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Evidence for shifting genetic structure among Caribbean giant barrel sponges in the Florida Keys

Lindsey K. Deignan[1,2] · Joseph R. Pawlik[1] · Susanna López-Legentil[1]

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Abstract
The giant barrel sponge *Xestospongia muta* is a dominant member of Caribbean reef ecosystems. Populations of *X. muta* that have been monitored annually in plots on Conch and Pickles Reefs in the Florida Keys increased by as much as 122% between 2000 and 2012, raising questions about the processes structuring these growing populations. Microsatellite markers for the closely related Pacific giant barrel sponge *X. testudinaria* were optimized for *X. muta* using individuals from Conch and Pickles Reefs (located 5.5 km apart). Further, within one plot on Conch Reef (AQS3 − 20 m depth), each individual of *X. muta* was mapped and genotyped to investigate fine-scale spatial genetic structuring. Significant spatial autocorrelation was detected at 2-m distance, but the dispersal distance and neighborhood size could not be determined, suggesting that recruitment extends beyond the plot. Finally, sponge samples from Conch Reef (15- and 20-m depth) and Pickles Reefs (15-m depth) were pooled into a single population for Bayesian cluster analyses. Results showed two distinct genetic clusters in the population, Clusters 1 and 2, with a near absence of Cluster 2 sponges among the largest individuals. Comparisons of the microsatellite data with mortality and recruitment data obtained from the plots revealed that the shifting genetic structure is due to disproportionate reproduction or recruitment of Cluster 2 sponges. The selective forces responsible for this genetic shift remain unclear, but it is further evidence of the dramatic changes occurring on coral reefs in the Anthropocene.

Introduction

Sponges are ecologically important components of coral reef ecosystems, particularly in the Caribbean where they may be the most abundant benthic organisms in terms of biomass (Loh and Pawlik 2014). Sponges provide structural complexity to reef habitats and are sources of food for reef fishes, sea turtles, and invertebrates (Pawlik 1983; Meylan 1988; Birenheide et al. 1993; Wulff 1995; Dunlap and Pawlik 1996; Diaz and Rutzler 2001). But with increasing reports that sponge populations are growing (Norström et al. 2009; McMurray et al. 2015; de Bakker et al. 2017), hypotheses have been advanced that link sponges to reduced resilience of reef-building corals on Caribbean reefs (Pawlik et al. 2016).

Genetic analyses of sponges frequently demonstrate high levels of local retention and self-recruitment within sponge populations (Calderón et al. 2007; Blanquer et al. 2009; Bell et al. 2014b; Chaves-Fonnegra et al. 2015; Giles et al. 2015; Richards et al. 2016). For two species in particular, the encrusting sponges *Crambe crambe* and *Scopalina lophyropoda*, fine-scale spatial genetic autocorrelation has been detected within a single reef plot at the scale of < 7 m (Calderón et al. 2007; Blanquer et al. 2009). These data suggested that for at least some sponge species recruitment was highly localized—a phenomenon that was described as philopatric larval dispersal (Calderón et al. 2007; Blanquer et al. 2009).

The Caribbean giant barrel sponge *Xestospongia muta* is the second most abundant sponge on Caribbean reefs in terms of percentage substratum cover, but likely the most

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✉ Lindsey K. Deignan
lindsey.deignan@gmail.com

[1] Department of Biology and Marine Biology, Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC 28409, USA

[2] Present Address: Singapore Centre of Environmental Life Sciences Engineering, Nanyang Technological University, 60 Nanyang Drive, SBS-01N-27, Singapore 637551, Singapore

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abundant sponge in terms of biomass (Zea 1993; Loh and Pawlik 2014). Giant barrel sponges can grow to more than a meter in height and diameter and live for hundreds of years (McMurray et al. 2008). Long-term monitoring of this species on reefs in the Florida Keys (USA) has resulted in the most comprehensive set of studies of growth and demographics for any sponge species (López-Legentil et al. 2008, 2010; McMurray et al. 2008, 2010, 2015; López-Legentil and Pawlik 2009; Deignan and Pawlik 2015). Additionally, this species has experienced significant recent population growth: from 2000 to 2012, the population of *X. muta* in monitored plots on reefs off the Florida Keys increased by 122% (McMurray et al. 2015), prompting questions about the genetic structure of these populations.

Xestospongia muta shares a general morphology with two described Pacific barrel sponges, *X. testudinaria* and *X. bergquistia*, and it was assumed that the Caribbean and Pacific giant barrel sponges shared a common genetic ancestry before the closing of the Isthmus of Panama about 3 million years ago (Keigwin 1978). However, recent genetic studies comparing *X. muta* to giant barrel sponges worldwide have revealed an arrangement inconsistent with typical phylogeographic patterns (Swierts et al. 2013, 2017; Setiawan et al. 2016a, b). Mitochondrial and nuclear DNA evidence suggest that giant barrel sponges diverged into a species complex prior to their geographic separation, and in the Caribbean, *X. muta* may consist of a complex of three species that occur in sympatry (Swierts et al. 2017). While these interesting developments address the broader distribution and diversity of giant barrel sponges, little is known about their genetic structure at within-reef scales, their relative demographic success over time, or their fine-scale dispersal patterns.

