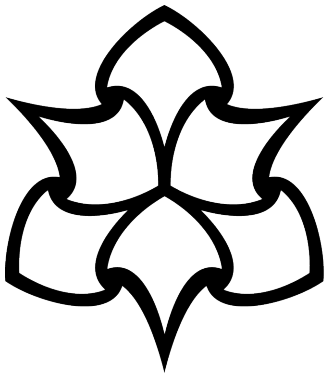


Using genetics to inform restoration and predict resilience in declining populations of a keystone marine sponge

Sarah M. Griffiths[1*], Evelyn D. Taylor-Cox[1,2], Donald C. Behringer[3,4], Mark J. Butler IV[5] & Richard F. Preziosi[1]

[1] Ecology and Environment Research Centre, Manchester Metropolitan University, Manchester, UK.
[2] Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, UK
[3] Fisheries and Aquatic Sciences, University of Florida, Gainesville, Florida, USA.
[4] Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA.
[5] Department of Biological Sciences, Old Dominion University, Norfolk, Virginia, USA.

[*]Corresponding author: Sarah Griffiths, sarah.griffiths@mmu.ac.uk

ORCHID IDs

Sarah M. Griffiths: 0000-0003-4743-049X
Evelyn D. Taylor-Cox: 0000-0002-3937-3643
Donald C. Behringer: 0000-0001-5244-471X
Mark J. Butler IV: 0000-0002-6052-6659
Richard F. Preziosi: 0000-0003-0468-6655

Acknowledgments

Many thanks for assistance in sampling to T. Pérez, C. Freeman, F. Clever, L. Gittens, the Forest and Marine Reserves Association of Caye Caulker (FAMRACC), the PACOTILLES campaign team and crew of the RV Antea, and the Old Dominion University and University of Florida 2014 field teams. Thanks to P. Briggs at the University of Manchester Bioinformatics Core Facility, and to N. Truelove for sequencing assistance. Many thanks to T. Pérez for providing helpful comments and discussion on the manuscript. This work was funded by a PhD studentship awarded to S. Griffiths by the Natural

Environment Research Council (NERC). Funding for the field collections in Florida was provided by a Nature Conservancy-NOAA Community-based Habitat Restoration Grant (GMT-ODU-091512) to M. Butler and D. Behringer. Fieldwork in Barbuda was funded by ship time awarded to T. Pérez and C. Fauvelot by Flotte Océanographique Française.

Field collection of sponge tissue in the Florida Keys was conducted under permits issued to M. Butler by the Everglades National Park (EVER-00401), the Florida Keys National Marine Sanctuary (FKNMS-2014-042), and the Florida Fish and Wildlife Conservation Commission (SAL-13-0582A-SR). Research collections in the Bahamas were conducted under a permit granted by the Bahamas Department of Marine Resources to M. Butler (MA&MR/FIS/17). Sampling in Belize was conducted under a permit granted by Belize Fisheries Department to S. Griffiths and R. Preziosi (0.00023-13). Samples in Barbuda were collected under permits issued to T. Pérez and C. Fauvelot as part of the PACOTILLES campaign (http://dx.doi.org/10.17600/15005300; http://dx.doi.org/10.17600/15005200).

Abstract

Genetic tools can have a key role in informing conservation management of declining populations. Genetic diversity is an important determinant of population fitness and resilience, and requires careful management to ensure sufficient variation is present. In addition, population genetics data reveal patterns of connectivity and gene flow between locations, enabling mangers to predict recovery and resilience, identify areas of local adaptation, and generate restoration plans. Here, we demonstrate a conservation genetics approach to inform restoration and management of the loggerhead sponge (*Spheciospongia vesparium*) in the Florida Keys, USA. This species is a dominant, habitat-forming component of marine ecosystems in the Caribbean region, but in Florida has suffered numerous mass mortality events. We developed microsatellite markers and used them to genotype sponges from 14 locations in Florida and a site each in the Bahamas, Belize and Barbuda. We found that genetic diversity levels were similar across all sites, but inbreeding and bottleneck signatures were present in Florida. Populations are highly structured at the regional scale, whilst within Florida connectivity is present in a weak isolation by distance pattern, coupled with chaotic genetic patchiness. Evidence of a weak barrier to gene flow was found in Florida among sites situated on opposite sides of the islands in the Middle Keys. Loggerhead sponge populations in Florida are vulnerable in the face of mass mortalities due to low connectivity with other areas in the region, as well as distance-limited and unpredictable local connectivity patterns. However, our discovery of Florida's high genetic diversity increases hope for resilience to future perturbations. These results provide valuable insight for sponge restoration practice in Florida.

Keywords (4-6)

Caribbean, conservation, Florida Keys, loggerhead sponge; Porifera, *Spheciospongia vesparium*

Introduction

Population declines in keystone species have a number of negative impacts on associated communities, ecosystem functioning, and the provision of ecosystem services (Sweeney et al 2004; Hicke et al 2012; Thomson et al 2015; Sorte et al 2017). Genetic factors are a significant determinant of population health and fitness, and can influence both the longevity of populations and the success of conservation strategies (Frankham 2005). However, genetic information is unavailable for the vast majority of species, and thus the application of conservation genetics theory to practice has been limited (Shafer et al., 2015; Taylor, Dussex & van Heezik, 2017).

