilates contributions from in-situ observations, shipboard campaigns and satellite imagery using the relatively fine-scale hydrodynamic NEMO ocean model (Madec 2008; version 3.1) to interpolate down to spatial scales of 1/12º (~10 km). This is considered over the year 2016. In Figures 3 and 4, a representative example of typical daily mean surface flow conditions has been selected for each of the seasons: winter (Jan - Mar), spring (Apr - Jun), summer (Jul - Sep) and autumn (Oct - Dec). In these figures, bathymetry and coastlines are determined from (GEBCO 2014), as above. To identify the location of submerged atolls, notably Pedro Bank and Formigas Bank, a red line marks the 100 m depth contour. Visualizations for the full year are available in Candy (2017).

It is striking in this region how variable in strength the ocean currents range, with relatively quiescent regions that remain robust in time, whilst in contrast very fast, strong flows of the order of $1 ms^{-1}$ appear that are confined to tight, narrow eddies and jets (see for example the south-westerly current passing south of Pedro Bank in (a) of Figures 3 and 4). Highly-variable and intense flows largely enter the regional waters of Jamaica from the deep waters of the south-west, advected by the Caribbean Current. These generally travel west and keep to the south of the island. To the north, flow generally follows the deep Cayman Trench westward or eastward, and structures are more stable and weaker than the south. In the shallower waters to the west and south-west of the island, the surface is relatively quiescent, protected by the steep bathymetry, where jets sometimes stream off to the north-west, passing the west side of Pedro Bank. This is also found to some degree to the east of the island. More generally the steep banks leading to the shallower waters of the ridge containing Haiti, Jamaica, and Serranilla Bank and beyond divide the hydrodynamical conditions between the north and south, acting as a barrier to interaction between the two.

Notably the very low surface velocity over Pedro Bank apparent in Figure 4 is an established feature throughout the year. Communities of *L. gigas* on the bank are relatively sheltered by the ≤ 0.1m/second flow speeds, and are not swept by the intense eddies and jets seen elsewhere in the region, on the order of 1m/second. This protection also means they are likely to receive larvae and genetic inputs, which strongly supports the isolation seen in the DAPC

results. In the flow fields shown in Figures 3 and 4, flows are seen to bring material from the south or west, and it is only in the winter example where there is potential for Pedro Bank to receive limited larvae from the north-eastern (NE) sites of the Jamaican mainland. This limited connectivity was also observed in the DAPC results.

Along the north coast of Jamaica, flow is generally to the east, in contrast to the strong westward flows seen in the south. Flow sometimes reverses to the west when jets spinning off the eddies to the south, but these reversals tends to be weak. This implies a trend in larvae distribution eastward along the north coast and the relative similarity seen between the Formigas Bank (FM), north-western (NW) and north-eastern (NE) sites and a connection to south eastern sites with flows often continuing to circle around to the Southern Shelf (e.g. (a) and (c) of Figures 3 and 4). Note that south eastern sites also receive material from the south (e.g. (b) of Figures 3 and 4) due to the strong jets, which supports its weaker relationship to the Formigas Bank (FM), north-western (NW) and north-eastern (NE) family of sites. The hydrodynamics also supports that reassignment from SO to NW is difficult and significantly less likely than to other sites, since north western sites are largely an upstream source to the other sites. Even during northward jet events such as seen in Figure 4b clockwise transport around the island is unlikely to pick up material form the south-eastern sites due to the shelter from the southern cap.

DISCUSSION

This is the first exclusive assessment of queen conch population structure in Jamaica's EEZ. Our findings have revealed that the queen conch population on the commercially important Pedro Bank is not only geographically isolated but receives limited gene flow from mainland and other historically important offshore populations within Jamaica's EEZ. An eighty-nine percent (89%) successful reassignment of individuals sampled at Pedro Bank, may suggest that it receives recruits from sources external to the EEZ, exclusive of those that supply the mainland. Alternatively, the population may rely primarily on local larval