Microsatellite analysis remains one of the most sensitive methods to establish intraspecific variation and connectivity patterns among sponge populations, particularly at small spatial scales (Duran et al. 2004, Calderón et al. 2007; Blanquer et al. 2009; Giles et al. 2015). Microsatellite markers developed for the closely related Pacific barrel sponge *X. testudinaria* were used in Indonesia and revealed that populations of *X. testudinaria* separated by 2–70 km were genetically different from one another (Bell et al. 2014a, b). In this study, we tested the usefulness of the microsatellite markers described by Bell et al. (2014a) for *X. muta* by genotyping two populations on reefs in the Florida Keys. Further, we investigated fine-scale genetic structure of this species using spatial autocorrelation analyses and by genotyping all of the individuals present in one of the 16-m diameter circular monitored plots for which we have long-term monitoring data (McMurray et al. 2010, 2015). This fine-scale genetic analysis builds on a study by Richards et al. (2016) using microsatellite markers to differentiate populations of *X. muta* across the Caribbean Sea that revealed a gradient of genetic differentiation along the Florida Keys reef tract correlated with the direction of surface currents. Giant barrel sponges are broadcast spawners that release negatively buoyant eggs that may cover the reef during reproductive events (Ritson-Williams et al. 2005), but their dispersal is subject to local flow conditions, and the extent to which eggs, sperm, and larvae survive when carried by water currents remains unknown. Here, we used microsatellite markers to look at the genetic structure of *X. muta* at reef-level spatial scales. Finally, we leveraged our long-term monitoring data set of individual *X. muta* in the Florida Keys (McMurray et al. 2010, 2015) to compare size-structured genetic data with mortality and recruitment data obtained from these same populations.

Methods

Microsatellite optimization for *X. muta*

Samples of *Xestospongia muta* (Schmidt 1870) were collected in June 2015 from two reefs in the Florida Keys, USA: 24 samples from Conch Reef (24°56′59″N; 80°27′13″W: ca. 15 m depth) and 55 samples from Pickles Reef (24°59′15″ N, 80°24′52″ W: ca. 15 m depth). This set of samples was used to test the usefulness of the 12 polymorphic microsatellite markers developed for the Pacific giant barrel sponge *X. testudinaria* by Bell et al. (2014a). After collection, sponge samples were immediately fixed in absolute ethanol and stored at − 20 °C. DNA extraction was conducted using the DNeasy Tissue and Blood extraction kit (QIAGEN) following manufacturer's instructions. A gradient PCR was first performed to optimize amplification for extractions of DNA from *X. muta* using the microsatellite primers published by Bell et al. (2014a). Amplifications were performed in a final volume of 25 µl using 12 µl BioLine MyTaq™ HS Red Mix, 10 µl molecular grade water, and 1 µl each of forward and reverse primers.

PCR amplification was done as follows: an initial 1 min denaturation step at 95 °C, followed by 30 cycles of 95 °C for 15 s, 50–60 °C for 15 s, and 72 °C for 10 s, and ending with a 1-min final extension at 72 °C. Sufficient amplification for all working loci was found at 56 °C. DNA sequencing was performed to confirm that the regions amplified by PCR contained microsatellite repeats. Amplified DNA was cloned using the TOPO TA cloning kit (Life Technologies). Bacterial colonies with a DNA insert in their plasmid (white colonies) were PCR amplified using the M13 Forward and M13 Reverse primers and the following method: an initial 10 min denaturation step at 95 °C, followed by 30 cycles of 95 °C for 30 s, 55 °C for 15 s, and 72 °C for 30 s, and ending with a 2-min final extension step at 72 °C. The resulting amplifications were sequenced using a 3130xl Genetic

Analyzer (Applied Biosystems) available at UNCW Center for Marine Science. Sequences were edited and aligned using Sequencher software and have been deposited in GenBank (Accession numbers KY858934 to KY858942).

All samples were genotyped to test for polymorphism among successfully amplified microsatellite loci. Forward primers were fluorescently labeled with FAM, NED, VIC, or PET (Life Technologies) and employed in Muliplex PCR to maximize efficiency of genotyping. Multiplex PCR amplification was performed in a final volume of 25 µl using 12 µl BioLine MyTaq™ HS Red Mix, 10 µl molecular grade water, and 2 µl of a mixture of the multiplex forward and reverse primers (Table S1). The PCR amplification profile consisted of an initial 2-min denaturation step at 95 °C, followed by 25 cycles of 95 °C for 30 s and 56 °C for 4 min. Genotyping was done on the same 3130xl Genetic Analyzer as above using the size standard GeneScan 600 LIZ. Peak Scanner 2 (Applied Biosystems) was used for length and allele scoring. Observed and expected heterozygosity, Fisher's exact test for Hardy–Weinberg equilibrium, and linkage disequilibrium with a Benjamini–Yekutieli correction were calculated using GENEPOP (Raymond and Rousset 1995; Rousset 2008). Allele richness and the fixation index (F_{IS}), commonly known as the inbreeding coefficient, were calculated in FSTAT 2.9.3.2 (Goudet 1995). MICRO-CHECKER (Van Oosterhout et al. 2004) was used to check for evidence of large allele dropout and scoring errors.