Declining populations are vulnerable to low genetic diversity due to the effects of genetic drift, in which rare alleles have a higher probability of being lost due to random chance in smaller populations. These effects are amplified considerably in populations that experience a rapid decline, or bottleneck, through which substantial genetic variation is randomly eliminated in a short space of time (Sbordoni et al 1986; Bellinger et al 2003; Bristol et al 2013). This threatens population survival, as genetic diversity is an important determinant of long-term population persistence (Frankham 2005). Indeed, high genetic diversity bolsters the resilience of populations, because they harbour a higher adaptive capacity with which to respond to perturbations such as disease, environmental change, or declining environmental conditions (Hughes & Stachowicz, 2004; Ehlers et al., 2008; Evans et al., 2017). Low genetic diversity is also related to inbreeding depression, where recessive deleterious alleles are more likely to combine within individuals and reduce fitness (Whitlock 2000; Reed and Frankham 2003; Charlesworth et al 2009), further compromising the long-term prospects for survival of the population.

Connectivity - the movement of individuals or propagules among populations - is an important counterforce against declining population size, low genetic diversity and local extinction. A well-connected population receives a regular supply of immigrants, thus boosting population size. Crucially, if these migrants successfully reproduce, they can help replenish the gene pool with new alleles, thus countering the effects of genetic drift through gene flow (Garant et al 2007; Saenz-

113 Agudelo et al 2011; Frankham 2015). Conversely, isolated populations with little connectivity are
114 more vulnerable to extinction due to limited immigration and gene pool restriction (van der Meer et al
115 2013). Assessing levels of genetic connectivity among geographical sites is therefore another key step
116 in managing vulnerable populations.
117
118 An interesting case study to apply such genetic information to conservation practice exists among
119 sponge populations in Florida Bay and the Florida Keys (USA). In nearshore hard-bottom habitats in
120 this area, sponges form a dominant component of benthic communities (Chiappone and Sullivan
121 1994; Tellier and Bertelsen 2008), and perform a number of vital functional roles and ecosystem
122 services. Given their high relative biomass, they provide the majority of architectural complexity and
123 habitat structure in the area (Herrnkind et al 1997). This is especially important given that Florida Bay
124 is a nursery area for a number of economically important fish and invertebrate species, including
125 snapper (*Lutjanus* spp.), stone crabs (*Menippe mercenaria*), and Caribbean spiny lobsters (*Panulirus*
126 *argus*). Several species of sponge are themselves the target of commercial fisheries in the region
127 (Butler et al. 2017). Moreover, sponge endosymbionts are important in creating soundscapes that form
128 an acoustic cue for larval settlement in a variety of taxa (Butler et al 2016). As filter feeders, sponges
129 drive nutrient cycling dynamics in the area (Fiore et al 2017; Hoer et al 2018; Valentine and Butler
130 2019), and contribute to the maintenance of water quality (Peterson et al 2006; Butler et al 2018).
131
132 Sponge communities in the Florida Keys have suffered a number of mass mortality events (Butler et
133 al 1995; Stevely et al 2010; Wall et al 2012) associated with recurring blooms of the cyanobacteria
134 *Synechococcus* spp. (Fourqurean and Robblee 1999; Berry et al 2015), as well as stochastic cold
135 weather events (Colella et al 2012) and storm damage (Stevely et al 2010). These mass mortalities
136 have had dramatic consequences for the ecosystem, including declines in local juvenile lobster
137 populations (Butler et al 1995; Herrnkind et al 1997), increased susceptibility to further
138 cyanobacterial blooms (Peterson et al 2006; Wall et al 2012) and diminished underwater soundscapes
139 predicted to impact larval recruitment from a variety of taxa (Butler et al 2016). Furthermore, sponge
140 population recovery is potentially forestalled by limited dispersal, as adults are sessile, and sponge

larvae are generally short-lived, with larval durations of a few hours to a few days before settlement (Maldonado 2006; Maldonado and Riesgo 2008).

Due to their keystone role in the ecosystem and the impacts of their decline, sponge restoration work has been undertaken in the area for a number of years, where healthy sponges have been fragmented and translocated to areas that have suffered mortalities (Butler et al 2016; Valentine and Butler 2019). However, cyanobacterial blooms and sponge mass mortalities continue to recur across different areas of the Florida Keys and Florida Bay. Coupled with work to identify the proximal causes of sponge mortality and the implementation of habitat improvement measures, understanding the genetic status of the populations is imperative for future restoration and management planning. In addition, investigating connectivity patterns will aid understanding of source-sink interactions across the Bay, and identify priority areas for restoration.

In this study, we investigated these topics in the loggerhead sponge, *Spheciospongia vesparium* (Lamarck, 1815). *Spheciospongia vesparium* is common throughout Florida Bay and has the largest biomass of all sponge species in the Bay (Tellier and Bertelsen 2008). It is also found on reefs and in lagoons throughout the Greater Caribbean region. Reproduction and larval biology have not yet been studied in *S. vesparium*, therefore limiting our ability to predict dispersal and population genetic pattners. However, studies of other Clionaidae species suggest that varied reproductive characteristics exist within the family: sexual and asexual reproduction have both been observed (Rosell and Uriz 2002; Schönberg 2002; Maldonado and Riesgo 2008), and similarly, gonochorism and hermaphroditism are also both found within the family (Piscitelli et al 2011; González-Rivero et al 2013). The Clionaidae are oviparous (i.e., broadcast spawning of both the sperm and eggs) (Ereskovsky 2018), and fertilization and larval development are mainly external, although in *Cliona vermifera* eggs are fertilized internally and the zygote released (Bautista-Guerrero et al 2014). Larvae are lecithotrophic (i.e., do not feed), and larval duration is short - in *Cliona viridis*, it was estimated at < 10 days (Mariani et al 2000). Clionaidae larvae have so far been observed to show weak swimming ability, with crawling behaviour common (Mariani et al 2000; Mariani et al 2001).