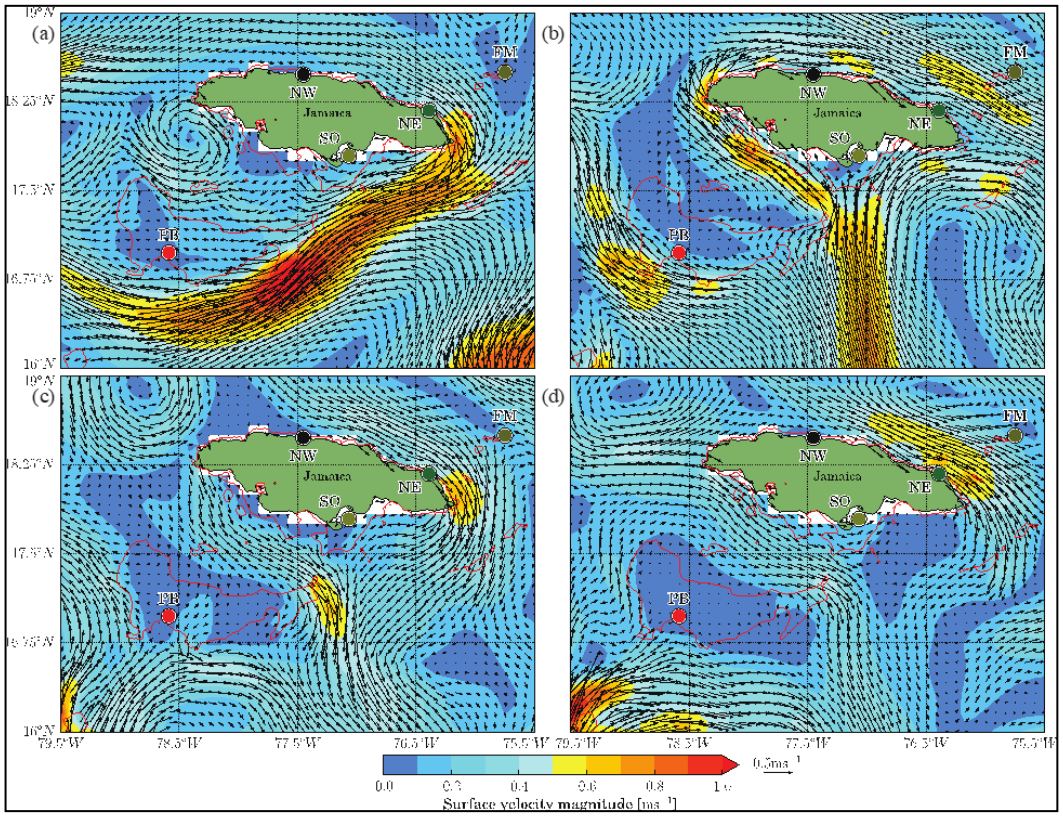

Figure 4. Typical daily mean surface currents of the coastal waters surrounding Jamaica, Pedro Bank and the sampled sites, for (a) winter (Jan - Mar) (b) spring (Apr - Jun) (c) summer (Jul - Sep) and (d) autumn (Oct - Dec) in the year 2016. Velocity data from the Mercator operational 1/12º global ocean physics and analysis data set (Mercator Ocean, 2017). Bathymetry and coastlines determined from GEBCO 2014, Weatherall (2015), as above. To identify the location of submerged atolls, notably Pedro Bank and Formigas Bank, a red line marks the 100m depth contour. Circle markers present a visualization of the DAPC results using the geographic sites criteria. Similar colours indicate that individuals from these sites are more similar and differences in colour indicate dissimilarity. The locations follow the same labeling and include: Pedro Bank (PB), Formigas Bank (FM), Northeast sites (NE), Northwest sites (NW) and Southeast sites (SE).

retention as suggested by the larval dispersal model of Schill et al. (2015). Our results support both claims, as observed within-relatedness for Pedro Bank was greater than expected. These results could be explained by chaotic genetic patchiness whereby the establishment of new recruits is successful or prevented by ecological and environmental factors (Eldon et al. 2016, Johnson and Black 1982, Watts et al. 1990) as observed in *L. gigas* populations of the Florida Keys by Campton et al. (1992). The geographic isolation of the bank could also result in significantly reduced gene flow from external sources allowing for genetic drift to occur resulting in the establishment of specific alleles and increase in genetic differentiation. Similarly, Machkour-M'Rabet et al. (2017) observed genetic differentiation of *L. gigas* at Cozumel Island in the Mexican Caribbean citing similar causes for this variation. In addition, Machkour-M'Rabet et al. (2017) suggests depth differentiation as a potential cause of genetic differentiation (Quattrini et al. 2015). However, more research is required to determine the application of this hypothesis to *L. gigas*. Quattrini et al. (2015) sampled octocoral species at depths between 340 m to 848 m, a substantial range when compared to *L. gigas*, commonly found at 30 m and very rare at a maximum of 75 m (McCarthy 2007). Briefly, *L. gigas* is depth limited as their food source is photosynthetic (Randall 1964) while the octocoral species studied by Quattrini et al. (2015) are not depth limited. As this study is exclusively focused on *L. gigas* population structure in Jamaica, it does not consider genetic samples from upstream populations external to Jamaica's EEZ. Therefore, concluding that the *L. gigas* populations on Pedro Bank are self-sustaining, based on the greater than expected within-relatedness is not appropriate. Further genetic parentage studies to include upstream populations external to Jamaica's EEZ are needed to confirm this. Consequently, unmasking the source of new recruitment into the populations on Pedro Bank is essential. It is therefore critical that Pedro Bank continue to be managed as one stock, with the identification and protection of important conch breeding and post-larval settlement habitats being a top priority to enhance recruitment. Pérez-Enriquez et al. (2011) and Machkour-M'Rabet et al. (2017) made similar recommendations of the self-sustaining population at Arrecife Alacranes in Mexico, which in that case experienced reduced gene flow from upstream populations. It is also critical to identify and collaborate with upstream territories to assess upstream populations as potential sources to Pedro Bank, as their mismanagement may potentially be detrimental to Jamaican stocks. We are reminded of the collapse of the conch fishery in the Florida Keys due to excessive harvesting in the 1970's, which has not recovered for over 30 years as their population relied mostly on self-recruitment (Delgado et al. 2004; Delgado et al. 2006). This could be the fate of Pedro Bank if it is similarly confirmed to also rely primarily on self-recruitment; and would require continued or even more careful management of the fishery.