Fine-scale spatial genetic analysis

The location of each individual *X. muta* within a 16-m diameter circular plot (AQS3) on Conch Reef at 20-m depth (24°56′59″N; 80°27′13 W) was triangulated by SCUBA divers and assigned to an *x*,*y* coordinate. All individuals of *X. muta* had been subjected to an annual census in this reef plot for demographic analysis over the period 2000–2013, with the exception of 2007, when bad weather prevented access to plots (McMurray et al. 2010, 2015). A total of 71 sponges within the plot were sampled and genotyped following the above protocol. Genetic diversity was calculated using GENEPOP (Raymond and Rousset 1995; Rousset 2008). Allele richness and the inbreeding coefficient (F_{IS}) were calculated in FSTAT 2.9.3.2 (Goudet 1995).

Spatial Pattern Analysis of Genetic Diversity (SPAGeDi) software (Hardy and Vekemans 2002) was used to assess fine-scale spatial genetic structure within the plot. Three methods were employed to evaluate spatial autocorrelation of the sponges: Rousset's *a* index, kinship coefficient index, and Moran's I. Rousset's *a* index is analogous to the commonly used population pairwise genetic distance $F_{ST}/(1–F_{ST})$ which uses regression with distance to assess isolation-by-distance among populations (Rousset 2000). The existence of spatial autocorrelation was also determined using a kinship coefficient index (Loiselle et al. 1995), which is independent of the samples being in Hardy–Weinberg equilibrium. Finally, Moran's (1948) is a metric similar to kinship estimates, but based on allogamy and dispersive spawning (Epperson 2005), both reproductive strategies employed by *X. muta*. Rousset's *a* and the kinship coefficient analyses were used to estimate dispersal distance (sigma, σ) and neighborhood size (Nb) by examining the slope of the autocorrelation coefficients in relation to the natural logarithm of the distance intervals (see Calderón et al. 2007). The distance intervals for the analysis were set at 1 m starting at 2 m from each sponge and extending to 14 m. A starting distance of 2 m was used to ensure that the area contained a minimum of 100 sponge comparison pairs. Each analysis included jackknifing over the loci to estimate variance, and 1000 permutations of location were used to calculate confidence intervals. To estimate gene dispersal (σ), the density of sponges was set as the total number of sponges/area of the plot ($71/201.062\ m^2 = 0.3531\ m^{-2}$).

Size-structured genetic analysis

For the size-structured genetic analysis, we pooled together data from 150 individuals collected in June 2015 from Pickles Reef (15 m depth), Conch Reef (15 m), and Conch Reef (AQS3; 20 m; Fig. S1) for which we had demographic information (McMurray et al. 2010, 2015). Conch Reef and Pickles Reef are located 5.5 km apart along the Florida Keys reef tract. Individuals from both sites were pooled together to allow for sufficient representation of sponges in each size category (McMurray et al. 2010). Number of alleles and observed and expected heterozygosity were calculated using GENEPOP (Raymond and Rousset 1995; Rousset 2008). Allele richness and the inbreeding coefficient (F_{IS}) were calculated in FSTAT 2.9.3.2 (Goudet 1995).

The size of each sponge was measured at the time of sampling using the method described by McMurray et al. (2008, 2010). In brief, each sponge osculum was measured to the nearest cm with a measuring tape and the volume calculated using the osculum/volume relationship described in McMurray et al. (2008, 2010). Sponges were classified into five size classes based on volume as in the demographic analyses of McMurray et al. (2010, 2015): Size Class I, $\leq 143.13\ cm^3$; Size Class II, $> 143.13\ cm^3$ but $\leq 1077.13\ cm^3$; Size Class III, $> 1077.13\ cm^3$, but $\leq 5666.32\ cm^3$; Size Class IV, $> 5666.32\ cm^3$ but $\leq 17\ 383.97\ cm^3$; and Size Class V, $> 17\ 393.97\ cm^3$. Fisher's exact test for Hardy–Weinberg equilibrium was calculated for each size class using GENEPOP (Raymond and Rousset 1995; Rousset 2008). An Analysis of Molecular Variance (AMOVA) with each size class as a group was conducted using Arlequin 3.5.2.2 (Excoffier and Lischer

2010), and pairwise F_{ST} values among size classes were calculated using the pegas package for R (Paradis 2010).