Here, we aimed to describe patterns of genetic diversity and genetic connectivity in *S. vesparium* at hard bottom sites across the Florida Keys. In addition, we sampled three other locations in the Greater Caribbean to act as comparative populations, and to observe drivers of population structure at the regional scale.

Methods

Sample collection and preservation

We collected *S. vesparium* samples from shallow water sites (< 2 m depth) in four main localities: the Florida Keys/ Florida Bay (USA), Abaco Island (Bahamas), Barbuda, and Caye Caulker (Belize) (Figure 1, Table 1). We sampled a number of sites across the Upper, Middle and Lower Florida Keys: 12 sites on the Florida Bay side of the Keys and 2 collection sites on the Atlantic side (Table 1, Figure 1). Our sites in Florida included both those that have previously been affected by cyanobacterial blooms and mortalities, and those that have not. At each site in Florida we sampled between 10 and 32 individuals (average of 18.6 ± 1.2 SEM), and in Abaco, Barbuda, and Caye Caulker we sampled 12, 20, and 10 individuals, respectively (Table 1). We avoided sites where restoration work had taken place in order to observe the natural patterns of population structure and genetic diversity as far as possible. We collected small tissue fragments (~ $2cm^3$) and immediately transferred the samples into 95% ethanol, which was renewed after 24 h.

Microsatellite development

For this study, we characterised twelve new tri- and tetra-nucleotide microsatellite loci (see Supplementary Material for full details of the methods). In brief, DNA from a single *S. vesparium* sample collected from Long Key (Bay-side) was sequenced using Illumina MiSeq 2 x 250 base pair technology. We then processed the sequence reads using the Palfinder Galaxy bioinformatics pipeline (Griffiths et al 2016) to quality filter the data, screen for microsatellites and design primers. We tested 36 loci, of which 12 could be successfully amplified and scored, and were subsequently used in this study.

197

198 **DNA extraction and genotyping**

199 We checked sponge tissue samples under a dissecting microscope to remove any visible
200 endosymbiotic invertebrates, and then extracted total DNA using the DNeasy® Blood and Tissue Kit
201 (Qiagen). We combined 10 of the 12 microsatellite primer pairs in two multiplex (5-plex) PCRs using
202 the fluorophores 6-FAM and HEX (Table S1), and ran two primer pairs, Vesp36 and Vesp9 in
203 singleplex PCRs due to problems encountered in multiplexing these loci. We utilized a three-primer
204 universal tail approach for fluorescent labelling PCR products, as described in Blacket et al. (2012)
205 and Culley et al. (2013). We carried out PCRs using the Type-it® Microsatellite PCR Kit (Qiagen)
206 with the following cycling conditions: 95°C for 5 minutes, 28 x (95°C for 30 seconds, 60°C for 90
207 seconds, 72°C for 30 seconds), 60°C for 30 minutes. For any amplification failures, PCRs were
208 repeated in singleplex reactions with lowered (50-59°C) annealing temperatures.

209

210 We sized PCR products by capillary electrophoresis using a 3730 DNA Analyzer (Thermo Fisher
211 Scientific) with GeneScan™ 500, 600 or 1200 LIZ® size standard (Thermo Fisher Scientific), or a
212 homemade ROX-based size standard. On all plates, we included both positive and negative controls.
213 We scored alleles using Genemapper® v3.7 software (Thermo Fisher Scientific), and corrected allele
214 sizes according to the positive controls to account for differences in allele length based on the
215 machine or size standard used. We then binned alleles using the R package 'MsatAllele' v1.02
216 (Alberto 2009) in RStudio v3.0.3 (R Core Team, 2014).

217

218 **Quality control and summary statistics**

219 We calculated the probability of linkage disequilibrium between pairs of loci using Genepop on the
220 Web v4.2 (Raymond and Rousset 1995; Rousset 2008), with p values corrected for multiple tests
221 using the false discovery rate procedure of Benjamini and Yekutieli (2001), as calculated using the R
222 function *p.adjust*. We estimated null allele frequency at each locus using the EM algorithm
223 (Dempster et al 1977) in FreeNA (Chapuis and Estoup 2007). As null alleles can cause overestimation
224 of F_{ST} values and levels of population differentiation (Chapuis and Estoup 2007), we conducted a post