The high dispersal potential of *L. gigas* has proven extremely influential on the populations sampled in the Jamaican EEZ and suggests the *L. gigas* populations of the Jamaican EEZ are clearly connected with weak differentiation between populations north and south of the island. Though on a much larger geographic scale, attempts to assess the genetic structure of *L. gigas* across the Caribbean yielded similar results of high connectivity with genetic differentiation among populations (Campton et al. 1992, Mitton et al. 1989, Morales 2004). Unfortunately, these studies did not sample populations in Jamaica's EEZ. However, examples of queen conch genetic assessments in Mexico (Pérez-Enriquez et al. 2011), the Florida Keys and Bimini (Campton et al. 1992) provide case studies on a smaller spatial scale comparable to Jamaica. Our findings revealed an increase in private alleles and expected heterozygosity in a westerly direction along the north coast from the Formigas Bank to north-western Jamaica, suggesting a reduction in gene flow in this direction. This result is supported by the dominant eastward surface flows experienced along the north coast for most of the year (see Figures 4). The expected heterozygosity observed at our sample sites is directly comparable to the nucleotide diversity reported by Pérez-Enriquez et al. (2011) which found a similar cline pattern along the Mexican Caribbean coast to the north of the Yucatán peninsula with no significant level of population differentiation, unlike the significant but weak level detected in our study. Machkour-M'Rabet et al. (2017) conducted a more recent study Broquet et al. (2013) suggests chaotic genetic patchiness as an explanation for patterns of genetic differentiation observed at such fine spatial scales in marine species with pelagic larvae. We suggest that in addition to this, the decades of commercial exploitation experienced by the species in Jamaica's EEZ and the wider Caribbean is among the myriad of causes for this reduction in gene flow (Allendorf et al. 2008).

Our findings as well as the literature (Morales 2004, Roberts 1997, Schill et al. 2015) suggests that the *L. gigas* populations sampled in Jamaica's EEZ, more so Pedro Bank, may operate as a sink, that is receiving recruits from multiple upstream sources external to the EEZ that influence nearshore and offshore populations at various levels (Stoner 1997). However, the number of migrants detected in our data were low at offshore banks and essentially non-existent nearshore. The commercial exploitation of the species would drastically reduce the effective population, significantly decreasing population size and genetic connectivity via larval dispersal throughout the fishery (Allendorf et al. 2008, Gascoigne and Lipcius 2004). The majority of individuals including migrants are probably being caught within the first 3 ½ - 4 years of life, before they are sexually mature (Brownell and Stevely 1981, Randall 1964) and have a chance to reproduce. This is possibly the case for near-shore populations found on the island shelf of Jamaica, with Aiken et al. (2006) reporting a virtual disappearance of these populations prior to 1990. The existing island shelf populations sampled are probably a combination of minimal, if any, local recruitment and some level of recruitment from upstream sources external to the Jamaican fishery. The latter may be the main reason why many commercially important species including *L. gigas* have not been completely exhausted from the island shelf despite decades