Finally, all sponges were ranked from the smallest to the largest volume within each of the five predetermined classes, and a Bayesian cluster analysis was performed using STRUCTURE software (Pritchard et al. 2000). The *K* values were set from 1 to 6 to account for the five size classes. For each analysis, a Markov Chain Monte Carlo (MCMC) simulation was set at 100,000 with a burn in of 25% and an admixture model. STRUCTURE Harvester (Earl and von Holdt 2012) was used to calculate Delta *K* and the best *K* value for the STRUCTURE analysis. The same analysis was repeated with samples separated according to their origin and revealed equivalent results (Fig. S2); therefore, only results obtained for the whole dataset are discussed herein.

In all instances, STRUCTURE results pointed to the existence of two main genetic pools or clusters (hereafter, Clusters 1 and 2). The number of alleles, observed and expected heterozygosity, and Fisher's exact test for Hardy–Weinberg equilibrium were calculated using GENEPOP for each genetic cluster (Raymond and Rousset 1995; Rousset 2008; Table S2). Recruitment dates for each sponge in Cluster 1 and 2 were obtained from the long-term monitoring data set (McMurray et al. 2010, 2015) and compared to each other to determine whether either cluster had experienced greater recruitment at a particular time. The recruitment date was defined as the year that each sponge was first recorded in the long-term monitoring database between 2001 and 2013 following the establishment of the monitored population. A paired *t* test was used to compare recruitment of Cluster 1 and 2 sponges for the entire study period (2001–2013) and then divided into two time periods, 2001–2006 and 2008–2013, to examine changes in recruitment of Clusters 1 and 2 sponges over time. Year 2007 was excluded from the analyses because no data were obtained that year (see above). A paired *t* test was also used to compare expected recruitment and actual recruitment (as defined above) for Cluster 2 sponges between 2001–2013, 2001–2006, and 2008–2013. Expected recruitment was calculated from the relative proportion of each cluster by sponge size in the population as a relative estimate of reproductive output (Table S3). Mortality data were obtained from McMurray et al. (2015) for the total population of all sponges monitored between 2000 and 2012 from plots at Conch Reef (15 and 20 m) and Pickles Reef. The data were organized by size class and divided into two time periods, mortality for 2000–2006 and 2006–2012 to compare mortality trends with genetic structure.

Results

Microsatellite optimization for *X. muta*

Eleven of the 12 primer pairs developed for *X. testudinaria* and *X. bergquistia* (Bell et al. 2014a) amplified successfully for *X. muta* (Table 1). Sequencing revealed the presence of microsatellite repeats for all amplified regions. However, Xesto 9 and 10 did not amplify consistently among samples and yielded fragment sizes that were off-range, so these two loci were not considered in further analyses. The remaining 9 microsatellites (Xesto 1–8, 11) amplified consistently for all of our *X. muta* samples and were further characterized and optimized for this species (Table 1). Some discrepancies with the information described in Bell et al. (2014a) were noted (Table 1). In particular, DNA sequencing revealed that the repeat motif for Xesto 6 was different than previously described, while Xesto 11 had opposite forward and reverse sequence reads than those reported by Bell et al. (2014a).

The number of alleles per locus ranged from 2 to 7 at Conch Reef, and the mean number of alleles per locus was 4.0 (± 2.0 SD; Table 1). For Pickles Reef, the number of alleles per locus ranged from 3 to 7, and the mean number of alleles per locus was 5.0 (± 2.0 SD; Table 1). For Conch Reef, 5 of the 9 microsatellites were in Hardy–Weinberg equilibrium, and 3 of the 9 microsatellites were in Hardy–Weinberg equilibrium for Pickles Reef. The remaining microsatellites were significantly outside Hardy–Weinberg equilibrium due to heterozygote deficiencies (Table 1). MICRO-CHECKER detected excess homozygotes for all microsatellites, except Xesto 2, Xesto 5, and Xesto 7. In addition, because of excess homozygosity, Xesto 4 also had the potential for scoring errors due to stuttering. However, none of the microsatellite loci showed evidence of large allele dropout. Linkage disequilibrium was detected for a few loci, particularly Xesto 8 and Xesto 11, likely as an indirect result of the excess homozygosity in the population. In order to investigate whether the potential for linkage between these loci could bias the spatial autocorrelation and Bayesian cluster analyses, we re-ran those analyses without Xesto 8 and Xesto 11. No differences in the results were observed either way, so only analyses including all of the microsatellite loci are presented herein.

Fine-scale spatial genetic structure

Fine-scale spatial genetic structure was detected within the shortest interval (up to 2 m) for Rousset's *a*, the kinship coefficient, and Moran's I (Fig. 1), indicating a higher

Table 1 Characterization of microsatellites for 55 samples form Pickles Reef and 24 samples from Conch Reef