225 hoc test to test the extent of any bias, as follows: We calculated average null allele frequencies for all
226 loci, and calculated global F_{ST} with and without ENA correction for null alleles (as implemented in
227 FreeNA). We then removed the locus with the highest null allele frequency and recalculated the
228 uncorrected and corrected global F_{ST} values. We repeated this systematically until just one locus
229 remained. This allowed us to observe the cumulative effects of each locus and their null allele loads
230 on F_{ST} by comparing the corrected and uncorrected values.
231
232 **Genetic diversity, inbreeding and bottlenecks**
233 We used Genodive v2.032b (Meirmans and Van Tiendener 2004) to calculate observed
234 heterozygosity (H_O) and gene diversity/ expected heterozygosity (H_S; Nei, 1987). We also tested for
235 probability of departure from Hardy-Weinberg Equilibrium (HWE) in Genodive using the AMOVA
236 (least squares) method and 50,000 permutations (p values corrected for multiple tests using the
237 Benjamini and Yekutieli method, calculated as previously). We calculated average allelic richness and
238 private allele richness rarefied to the lowest sample size (maximum g = 10) in ADZE v1.0 (Szpiech et
239 al 2008). We repeated these analyses with all the Florida sites grouped as one population and each
240 separately.
241
242 We estimated inbreeding coefficients (Avg Fi) in INEst v2.1 (Chybicki and Burczyk 2009), correcting
243 for the presence of null alleles. The program includes three possible parameters that can affect
244 inbreeding coefficient estimation: null alleles ('n'), inbreeding ('f') and genotyping failure ('b'). We
245 ran the individual inbreeding model (IMM) for all combinations of these parameters and calculated
246 the Deviance Information Criteria (DIC) for each run to determine the best model fit for the data. We
247 ran the model using 500,000 Monte Carlo Markov Chain (MCMC) cycles with 50,000 burnin cycles.
248
249 We used INEst to find evidence of genetic signatures of recent population bottleneck events. The
250 program implements two tests; the first identifies heterozygosity excesses in respect to allelic richness
251 (Cornuet and Luikart 1996), and the second identifies M-ratio (mean ratio of allelic richness to allelic
252 size range) deficiencies (Garza and Williamson 2001). Both phenomena have been observed when

253 populations experience rapid reductions in size. We used the two-phase mutation model, and tested
254 significance using a Wilcoxon signed-rank test with 1000 permutations.
255
256 **Genetic connectivity patterns**
257 We estimated genetic differentiation among sites by calculating pairwise F_{ST} (Wright 1943; Wright
258 1949) and *D* (Jost 2008) in Genodive v2.032b. For F_{ST} values, we tested their significance in
259 Genodive using 50,000 permutations, and corrected significance for multiple tests using B-Y
260 correction as described above.
261
262 We used two different approaches to infer the number of population clusters ('*K*') in the data. Firstly,
263 we used the Bayesian individual-based assignment model implemented in the 'Geneland' package
264 v.4.0.8 (Guillot et al 2005; Guillot et al 2008) in RStudio, which uses spatial and genetic data to infer
265 *K* and calculate the probability of individual assignment. Due to the assumptions of this model, we
266 used only the seven loci that did not deviate from HWE in the majority of the sites, and deleted
267 samples in which missing data was present in the majority of the HWE loci (n = 285). We first ran the
268 no-admixture model to obtain estimates of cluster membership and allele frequencies. We used the
269 uncorrelated allele frequencies, spatial and null allele models, and ran the program with 1,000,000
270 MCMC iterations, 100 thinning and 1000 burnin, and uncertainty on coordinates set to 0.0005. We set
271 the maximum number of nuclei to 855, and the maximum rate of the Poisson process to 285. We
272 repeated this with *K* ranging from 1 to 17, with 10 independent runs for each value of *K*. We then ran
273 the admixture model using the estimates obtained from the no-admixture run with the highest average
274 posterior probability. For the admixture model, we used 1,000,000 MCMC iterations, a thinning of
275 100, and a burnin of 1000. We extracted the q-matrix of estimated individual membership proportions
276 to each of the detected clusters, and used Distruct v1.1(Rosenberg 2004) to graphically display the
277 results.
278
279 We used Flock v3.1 (Duchesne and Turgeon 2012) as an alternative method to infer membership to
280 population clusters. This method estimates *K* and partitions samples into *K* clusters based on iterated

281 reallocation, uses no *a priori* information on sampling location, and does not assume populations are
282 in HWE. We tested *K* from 1 to 17 in 50 independent runs per value of *K*, and ran each model with 20
283 iterations (i.e. 20 rounds of reallocation). We used plateau analysis based on log likelihood difference
284 (LLOD) scores, as described by Duchesne and Turgeon (2012), to infer the most likely value of *K*.
285 We carried out hierarchical clustering approaches for both the Geneland and Flock analyses by first
286 running the models using all sites, and then repeating the process on any multi-site clusters identified.
287
288 We used Discriminant Analysis of Principle Components (DAPC) (Jombart et al 2010) as
289 implemented in the package 'adegenet' v.2.1.1 (Jombart 2008) in RStudio to examine genetic
290 variation among the sites based on allele frequencies. We used the function *optim.a.score* to calculate
291 the optimum number of principle components (PCs) to retain in the analysis to prevent over-fitting of
292 the model, whilst preserving the maximum discriminability. We included all sites in the first instance,
293 and then conducted a further analysis on the Florida sites alone to examine the presence of fine-scale
294 structure.
295
296 We carried out a Principle Coordinates Analysis (PCoA) in GenAlEx v6.502 (Peakall and Smouse
297 2012) using null allele corrected pairwise F_{ST} values as calculated previously. We carried out the
298 analysis first on all sites, and then on only the Florida sites. We repeated the analysis using Jost's *D* to
299 confirm robustness of the results.
300
301 We used an analysis of molecular variance (AMOVA) to examine the partitioning of genetic variation
302 within and among individuals and sites. We included a grouping factor for the Florida sites in order to
303 estimate variation among sites within Florida, and among Florida, Bahamas, Barbuda and Belize
304 within the analysis. We carried out the AMOVA in Genodive v3.0.0 using the infinite allele model.
305
306 We looked for evidence of barriers to gene flow among the Florida and Bahamas sites using the
307 software Barrier v2.2 (Manni et al 2004). We excluded the Barbuda and Belize sites from this analysis
308 because of the large geographic distances separating them from the other sites, as this does not offer