of intense fishing pressure (Roberts 1997). This observation, along with the implementation of conservation measures by the Government of Jamaica, have prevented the complete exhaustion of this important resource (Aiken et al. 2006). It is also possible that migration via larval dispersal from external sources is not sufficient to replenish Jamaica's nearshore populations after complete exhaustion (Schill et al. 2015), since conch require a certain density to copulate with no mating or spawning activity observed at a mean density of < 56 adult conch/ha (Stoner and Ray-Culp 2000). With the recommended density required to ensure adequate mating at a level of ≥ 100 adult conch/ha; densities below this significantly impair recruitment (Queen Conch Expert Workshop Group 2012). Mean densities on the island shelf observed by the authors in this case study have not exceeded 30 adult conch/ha. Therefore, the Allee effect, also detected by Baker et al. (2016) in Puerto Rico and Stoner and Ray-Culp (2000) in the Bahamas, is evident in Jamaica's nearshore populations. As it relates to Pedro Bank, most genetic outliers detected are possibly the reason for the higher levels of genetic differentiation observed. This continues to provide evidence that Pedro Bank populations may receive recruits from external sources not limited to those that supply nearshore/mainland sites and that this population may experience some level of local retention, both of which are supported by our analysis of the local surface flows in the region.

Biophysical models simulating coral larval dispersal across the Caribbean have demonstrated that Jamaica has connectivity with multiple upstream territories (Schill et al. 2015). These findings suggest potential *L. gigas* recruitment from multiple sources into the Jamaican fishery, with the dominant east to west flow of the Caribbean Current being the backbone of this recruitment. This is supported by the more in-depth study of the hydrodynamics presented. Additionally, though based on coral larvae, the Schill et al. (2015) model uses biological parameters similar to *L. gigas* veliger larvae, making it useful in the assessment of *L. gigas* spatial genetic connectivity. This research identifies potential larval sources external to the Jamaican territory. In addition, it highlights that the north and south coast potentially receive recruitment from different sources but not exclusively. Briefly for example, the north coast has weak connections with territories such as the Bahamas and Turks and Caicos by way of the Windward Passage which would not necessarily have a demographic impact on south coast populations. The same applies to the south coast with low connections with Venezuela, Panama, and the Netherland Antilles (Aruba, Bonaire, and Curacao), whereas low connections with the Eastern Caribbean apply to Jamaica's entire EEZ. Cuba and Haiti have the strongest connections with Jamaica. This provides some support for our hypothesis that populations in Jamaica's EEZ receive recruits from external sources.

The results of our study, like others based on indirect measures of genetic differentiation (Fst), must be taken with caution. Low Fst values are common to marine populations due to relatively higher levels of connectivity through larval transport by ocean circulation patterns between populations, increasing gene flow (Ward et al. 1994). However, low but statistically significant Fst values have been observed in other species such as the Atlantic cod (*Gadus morhua*), walleye pollock (*Theragra chalcogramma*), and Atlantic herring (*Clupea harengus*) but deciphering if this statistical significance holds ecological significance presents its challenges (Bekkevold et al. 2005, Knutsen et al. 2011, O'Reilly et al. 2004). Many theories have been put forward and discussed to account for this phenomenon with Waples (1998) providing strategies to overcome such challenges. We applied these strategies by genotyping nine microsatellite loci and conducting random sampling of individuals across the EEZ at twelve sites as well as the implementation of the discriminant analysis of principal components, which is not based on any theoretic genetic assumptions.

Management Recommendations for the Queen Conch Fishery in Jamaica

Despite not knowing the direct drivers responsible for the weak differentiation exhibited by *L. gigas* population in Jamaica's EEZ, it is of paramount importance that Jamaica's queen conch populations on Pedro Bank continue to be managed as one stock, potentially relying on local and external recruitment. Efforts should therefore be focused on identifying and protecting the important breeding and post-larval settlement habitats present, to enhance the recruitment of future generations of queen conch. Research has already begun in this regard with the development of a species distribution model for queen conch on Pedro Bank by Morris (2016). This model utilises the four abundance surveys conducted over the last 15 years in conjunction with environmental variables such as depth, substrate and primary production allowing for the detection of important conch habitats. Additionally, the managers of Pedro Bank must continue to monitor populations to ensure that densities are well above the minimum required for adequate mating (≥100 adult conch/ha) to prevent the Allee effect and the collapse of the fishery. Further genetic studies are also needed to determine the role of Pedro Bank in the local and regional connectivity patterns of *L. gigas*. Additional measures should also be put in place to ensure the protection of potential upstream offshore habitats within Jamaica's EEZ such as the Formigas Bank and the Morant Cays (not sampled in this study); protecting important conch breeding grounds and post-larvae settlement habitat that will enhance recruitment of downstream populations within the EEZ. Our study supports this initiative as the central position of Formigas Bank (FM) in the DAPC plot (Figure 2) suggests it is a potential source to other downstream sample sites, and the surface flow analysis supports the hypothesis of downstream migration to the southern shelf sites. The recovery of those populations present on the island shelf is possible, due to potential larval transport from upstream sources, within and external to the EEZ. However, to determine recruitment potential, larval density assessments and parentage analysis are required to determine their impact on Jamaican populations. Moreover, further research into the local hydrodynamic patterns that prevail throughout the EEZ together with more rigourous approaches to modelling the Lagrangian advection of