		Pickles							Conch Reef						
Locus	Repeat motif	Size Range (bp)	N_A	AR	H_o	H_E	P_{HWE}	F_{IS}	Size Range (bp)	N_A	AR	H_o	H_E	P_{HWE}	F_{IS}
Xesto 1	$(AAC)_9$	213–233	6	5.086	0.564	0.75	0.0000	**0.248**	213–233	7	6.331	0	1	0.0012	**0.402**
Xesto 2	$(CCTGAG)_8$	287–311	5	4.833	0.545	0.5	0.1722	− 0.084	293–311	3	3	0	0	0.4920	0.111
Xesto 3	$(AT)_{14}$	107–129	7	5.898	0.582	0.61	0.0000	0.046	107–129	6	5.336	0	1	0.0004	**0.353**
Xesto 4	$(AG)_6$	204–212	5	4.452	0.309	0.48	0.0000	**0.353**	204–212	5	4.605	0	0	1.0000	− 0.158
Xesto 5	$(TAA)_4(GA)_9$	174–186	7	5.169	0.545	0.58	0.9092	0.058	174–184	6	5.601	1	1	0.5258	− 0.166
Xesto 6	$(AGG)_6$	113–119	3	2.849	0.236	0.36	0.0002	0.346	113–119	3	2.995	1	1	0.4218	0.021
Xesto 7	$(TCT)_5$	80–86	3	2.581	0.127	0.18	0.0599	0.303	80–86	2	2	0	0	1.0000	− 0.150
Xesto 8	$(AT)_9$	188–198	4	3.419	0.109	0.34	0.0000	**0.685**	188–198	3	2.708	0	0	0.0000	**0.906**
Xesto 11	$(TC)_7$	161–171	3	2.309	0.018	0.32	0.0000	**0.943**	161–171	4	3.687	0	0	0.0000	**0.643**

N_A number of alleles, *AR* allele richness H_o observed heterozygosity, H_E expected heterozygosity, *p* value for the exact test Hardy–Weinberg equilibrium (P_{HWE}), inbreeding coefficient F_{IS} with significant values in bold

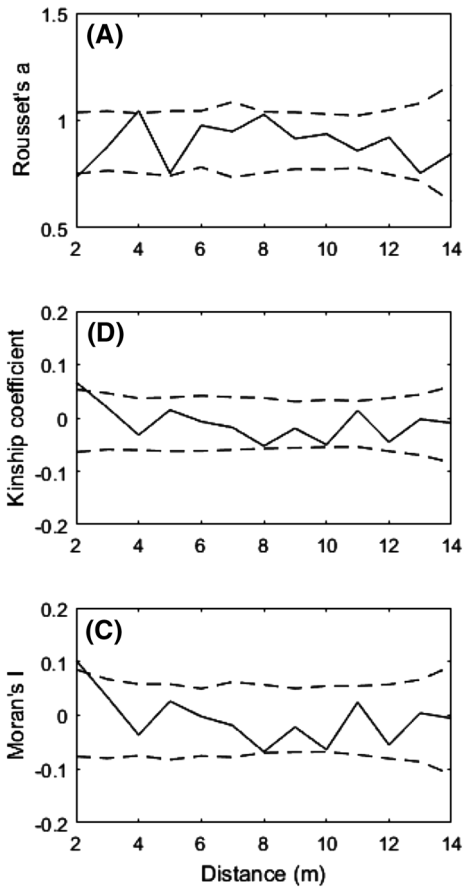

Fig. 1 Spatial autocorrelation analyses **a** Rousset's *a*, **b** Kinship coefficient, and **c** Moran's I. The dashed lines represent the 95% confidence intervals and the distances on the x-axis represent the highest extent of the distance interval

degree of inbreeding, or the breeding of closely related individuals, than expected at that scale. Accordingly, samples within this plot (AQS3) were characterized by values for expected heterogeneity that were overall higher than for observed heterozygosis and high inbreeding coefficients (Table 2). Negative spatial autocorrelation was also detected at 3–4 m for Rousset's *a*, but not for the kinship coefficient or Moran's I. For both Rousset's index and the kinship coefficient there was no convergence of the slopes of the autocorrelation coefficients with the natural logarithm of the distance, making it impossible to determine the dispersal parameter (sigma; σ), and the neighborhood size (Nb). A lack of convergence indicates that the dispersal distance for *X. muta* exceeds the plot examined (16 m diameter).

Size-structured analysis

There were 43 sponges classified in Size Class I, 25 sponges in Size Class II, 24 sponges in Size Class III, 23 sponges in

Table 2 Characterization of 9 microsatellites for 71 samples collected from plot AQS3 on Conch Reef and for all 150 samples pooled together from Pickles Reef, Conch Reef, and plot AQS3 on Conch Reef