an appropriate theoretical framework to search for oceanographic barriers. Barrier uses the spatial coordinates of the sampling sites and Delauney triangulation to partition geographic space into polygons, creating a Voronoï tessellation map with each site contained within a single polygon whose edges border neighbouring adjacent sites. Monmonier's (1973) maximum difference algorithm then uses this tessellation map along with a genetic distance matrix (Jost's *D*) to detect genetic discontinuities among neighbouring sites. We assessed the robustness of the computed barriers by repeating analysis on 100 resampled bootstrap *D* matrices. We created the resampled bootstrap matrices in the R package 'diveRsity' v1.9.90 (Keenan et al 2013). We computed increasing numbers of barriers until bootstrap support fell below 50%, reaching a maximum of three barriers. Following computation of barriers, we used AMOVAs to examine the partitioning of genetic variation across barriers.

To test the presence of genetic isolation by distance (IBD) within Florida, we performed a Mantel test to detect association between matrices of linearised pairwise genetic distances ($F_{ST}/[1-F_{ST}]$) and the logarithm of geographic distances. We calculated least-cost oceanographic distances between sites (i.e., the shortest distance possible, excluding landmasses) using 'marmap' v0.9.5 (Pante and Simon-Bouhet 2013) in RStudio, and carried out the Mantel tests in 'ade4' v1.7-10 (Dray et al 2007) in RStudio, with 9999 permutations to calculate significance.

We used Geneclass2 v2 (Piry et al 2004) to detect first generation migrants among the sampling locations, and their putative population origins. We used the Bayesian criteria of Rannala and Mountain (1997) for likelihood estimation, and the Monte Carlo method of Paetkau et al. (2004) for probability computation, with the L_{home} criterion, as source populations for all individuals were unlikely to have been sampled. We used a significance threshold of $p < 0.01$ and carried out simulations with 10,000 individuals.

Results

337 **Quality control and summary statistics**

338 In total, we collected samples from 326 individuals across 17 sites (Table 1). Twenty-two samples
339 were removed from the final dataset due to amplification failure in over 50% of the loci, leaving 304
340 individuals. Two individuals from the Lakes Passage had identical genotypes, one of which was
341 removed from the dataset for analysis, yielding 303 individuals. Following correction for multiple
342 tests, no significant linkage disequilibrium was found between pairs of loci. Null allele frequency was
343 high in some markers (Tables S1, S2); however, post hoc analysis showed that the null allele-
344 corrected global F_{ST} value was only marginally higher (+ 0.002) than the uncorrected value when all
345 loci were included in the analysis (Table S2). Furthermore, the difference between the uncorrected
346 and corrected F_{ST} did not increase as more loci were added (r^2 = -0.03608, p = 0.4504), and therefore
347 all loci were retained for the population genetics analysis. The number of alleles per locus over all
348 sites ranged from 4 (Vesp23) to 27 (Vesp30) (Table S1).
349
350 **Genetic diversity, inbreeding and bottlenecks**

351 Genetic diversity (allelic richness and gene diversity) was slightly lower at the Florida and Barbuda
352 sites compared to the Bahamas and Belize. However, overlapping error bars among many of the sites
353 indicate that this is only significant for a few Florida sites (Table 2, Fig 2, Table S3). Genetic
354 diversity can therefore be considered to be the same across Florida and non-Florida sites. Average
355 rarefied allelic richness ranged from 3.408 (Pigeon Key) to 4.399 (Long Key – Bay-side) and gene
356 diversity (H_S) ranged from 0.569 (in Pigeon Key) to 0.735 (in Belize) (Figure 2, Table 2, Table S3).
357
358 Observed heterozygosity over all loci varied from 0.251 (Craig Key - Atlantic) to 0.504 (Bahamas)
359 (Table 2, Table S3). All sites had lower than expected levels of heterozygosity (Table 2, Table S3),
360 and significant departures from HWE were found in a number of loci and populations (Table S4). The
361 DIC analysis in INEst determined either the 'nfb' (null allele, inbreeding and genotyping failure) or
362 'nb' (null allele and genotyping failure) models to be the best fit for the sites in this study (Table 2).
363 This indicates that null alleles and genotyping failure would affect inbreeding coefficient estimations
364 in all the sites, but in ten of the sites, inbreeding was also an influential component of the model. The