larvae are critical, as this directly impacts larval dispersal patterns and settlement. Tracking these patterns and observing where they intersect with suitable post-larvae settlement habitat will provide a framework for selecting sites for Special Fishery Conservation Area designation. This method has proven successful in enhancing the Honduran lobster fishery by combining spatially explicit population models and the life history of the species, involving ontogenetic migration, larval and adult movements (Chollett et al. 2016). Comparing the genetics of *L. gigas* veliger larvae and juveniles present will decipher if recruits are settling successfully within a Jamaican habitat or if it is unsuitable due to anthropogenic factors such as water pollution, climate change and fishing pressure, that may have degraded it (Glazer and Quintero 1998, National Oceanic and Atmospheric Administration 2014).

Conclusion

Our study of the *L. gigas* populations in the Jamaican fishery agrees with the early findings of Mitton et al. (1989) and Campton et al. (1992), implying weak but statistically significant differentiation throughout the Jamaican EEZ. Increased efforts must be made to ensure sustainable management of the commercially threatened populations on Pedro Bank. Despite being exposed to intense fishing pressure over the last three decades, external recruitment into Jamaica's EEZ is possibly the only factor preventing complete exhaustion of fishable resources on the island shelf, including the queen conch. We highlight the need for studies into mapping crucial habitat patterns and identifying key pathways (with coherent Langrangian structures, for example) that affect Jamaica's EEZ as this information can be used to design Special Fishery Conservation Areas that can increase yield while maintaining the integrity of the populations present. Collaborating with upstream territories to determine the extent of their impact on recruitment into Jamaica's EEZ. The long term genetic monitoring of *L. gigas* population structure would provide up-to-date information on any threats to diversity to this intensely exploited resource.

ACKNOWLEDGEMENTS

We thank Charlene Thomas of the Fisheries Division of the Ministry of Industry Commerce, Agriculture and Fisheries for her assistance in sample collections across Jamaica. We would also like to thank the reviewers of this publication for their constructive feedback. This work was supported by the Smithsonian/ Link Foundation, the University of the West Indies, Mona Campus and the Netherlands Organization for Scientific Research (NWO, grant number 858.14.061).

LITERATURE CITED

Aiken, K., A. Kong, S. Smikle, R. Appeldoorn, and G. Warner. 2006. "Managing Jamaica's Queen conch Resources." *Ocean & Coastal Management* **49**(5-6):332 - 341. doi: 10.1016/j.ocecoaman.2006.03.004.

Aiken, K., S. Smikle, R. Mahon, and R. Appeldoorn. 1999. The Queen conch Fishery on Pedro Bank, Jamaica: Discovery, Development, Management. *Ocean & Coastal Management* **42**:1069 - 1081.

Alberto, F. 2009. MsatAllele_1. 0: An R Package to Visualize the Binning of Microsatellite Alleles. *Journal of Heredity* **100**(3):394 - 397.

Allendorf, F.W., P.R. England, G. Luikart, P.A. Ritchie, and N. Ryman. 2008. Genetic Effects of Harvest on Wild Animal Populations. *Trends in Ecology & Evolution* **23**(6):327 - 337.

Alvera-Azcárate, A., A. Barth, and R.H. Weisberg. 2008. The surface circulation of the Caribbean Sea and the Gulf of Mexico as inferred from satellite altimetry. *Journal of Physical Oceanography* **39** (3):640 - 657.

Andrade, C.A., and E.D. Barton. 2000. Eddy development and motion in the Caribbean Sea. *Journal of Geophysical Research: Oceans* **105** (C11):26191 - 26201.

Baker, N., R.S. Appeldoorn, and P.A. Torres-Saavedra. 2016. Fishery-Independent Surveys of the Queen conch Stock in Western Puerto Rico, with an Assessment of Historical Trends and Management Effectiveness. *Marine and Coastal Fisheries* **8**(1):567 - 579.

Bekkevold, D., C. André, T.G. Dahlgren, L.A.W. Clausen, E. Torstensen, H. Mosegaard, G.R. Carvalho, T.B. Christensen, E. Norlinder, and D.E. Ruzzante. 2005. Environmental Correlates of Copulation Differentiation in Atlantic Herring." *Evolution* **59**(12):2656 - 2668.

Benjamini, Y. and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological)* **13**:3261 - 3273.