Locus	Plot AQS3 (Conch Reef 20 m)						All samples					
	Size Range (bp)	N_A	AR	H_o	H_E	F_{IS}	Size Range (bp)	N_A	AR	H_o	H_E	F_{IS}
Xesto 1	213–233	6	5.139	0.549296	0.764259	**0.274**	213–233	7	5.24	0.54	0.758149	**0.285**
Xesto 2	278–323	9	7.282	0.535211	0.592056	0.104	278–323	9	6.393	0.506667	0.528629	0.045
Xesto 3	107–143	9	6.406	0.450704	0.617176	**0.282**	107–143	9	6.184	0.486667	0.610865	**0.209**
Xesto 4	202–212	6	5.067	0.211268	0.624313	**0.681**	202–212	6	5.135	0.286667	0.542787	**0.480**
Xesto 5	174–186	7	5.37	0.478873	0.66337	**0.275**	174–186	7	5.365	0.526667	0.618863	**0.147**
Xesto 6	113–119	3	2.972	0.295775	0.466917	**0.365**	113–119	3	2.93	0.313333	0.446397	**0.297**
Xesto 7	80–89	4	3.254	0.126761	0.279403	**0.508**	80–89	4	2.813	0.153333	0.239717	**0.329**
Xesto 8	188–198	5	4.517	0.15493	0.478673	**0.677**	188–198	5	4.023	0.12	0.42495	**0.718**
Xesto 11	161–171	4	3.234	0.042254	0.507841	**0.944**	161–171	5	3.434	0.053333	0.434983	**0.892**

N_A number of alleles, *AR* allele richness, H_o observed heterozygosity, H_E expected heterozygosity, inbreeding coefficient F/swith significant values in bold

Table 3 *P* values for the exact test for Hardy–Weinberg equilibrium for each size class and each microsatellite marker

	Size class I	Size class II	Size class III	Size class IV	Size class V
Xesto 1	0.0000*	0.0000*	0.0005*	0.1124	0.0580
Xesto 2	0.0000*	0.0035*	0.0042*	0.9048	0.9336
Xesto 3	0.0000*	0.0013*	0.0035*	0.2342	0.1519
Xesto 4	0.0000*	0.0000*	0.0000*	0.0431*	0.0391*
Xesto 5	0.6887	0.0076*	0.2037	0.4827	0.1248
Xesto 6	0.0001*	0.0042*	0.0002*	0.2132	0.1579
Xesto 7	0.0015*	0.2407	0.1474	0.0289*	1.0000
Xesto 8	0.0000*	0.0000*	0.0001*	0.0225*	0.0136*
Xesto 11	0.0000*	0.0000*	0.0000*	0.0226*	0.0143*

*Significant departure of Hardy–Weinberg equilibrium (P_{HWE})

Table 4 Analysis of molecular variance (AMOVA) using size class as population and allsamples pooled

Source of variation	Sum of squares	Variance components	Percentage variation
Among classes	46.504	0.16219	6.63418
Among individuals within classes	641.887	2.28260	93.36582
Total	688.391	2.44479	

Table 5 Pairwise F_{ST} values by Size Class for all samples pooled together

Size class	I	II	III	IV
II	0.0063	–	–	–
III	0.0175	0.0267	–	–
IV	0.0538*	0.0784*	0.0301	–
V	0.0561*	0.0746*	0.0236*	0.0078

*Significant differentiation

Size Class IV, and 35 sponges in Size Class V. The number of microsatellites in Hardy–Weinberg equilibrium increased with sponge size class (Table 3). In Size Class I and II, there was only one microsatellite in Hardy–Weinberg equilibrium, while by Size Class V there were 6. The analysis of molecular variance (AMOVA) showed that most of the variation was among individuals within size classes (Table 4). Pairwise F_{ST} values revealed significant differentiation between the smallest and largest size classes (Table 5).

The Bayesian clustering analysis using STRUCTURE showed that there were two main genetic pools or clusters ($\Delta K = 1458.9308$; Fig. 2). The genetic cluster with more sponges in it, Cluster 1 (in green), contained individuals from all five size classes (Fig. 2). The second genetic cluster, Cluster 2 (in red), mostly contained sponges classified within the three smallest size classes (≤ 5666.32 cm^3), with the exception of two individuals (one classified in class IV and one in class V; Fig. 2), both of which were in the Conch Reef plot at 20-m depth (AQS3, Fig. S2).

There were 55 sponges from Cluster 1 and 10 sponges from Cluster 2 present in the monitored plots in 2000

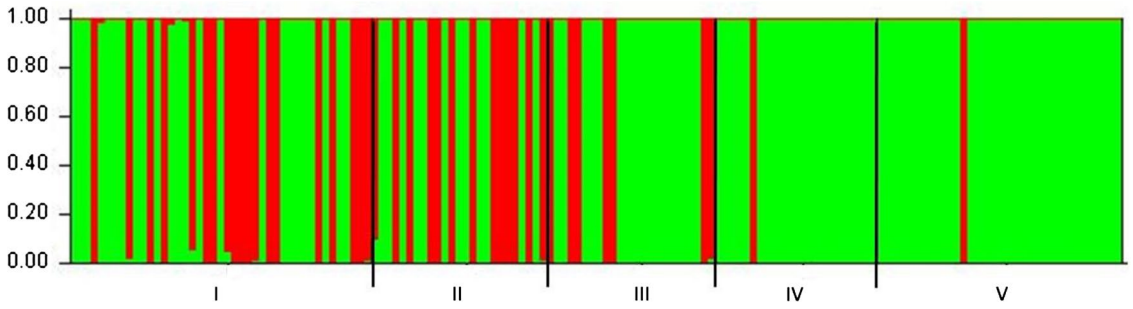

Fig. 2 STRUCTURE bar plot assignment of the 150 individuals from Pickles Reef (15 m depth), Conch Reef (15 m), and Conch Reef (AQS3—20 m) ranked in order from smallest to largest volume to each of the two genetically differentiated clusters (Delta $K = 1458.9308$). Cluster 1: Green, Cluster 2: Red. The x-axis shows the Size Classes I–IV