365 null allele-corrected inbreeding coefficients were positive in all locations, ranging from 0.036
366 (Bahamas) to 0.343 (Craig Key - Atlantic). However, the posterior 95% probability intervals included
367 zeros at all sites, and therefore F_{IS} cannot be considered to be significantly above zero. When the
368 Florida sites were grouped together as a single population, however, the posterior 95% probability
369 interval was above zero, which may indicate significant inbreeding across the area.
370
371 We found deficiencies in M-ratios at four sites, indicating the presence of recent bottleneck events
372 (Boca Chica Channel, $p = 0.0385$; Little Crane Key, $p < 0.001$; Craig Key (Atlantic, $p < 0.001$; Long
373 Key (Atlantic), $p < 0.001$). However, none of the sites showed significant heterozygote excess in
374 comparison to allelic richness.
375
376 **Genetic connectivity patterns**
377 Pairwise F_{ST} ranged from -0.019 (no differentiation) between Craig Key (Atlantic) and Long Key
378 (Atlantic), to 0.273 (great differentiation) between Pigeon Key and Barbuda (Table 3). Among the
379 four regional locations (Florida, Bahamas, Barbuda, Belize), F_{ST} values were large and significant,
380 showing strong differentiation. Among sites within Florida, F_{ST} values were lower (≤ 0.116), but
381 significant differentiation was present between many pairs of sites. In general, higher differentiation
382 could be observed among Upper and Lower Keys sites than comparisons involving the Middle Keys
383 sites, but patchiness can be observed throughout. Patterns of D were similar, and ranged from -0.035
384 (between the Craig Key and Long Key Atlantic sites, as previously) to 0.668 (between the Bahamas
385 and Waltz Key) (Table 3). Private alleles were present at many sites (Table 2, Table S3), and average
386 private allelic richness was higher among the non-Florida sites (Table 2, Figure 2).
387
388 Using Geneland, K=4 was found for each independent run, with each regional location forming a
389 separate population cluster (Figure 3). In contrast, Flock showed strong evidence for K=2. Samples
390 were broadly partitioned into a Florida cluster and a cluster comprising individuals from Belize,
391 Barbuda and the Bahamas. Two individuals from Florida (Craig Key Atlantic and Lakes Passage) fell
392 into the Belize, Barbuda and the Bahamas cluster; otherwise, clustering was concurrent with sampling

393 locations. When a second Flock analysis was carried out on the Barbuda, Belize and Bahamas cluster,
394 the samples were partitioned into K=3 concurrent with sampling locations. When repeating the
395 models for only the Florida samples, the Geneland model was unable to converge, indicating that K=1
396 or the presence of strong isolation by distance in the data. Similarly, no plateau was obtained in Flock,
397 indicating K=1.
398
399 The DAPC showed clear separation of the Barbuda and Bahamas sites from all other sites (Figure 4a).
400 All the Florida sites clustered together, with inertia ellipses showing substantial overlap among sites.
401 The Belize site clustered closely to the Florida sites, with some Belize samples showing overlap with
402 the Florida point cloud. In the Florida-only DAPC analysis, no clustering patterns were present, but
403 points from sites more closely situated geographically tended to be closer together in the DAPC plot
404 (Figure 4b).
405
406 In the PCoA carried out on all sites (Figure 5a), the first axis separated Florida from the Bahamas,
407 Belize and Barbuda, and the second separated the Upper Keys from the Lower Keys and Atlantic side
408 of the Middle Keys; the Bay side Middle Keys were distributed among both. In the Florida-only
409 PCoA (Figure 5b), points were distributed in a loose isolation by distance fashion, but notably the
410 sites on the Atlantic side of the Middle Keys (Long Key and Craig Key) were clustered with Waltz
411 Key, and separated from the sites on the Bay side of the Middle Keys. When the analysis was
412 replicated with Jost's D instead of F_{ST}, the patterns observed were very similar (data not shown).
413
414 The AMOVA showed that 18.2% of the total variation was found among the four main locations,
415 while 2.4% was found among the sites within Florida. 30.9% of variation was found among
416 individuals within sites, while 48.5% was within individuals (Table 4).
417
418 Barrier software suggested the presence of two barriers with high bootstrap support: the first was a
419 barrier between Florida and the Bahamas, with a bootstrap score of 100%. A second barrier separated
420 the Atlantic sites from their adjacent Bay-side sites in the Middle Keys in Florida (Figure 6). As

barriers are computed based on the tessellation map, this barrier comprised a number of polygon edges, which showed bootstrap support ranging 39-99% (Figure 6). A further barrier was estimated to separate the Middle and Lower Keys sites, however, this had low bootstrap support (10-54 %) (Figure 6). AMOVA analysis of sites separated by barriers showed that a large proportion of genetic variation was present between Florida and the Bahamas (20.1% of genetic variation, F_{ST} = 0.201), while only a small amount of genetic variation was found between the Atlantic and adjacent Bay-side sites in the Middle Keys (3.3% of genetic variation, F_{ST} = 0.033) (Table 4). In both cases, more variation was found across the barrier than among sites on the same side of the barrier.

Isolation by distance within Florida was significant, but the effect size was relatively small (r = 0.229, p = 0.031) (Figure 5). We obtained comparable results when repeating the analysis with Jost's *D* (r = 0.225, p = 0.033).

Three putative first generation migrants were detected. All potential migrants were found within Florida sites, and all originated from other Florida sites. Two migrants were found at Long Key (Bay), with origins from Waltz Key (p = 0.0007; distance 89 km) and Fiesta Key (p = 0.0019, distance 5 km), and the third migrant was found at Little Crane Key with inferred origins of Kemp Channel (p < 0.0001, distance 12 km).