Broquet, T., F. Viard, and J.M. Yearsley. 2013. Genetic Drift and Collective Dispersal can Result in Chaotic Genetic Patchiness. *Evolution* **67**(6):1660 - 1675.

Brownell, W.N. and J.M. Stevely. 1981. The biology, fisheries and the management of queen conch, *Strombus gigas*. *Marine Fishery Review* **43**(7):1 - 12.

Campton, D.E., C.J. Berg Jr., L.M. Robison, and R.A. Glazer. 1992. Genetic Patchiness Among Populations of Queen conch *Strombus gigas* in the Florida Keys and Bimini. *Fishery Bulletin* **90**(2):250 - 259.

Candy, A.S. 2017. Daily mean surface currents of the regional Caribbean Sea surrounding Jamaica and its coastal waters in the year 2016. doi: 10.6084/m9.figshare.5493163.

Chapuis, M-P. and A. Estoup. 2007. Microsatellite Null Alleles and Estimation of Population Differentiation. *Molecular Biology and Evolution* **24**(3):621 - 631.

Chollett, I., L. Garavelli, S. O'Farrell, L. Cherubin, T.R. Matthews, P.J. Mumby, and S.J. Box. 2016. A Genuine Win-Win: Resolving the "Conserve or Catch" Conflict in Marine Reserve Network Design. *Conservation Letters*. doi: 10.1111/conl.12318.

Delgado, G.A, C.T. Bartels, R.A. Glazer, N.J. Brown-Peterson, and K.J. McCarthy. 2004. Translocation as a Strategy to Rehabilitate the Queen conch (*Strombus gigas*) Population in the Florida Keys. *Fishery Bulletin* **102**(2):278 - 288.

Delgado, G.A., R.A. Glazer, D.Hawtof, D. Aldana Aranda, L.A. Rodríguez-Gil, and A. de Jesús-Navarrete. 2006. Do Queen conch (*Strombus gigas*) Larvae Recruiting to the Florida Keys Originate from Upstream Sources? Evidence from Plankton and Drifter Studies. *Caribbean Connectivity: Implications for Marine Protected Area Management* **9**:29.

Eldon, B., F. Riquet, J. Yearsley, D. Jollivet, and T. Broquet. 2016. "Current Hypotheses to Explain Genetic Chaos under the Sea. *Current Zoology* **62**(6):551 - 566.

Excoffier, L., and H.E.L. Lischer. 2010. Arlequin Suite ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows. *Molecular Ecology Resources* **10**:564 - 567.

Gascoigne, J., and R.N. Lipcius. 2004. Conserving Populations at Low Abundance: Delayed Functional Maturity and Allee Effects in Reproductive Behaviour of the Queen Conch *Strombus gigas*. *Marine Ecology Progress Series* **284**:185 - 194.

GEBCO. 2014. *General Bathymetric Chart of the Oceans*. https://www.gebco.net.

Glazer, R.A. and I. Quintero. 1998. Observations on the Sensitivity of Queen conch to Water Quality: Implications for Coastal Development. *Proceedings of the Gulf Caribbean Fisheries Institute* **50**:78 - 93.

Hellberg, M.E., R.S. Burton, J.E. Neigel, and S.R. Palumbi. 2002. Genetic Assessment of Connectivity among Marine Populations. *Bulletin of Marine Science* **70**(1):273 - 290.

Johnson, M.S. and R. Black. 1982. Chaotic Genetic Patchiness in an Intertidal Limpet, *Siphonaria* sp. *Marine Biology* **70**(2):157 - 164.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant Analysis of Principal Components: A New Method for the Analysis of Genetically Structured Populations. *BMC Genetics* **11**(1):1 - 15. doi: 10.1186/1471-2156-11-94.

Knutsen, H., E.M. Olsen, P.E. Jorde, S. Heiberg Espeland, C. Andre, and N.C. Stenseth. 2011. Are Low but Statistically Significant Levels of Genetic Differentiation in Marine Fishes 'Biologically Meaningful'? A Case Study of Coastal Atlantic Cod. *Molecular Ecology* **20** (4):768 - 783. doi: 10.1111/j.1365-294X.2010.04979.x.

Loiselle, B.A., V.L. Sork, J. Nason, and C. Graham. 1995. Spatial Genetic -Structure of a Tropical Understory Shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* **82**:1420 - 1425.

Machkour-M'Rabet, S., J. Cruz-Medina, F.J. García-De León, A. de Jesús -Navarrete, and Y. Hénaut. 2017. Connectivity and genetic structure of the queen conch on the Mesoamerican Reef. *Coral Reefs*:1 - 14.

Madec, G. 2008. *NEMO Ocean Engine."* Note du Pôle de modélisation, Institut Pierre-Simon Laplace (IPSL), France, No 27, ISSN No 1288 -1619, 2008.