(Table S2). From 2001–2013, recruitment of Cluster 1 sponges was significantly higher than recruitment of Cluster 2 sponges (Paired t test, $t11 = 2.9888$, $P = 0.01232$), with 53 sponges recruiting into Cluster 1 and 31 sponges recruiting into Cluster 2. Accordingly, during the 2001–2006 period recruitment of Cluster 1 sponges was significantly higher than recruitment of Cluster 2 sponges (Paired t test, $t5 = 3.1623$, $P = 0.02503$). During the 2008–2013 period there was no significant difference in observed recruitment of Cluster 1 and 2 sponges (Paired t test, $t5 = 1.2247$, $P = 0.2752$). However, the foregoing analyses did not take sponge size and reproductive output into consideration: when differences in the size classes of sponges for each cluster were taken into account and similar analyses were performed using expected recruitment values based on relative reproductive output as a function of sponge size (Table S3), recruitment of Cluster 2 sponges was significantly higher than expected for 2001–2013 (Paired t test, $t11 = 3.9734$, $P = 0.002183$). For 2001–2006, there was a significant difference between the observed and expected recruitment of Cluster 2 sponges (Paired t test, $t5 = 2.5926$, $P = 0.4869$), and this trend increased in 2008-2013, with the observed recruitment significantly higher than the expected recruitment (Paired t test, $t5 = 3.0541$, $P = 0.02829$). These data reveal a shift in relative recruitment of sponges belonging to Cluster 2, with increased recruitment success in the most recent years.

Consistent with broadcast spawning animals, mortality was greatest in the smallest size class (Size Class I) and decreased with increasing sponge size during the 2000–2012 period (Table S4). However, during the 2000–2006 period, the second highest levels of mortality occurred within the largest size class (Size Class V). Accordingly, the higher mortality rates of both the smallest and largest sponges resulted in the mid-sized sponges having the lowest overall mortality.

Discussion

Nine of the 12 microsatellite loci characterized by Bell et al. (2014a) for the Pacific giant barrel sponge *Xestospongia testudinaria* were successfully optimized for analyzing the fine-scale spatial genetic structure of the Caribbean giant barrel sponge *X. muta* in the Florida Keys. As many as six microsatellites per population were not in Hardy–Weinberg equilibrium and high inbreeding coefficients (F_{IS}) were recorded in most cases. Both the lack of Hardy–Weinberg equilibrium for some microsatellites and high F_{IS} values are common in sponges (e.g., Riesgo et al. 2014; Taboada et al. 2015), yet these same microsatellite markers were subsequently successfully used to infer genetic structure and gene flow among populations (e.g., Chaves-Fonnegra et al. 2015; Riesgo et al. 2016).

Population genetic studies have revealed that gene flow patterns for *X. muta* follow Caribbean and Florida surface currents (López-Legentil and Pawlik 2009; Richards et al. 2016). Along the Florida Keys reef tract, there is a gradient of genetic differentiation, suggesting gene flow occurs across adjacent reefs (Richards et al. 2016). We found evidence of closely related individuals settling near each other at the smallest scales (< 2 m). Under low flow conditions, larvae may recruit near their parents or siblings, likely explaining the particularly high F_{IS} values and heterozygote deficiencies reported for the deeper plot on Conch Reef at 20 m depth (AQS3), which tends to experience less flow than shallower sites. Overall, however, the dispersal distance and neighborhood size could not be determined, indicating that recruitment mostly occurred outside the monitored 16 m diameter plot. Together with previous studies (López-Legentil and Pawlik 2009; Richards et al. 2016), our results confirm that recruitment and gene flow occur regularly among adjacent populations within the same reef tract.

The Bayesian clustering analyses revealed that two distinct genetic clusters (1 and 2) coexist in the plots on Conch and Pickles Reefs in the Florida Keys with almost no mixing between them. These results are consistent with a previous study using other microsatellite markers that revealed two genetic pools in the Florida Keys and a total of up to 5 for the whole Caribbean (Richards et al. 2016). More recently, another genetic study using both mitochondrial (COI) and nuclear (ATP6, ATPsβ) DNA sequence data concluded that the Caribbean giant barrel sponge *X. muta* is part of a species complex containing at least three reproductively isolated species (Swierts et al. 2017). Further research will be required to determine how the microsatellite data presented herein and in Richards et al. (2016) relate to the mitochondrial and nuclear DNA data reported in Swierts et al. (2017).