Discussion

Genetic diversity and bottlenecks

Genetic diversity was similar throughout all of the sites sampled in Florida and the Caribbean, and was comparable to levels observed in other demosponge species (Chaves-Fonnegra et al 2015; Riesgo et al 2019). This implies that recurring mass mortality events have not significantly reduced genetic diversity in Florida, however, pre-mortality data is not available to confirm this hypothesis. Nevertheless, this study does provide a baseline with which future assessments of genetic diversity can be compared. We did not find signatures of genetic bottlenecks in sites that have been affected by

449 cyanobacterial blooms. However, bottleneck signatures were present in four Florida sites that have
450 not been affected by cyanobacterial blooms. These sites may have suffered unrecorded mortality
451 events due to a different cause, such as disease, climatic variation, or hurricane disturbance. As
452 sponges rapidly disappear once dead, and leave no visible skeleton, mass mortalities in sponges can
453 be overlooked unless specific, regular monitoring is undertaken (Wulff 2006).
454
455 Levels of genetic diversity (allelic richness, gene diversity) in other sponge populations that have
456 experienced mass mortalities vary by species. *Spongia officinalis* has high genetic diversity with no
457 bottleneck signatures (Dailianis et al. 2011), whilst the opposite was found for a congener: *S. lamella*
458 (Pérez-Portela et al 2015). In *Ircinia fasciculata*, evidence of bottlenecks have been found at many
459 (but not all) sites known to have suffered mortalities (Riesgo et al 2016). In other species within the
460 Florida reef tract, such as the coral *Acropora cervicornis* and sea urchin *Diadema antillarum*, genetic
461 diversity was similar to other Caribbean sites tested (Chandler et al 2017; Drury et al 2017), despite
462 mass mortality events. High genetic diversity, despite recent mass mortalities, may be due to high
463 levels of connectivity with other sites. This would provide a pathway for re-colonisation and would
464 increase the effective population size (N_e), protecting the population against the effects of genetic drift
465 (Dailianis et al 2011; Riesgo et al 2016). However, high variance in reproductive success can occur in
466 broadcast spawning marine invertebrates, reducing N_e, and thus increasing vulnerability to bottlenecks
467 (Hedgecock 1994).
468
469 Similarity in genetic diversity across all sampling sites implies that *S. vesparium* in Florida may still
470 have sufficient genetic variation for resilience against future stressors. In the Florida Keys, those
471 include anthropogenic effects on water quality and global climate change (Wall et al 2012; Kearney et
472 al 2015; Butler and Dolan 2017), as well as further cyanobacteria blooms. However, bottleneck
473 signatures in some sites suggest that genetic diversity may have been previously lost due to unknown
474 causes, and therefore caution should be exercised in management to prevent possible reductions in
475 genetic variation.
476

Inbreeding and null alleles

Observed heterozygosity was lower in Florida and Barbuda than Belize and the Bahamas. However, all sites showed excesses in homozygosity, and departures from Hardy Weinberg equilibrium were present across loci and sites. This phenomenon can be caused by inbreeding, but it can also be attributed to the presence of null alleles, which were found in a number of loci and across all sampling sites in our study. Null alleles are caused by mutations in primer binding regions that prevent primers from binding, and subsequently cause amplification failure in PCR, either in both alleles (resulting in missing data), or for only one allele (resulting in false homozygotes). High null allele frequencies are commonly found in sponge microsatellite studies (Dailianis et al 2011; Guardiola et al 2012; Chaves-Fonnegra et al 2015; Pérez-Portela et al 2015; Guardiola et al 2016; Richards et al 2016), suggesting that the problem may be common in the phylum, and is a known issue in other taxa (e.g., molluscs and insects; Chapuis & Estoup, 2007). To reduce the impact of null allele bias on our estimates of inbreeding, we corrected F_{IS} values for null alleles.

We found positive F_{IS} values in all populations when corrected for null alleles, but this was not statistically significant in any of the sites when tested individually, potentially due to small sample sizes. Our genetic clustering analyses concluded that Florida was a single population, which enabled us to group the Florida sites for more statistical power and a significant positive mean F_{IS} value. This suggests the presence of inbreeding in Florida *S. vesparium* populations, although the large 95% posterior probability intervals at the individual site level preclude a more fine-scale spatial assessment. Inbreeding has negative implications for fitness, thus our results highlight a potential concern for the health, reproductive success and longevity of *S. vesparium* in Florida.

Inbreeding is often characteristic of populations that have experienced declines. Hence, the positive F_{IS} values we observed for *S. vesparium* in Florida may be due to mass mortality events, coupled with limited regional-scale connectivity to replenish the gene pool. However, high inbreeding coefficients are widespread in the Porifera (Guardiola et al 2012; Bell et al 2014; Chaves-Fonnegra et al 2015; Pérez-Portela et al 2015; Giles et al 2015; Padua et al 2017). This suggests that the positive F_{IS} values