McCarthy, K.. 2007. *A Review of Queen conch (Strombus gigas) Life-History.* Miami, Florida. Sustainable Fisheries Division. http://sedarweb.org/docs/wpapers/S14DW04%20Conch%20life%20history.pdf.

Meirmans, P.G. and P.H. Van Tienderen. 2004. GENOTYPE and GENODIVE: Two Programs for the Analysis of Genetic Diversity of Asexual Organisms. *Molecular Ecology Notes* **4**:792 - 794.

Mercator Ocean. 2017. *Operational 1/12° global ocean physics and analysis data set". Version: global-analysis-forecast-phy-001-024, Copernicus marine environment monitoring service.* https://marine.copernicus.eu/documents/PUM/CMEMS-GLO-PUM-001-024.pdf.

Mitton, J.B., C.J. Berg Jr., and K.S. Orr. 1989. Population Structure, Larval Dispersion and Gene Flow in the Queen conch, *Strombus gigas*, of Caribbean. *Biological Bulletin* **177**:356 - 362.

Morales, F. *Metapopulation Structure of the Queen conch,* Strombus gigas *(Linne, 1758) Throughout the Intra-Americas Sea. 2004*. (PhD diss.), Florida Institute of Technology, Melbourne, Florida USA.

Morris, R.A. 2012. *Jamaica National Queen Conch Report.* Fisheries Division, Ministry of Agriculture and Fisheries, Kingston, Jamaica. http://www.strombusgigas.com/Meeting%20Panama/Queen%20Conch%20Meeting%20(23%20October)/Jamaica/National%20Conch%20Report_CFMC_WECAFC_QCWG_2012_2.pdf.

Morris, R.A. Distribution of Queen conch (*Strombus gigas*) on the Pedro Bank, Jamaica: Descriptive and Predictive Distribution Models. 2016. (MSc) University of Iceland, Reykjavík, Iceland.

National Oceanic and Atmospheric Administration. 2014. *Queen conch, Strombus gigas (Linnaeus 1758) Status Report.* http://www.fisheries.noaa.gov/pr/species/Status%20Reviews/queen_conch_sr_2014.pdf.

O'Reilly, P.T., M.F. Canino, K.M. Bailey, and P. Bentzen. 2004. Inverse Relationship between FST and Microsatellite Polymorphism in the Marine Fish, Walleye Pollock (*Theragra chalcogramma*): Implications for Resolving Weak Population Structure. *Molecular Ecology* **13**(7):1799 - 1814.

Paris, C.B., D. Aldana-Aranda, M. Perez-Perez, and J.T. Kool. 2008. Connectivity of Queen conch, *Strombus gigas*, Populations from Mexico." Paper presented at the 11th meeting for the *International Coral Reef Symposium*, Ft. Lauderdale, Florida USA.

Peakall, R. and P.E. Smouse. 2012. GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research-An Update. *Bioinformatics* **28**:2537 - 2539.

Pérez-Enriquez, R., F.J. Garcia-Rodriguez, G. Mendoza-Carrion, and C. Padilla. 2011. Geographical Variation in the Genetic Diversity and Composition of the Endangered Queen conch *Strombus gigas* (Mesogastropoda: Strombidae) from Yucatán, México. *México Revista de Biología Tropical* **59**(3):1115 - 1126.

Pew, J., P.H. Muir, J. Wang, and T.R. Frasier. 2015. Related: an R package for Analysing Pairwise Relatedness from Codominant Molecular Markers. *Molecular Ecology Resources* **15**:57 - 561. doi: 10.1111/1755-0998.12323.

Prada, M., E. Castro, E. Taylor, V. Puentes, R. Appeldoorn, and N. Daves. 2008. Non-Detrimental Findings for the Queen conch (*Strombus gigas*) in Colombia. Paper presented at the meeting for the *NDF Workshop Case Studies WG*.

Qu, W., H. Morris, and J. Shilling. 2001. T21 Jamaica: A Tool For Long-term Sustainable Development Planning. *International Journal of Sustainable Development and Planning* **6**(2):135 - 151.

Quattrini, Andrea M, Iliana B Baums, Timothy M Shank, Cheryl L Morrison, and Erik E Cordes. 2015. Testing the depth-differentiation hypothesis in a deepwater octocoral." Paper presented at the 282th meeting for the *Proceedings of the Royal Society B*. **282** (1807):20150008.

Queen Conch Expert Workshop Group. 2012. *Recommendations of the Queen conch Expert Workshop.* United States of America, Miami, Florida USA. https://cites.org/sites/default/files/common/cop/16/doc/QCEW_Meeting_Report_Recommendations.pdf.