The novelty of the present study lies in the coupling of the genetic analyses with the demographic data from time-series monitoring of the individual sponges within plots since 2000. Sponges in Cluster 2 constituted approximately 50% of the sponges in the smaller size classes, but this cluster was very rare in the larger and older size classes. By combining data on sponge size (a proxy for reproductive output; see below), recruitment and mortality with the Bayesian analysis of the genetic data, it is clear that there has been a shift in relative recruitment in favor of Cluster 2 sponges, despite greater reproductive potential among Cluster 1 sponges. While Cluster 1 sponges continue to recruit into the population, their proportional recruitment is surprisingly low considering both the evidence for localized recruitment and the expectation that Cluster 1 sponges should produce the vast majority of propagules, as they predominate among the largest sponge size classes.

As with other modular and colonial organisms, sponge reproductive output increases as a function of increasing size (Babcock 1991; Uriz et al. 1995; Hall and Hughes 1996; Whalan et al. 2007; Abdo et al. 2008; Wahab et al. 2014, 2017). This was directly observed by us during several spawning events over the nearly two decades of this time-series monitoring program, with female *X. muta* producing a volume of flocculent egg mass in proportion to sponge volume (Ritson-Williams et al. 2005). Accordingly, *X. muta* in size classes IV and V should account for approximately 95% of the total recruitment observed in any given year, as determined by McMurray et al. (2017). Given that our samples contained only two Cluster 2 sponges out of 58 total sponges in the largest two size classes, we would expect to see very low representation of Cluster 2 sponges among new recruits (see calculations in Table S3). However, Cluster 2 sponges represented approximately 50% of the observed recruitment into the smaller size classes. The high recruitment of Cluster 2 sponges despite their low representation in the larger size classes suggests either a decrease in the expected reproductive output or recruitment of Cluster 1 sponges, or enhanced reproductive success or recruitment of Cluster 2 sponges.

The mortality data corroborate the conclusion that the genetic structure of the population is shifting, rather than stable. Demographic data collected during the long-term monitoring efforts (McMurray et al. 2010, 2015) revealed that mortality followed the pattern typical of broadcast spawning organisms, with the smallest individuals experiencing the greatest mortality (Babcock 1985; Smith 1992; Wilson and Harrison 2005). The only exception to this pattern occurred during the 2000–2006 period, when there was an unusually high mortality rate for the largest size class (V) in 2005 because of a pathogenic syndrome called sponge orange band (SOB; Cowart et al. 2006). However, this mortality event occurred at least 3 years before the enhanced recruitment of Cluster 2 sponges into size classes I and II observed during the 2008–2013 period (Table S4). Following the age-growth curves calculated by McMurray et al. (2008), sponges in Size Class I are 0–2 years old and those in Size Class II are 2—6 years old. The mass mortality event caused by SOB was recorded in 2005, but the increase in recruitment for Cluster 2 was not observed until 2008 and continued in 2009, 2010, 2011, 2012, and 2013. This means that the Cluster 2 sponges that recruited during 2008–2013 could not have been the progeny of hypothetical Cluster 2 parent sponges that differentially (relative to Cluster 1 sponges) died of SOB in 2005.

Whether the recent increase in Cluster 2 sponges is due to impaired reproductive output or recruitment of Cluster 1 sponges or to an unidentified selective force favoring Cluster 2 sponges is not currently known. One possibility is that larger size class Cluster 2 sponges are more common at depths greater than the plots sampled in this study, and that these deep-water sponges serve as a source of recruits to shallower depths, perhaps delivered by internal waves that are known to move across Conch Reef (Leichter et al. 1998, 2005, 2014); however, if this is true, the recruitment data indicate that this mechanism of delivery of Cluster 2 recruits must have increased since 2008. Other studies have demonstrated genetic differentiation with depth in the Florida Keys for the excavating sponge *Cliona delitrix* (Chaves-Fonnegra et al. 2015) and for the corals *Montastraea cavernosa* and *Porites astreoides* (Serrano 2013; Serrano et al. 2014). The differentiation is thought to be attributable to currents along the Florida Keys reef tract and asynchronous spawning between shallow and deep populations (Serrano 2013; Serrano et al. 2014; Chaves-Fonnegra et al. 2015). Additional genetic sampling will be required to determine whether populations of sponges at greater depths have a higher proportion of Cluster 2 sponges.

This is the first study to incorporate long-term demographic data with genetic analyses to reveal a shifting genetic

structure in a sponge population. Additional monitoring and genetic sampling will be needed to determine whether the observed genetic shift continues, and whether the increase in Cluster 2 sponges is associated with a particular selective force, an influx of recruits from deeper reefs, or is a consequence of reduced reproduction or recruitment of Cluster 1 sponges. With the growing awareness that sponges play an outsized role in carbon and nutrient cycling on Caribbean reefs (Southwell et al. 2008; de Goeij et al. 2013; McMurray et al. 2016, 2018; Pawlik et al. 2016, 2018), it is important to understand how the genetic structure of sponge populations may be shifting in response to climate change and other anthropogenic stressors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable guidelines for the collection of sponge tissue samples were followed under permit FKNMS-2009-126-A1.

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