Randall, J.E. 1964. Contributions to the Biology of the Queen conch, *Strombus gigas*. *Bulletin of Marine Science* **14**:247295.

Roberts, C.M. 1997. Connectivity and Management of Caribbean Coral Reefs. *Science* **278**:1454 - 1457. doi: 10.1126/science.278.5342.1454.

Schill, S.R., G.T. Raber, J.J. Roberts, E.A. Treml, J. Brenner, and P.N. Halpin. 2015. No Reef is an Island: Integrating Coral Reef Connectivity Data into the Design of Regional-Scale Marine Protected Area Networks. *PLoS One* **10**(12):e0144199. doi: 10.1371/journal.pone.0144199.

Stoner, A.W, and M. Ray-Culp. 2000. Evidence for Allee Effects in an Over-Harvested Marine Gastropod: Density-Dependent Mating and Egg Production. *Marine Ecology Progress Series* **202**:297 - 302.

Stoner, A.W. 1997. The Status of Queen conch, *Strombus gigas*, Research in the Caribbean. *Marine Fishery Review* **59**(3):14 - 22.

Sunucks, P. 2000. Efficient Genetic Markers for Population Biology. *Tree* **15**(5):199 - 203.

Tewfik, A. 1997. An Assessment of the Biological Characteristics, Abundance and Potential Yield of the Queen conch (*Strombus gigas*) Fishery on the Pedro Bank of Jamaica. (MS. Sc) Acadia University, Wolfville, Canada,

Thiele, S. 2001. *Queen conch Fishery and Their Management in the Caribbean.* TRAFFIC Europe, Technical Report to the CITES Secretariat, Cambridge, UK. http://www2.cedarcrest.edu/academic/bio/jcigliano/MFEC/traffic_europe_report.pdf.

Truelove, N.K. 2014. *The Conservation Genetics of Ecologically and Commercially Important Coral Reef Species*. (PhD diss.) University of Manchester, Manchester, United Kingdom.

Truelove, N.K., L. Fai Ho, R.F. Preziosi, and S.J. Box. 2016. Validation and Characterization of Thirteen Microsatellite Markers for Queen conch, *Lobatus gigas*. *PeerJ Preprints* **4**:e2559v1.

U.S. Department of State. 2004. *Limits in the seas no. 125: Jamaica's maritime claims and boundaries.* https://www.state.gov/documents/organization/57677.pdf .

Van Oosterhout, C, W.F. Hutchinson, D.P.M. Wills, and P. Shipley. 2004. Micro-checker: Software for Identifying and Correcting Genotyping Errors in Microsatellite Data. *Molecular Ecology Notes* **4**(3):535 - 538. doi: 10.1111/j.1471-8286.2004.00684.x.

Wang, J. 2002. An Estimator for Pairwise Relatedness using Molecular Markers." *Genetics* **160**:1203 - 1215.

Waples, R.S. 1998. Separating the Wheat from the Chaff: Patterns of Genetic Differentiation in High Gene Flow Species. *Journal of Heredity* **89**(5):438 - 450.

Ward, R.D., M. Woodmark, and D.O.F. Skibinski. 1994. A Comparison of Genetic Diversity Levels in Marine, Freshwater and Anadromous Fishes. *Journal of Fish Biology* **44**(2):213 - 232.

Watts, R.J., M.S. Johnson, and R. Black. 1990. Effects of Recruitment on Genetic Patchiness in the Urchin *Echinometra mathaei* in Western Australia. *Marine Biology* **195**(1):145 - 151.

Weatherall, P., K.M. Marks, M. Jakobsson, T. Schmitt, S. Tani, J.E. Arndt, M. Rovere, D. Chayes, V. Ferrini, and R. Wigley. 2015. A new digital bathymetric model of the world's oceans. *Earth and Space Science* **2**(8):331 - 345.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the Analysis of Population Structure. *Evolution* **38**:1358 - 1370.

Zamora-Bustillos, R., R. Rodriguez-Canul, and F.J. Garcia De Leon. 2007. Isolation and Characterization of Eight Polymorphic Microsatellite Markers from Pink conch (*Strombus gigas*). *Molecular Ecology Notes* **7**(4):597 - 599. doi: 10.1111/j.1471-8286.2007.01687.x.

Zamora-Bustillos, R, R. Rodríguez-Canul, de León F.J. García, and C.J. Tello. 2011. Genetic Diversity in Two Populations of the Snail *Strombus gigas* (Gastropoda: Strombidae) from Yucatan, Mexico, using Microsatellite. *Revista de Biologia Tropical* **59**(3):1127 - 1134.