505 for Porifera may be general characteristics of the phylum, perhaps associated with high philopatry due
506 to limited larval dispersal. Additionally, sponges in the Clionaidae family can be simultaneously
507 hermaphroditic (contain both eggs and sperm at the same time) (Piscitelli et al 2011), so self-
508 fertilisation, or selfing, could theoretically be possible; however there is currently no recorded
509 evidence of selfing in the Porifera phylum.
510
511 Positive F_{IS} values can also be caused by excess homozygosity driven by Wahlund effects. These
512 effects can occur when there is population structure within a site or group, and can be caused by
513 reproductive asynchronicity or recruitment of different genetic cohort (Duran et al 2004; Chaves-
514 Fonnegra et al 2015; Riesgo et al 2016). With this in mind, it is difficult to fully gauge the
515 implications of positive F_{IS} for population health.
516
517 **Genetic connectivity patterns**
518 *Spheciospongia vesparium* exhibited strong population structure at the regional (Caribbean) spatial
519 scale, indicating that connectivity among sponge populations in the four countries we sampled is low.
520 These results are congruent with those of other sponge species, which exhibit high differentiation at
521 large spatial scales in the Caribbean (López-Legentil and Pawlik 2009; Chaves-Fonnegra et al 2015;
522 de Bakker et al 2016; Richards et al 2016; DeBiasse et al 2016), but also in other regions (Duran et al
523 2004; Xavier et al 2010; Pérez-Portela et al 2015; Riesgo et al 2016; Brown et al 2017; Padua et al
524 2017; Taboada et al 2018; Riesgo et al 2019). Dispersal in marine species is affected by a number of
525 factors and the complex interactions between them (Cowen et al 2006; Cowen and Sponaugle 2009),
526 including ocean current patterns and life history characteristics such as pelagic larval duration, larval
527 behaviour, and reproductive strategies (Butler et al 2011; Selkoe and Toonen 2011; Kough and Paris
528 2015; Coelho and Lasker 2016). Although reproductive and larval traits for *S. vesparium* are not
529 known, sponge larvae generally have short pelagic larval durations, limiting their dispersal capacity.
530 This includes previously studied members of the Clionaidae family (Warburton 1966; Mariani et al
531 2000; Mariani et al 2001), to which *S. vesparium* belongs. Furthermore, Clionaidae larvae have been

found to exhibit weak swimming ability, and commonly crawl (Mariani et al 2000; Mariani et al 2001), further minimizing dispersal capacity.

Our results on the population structure of *S. vesparium* are consistent with expectations of connectivity as determined by regional ocean current patterns. Our analyses indicated the presence of a barrier to gene flow between the Florida sites and Abaco in the Bahamas, which concurs with patterns found in genetic studies of other sponges (López-Legentil and Pawlik 2009; Richards et al 2016; DeBiasse et al 2016) and corals (Brazeau et al 2005; Baums et al 2010), and biophysical modelling predictions of fish and lobster larvae (Cowen et al 2006; Truelove et al 2017). This break is likely due to the strong Florida Current, which runs between the Bahamas and Florida, and can act as a strong barrier to dispersal.

Genetic differentiation was much larger between Florida and Abaco than between Florida and Belize, despite the geographic distance being much larger for the latter pair, as shown by genetic distance calculations and the DAPC analysis. Connectivity between Florida and Belize could be aided by the Caribbean Current and Loop Current, which can support larval transport from Belize towards Florida in as little as 7 to 10 days (Muhling et al 2013). This is likely to be higher than the larval duration of most sponge species, but locations 'upstream' from Florida, such as the Yucatán Peninsula, could act as intermediate 'stepping stones' to aid gene flow between these areas, as appears the case for some marine diseases (Kough et al 2015), thus reducing genetic differentiation.

Connectivity across the Florida Keys

Florida formed a single genetic cluster in our analyses (Geneland, Flock, DAPC), and the AMOVA showed that only 2.4% of the total genetic variation in the dataset was among sites in Florida. These results indicate that some level of connectivity is present across the Keys. We also found evidence of recent migration between Florida sites in our first generation migration analysis. According to genetic distance and PCoA analyses, sites such as Long Key (Bay-side) in the Middle Keys and Boca Chica Channel in the Lower Keys appeared well-connected to sites throughout the Florida Keys range. The

560 complex currents found across the Florida Keys are likely to aid in connectivity among disparate sites.
561 Although the main current dominating the area is the north-easterly running Florida Current, there are
562 many local oceanographic processes that can affect larval dispersal patterns. Westerly running counter
563 currents arise as a result of downwelling winds and offshore eddies and gyres (Lee and Williams
564 1999; Yeung et al 2001; Kourafalou and Kang 2012), and eddies themselves also drive connectivity in
565 the area (Sponaugle et al 2005). Connectivity is also influenced by a species' life history.
566 Reproduction of *S. vesparium* has not been described, however, oviparity occurs in some members of
567 the Clionaidae family (Maldonado and Riesgo 2008; González-Rivero et al 2013); if *S. vesparium* is
568 also oviparous, additional dispersal of the gametes before fertilization may increase connectivity over
569 longer distances compared to viviparous sponges. However, in other oviparous sponges, egg masses
570 have been observed to stick to the substrate close to the mother sponge due to their envelopment in an
571 adhesive material (Mariani et al 2001). Furthermore, fertilization rates generally decrease over
572 increasing gametic dispersal distances in broadcast spawners (Levitan 1991; Lauzon-Guay and
573 Scheibling 2007). However, even a relatively small proportion of far-dispersing eggs that get
574 successfully fertilized could increase the genetic connectivity between populations (Trakhtenbrotl et
575 al 2005).
576
577 Despite evidence of connectivity, there was still population structure among the Florida sites,
578 demonstrating that the area does not form a completely panmictic population. Isolation by distance
579 accounted for some of the structure across sites: genetic similarity decreases with geographic distance.
580 This suggests that distance-limited dispersal influences population structure on smaller (< 160 km)
581 spatial scales and is again likely to be due to the short pelagic larval duration found in sponges.
582
583 The Barrier analysis suggested a barrier to gene flow between adjacent Atlantic and Bay-side sites in
584 the Middle Keys. The AMOVA confirmed that more genetic variation was found across the barrier
585 than among sites on the same side of the barrier. In addition, the Florida-only PCoA showed the
586 Atlantic sites (along with Waltz Key) separated from the rest of the sites by the second axis. These
587 results suggest that dispersal through the channels between the islands of the Keys archipelago is

588 limited, at least in the Middle Keys where we sampled. This is somewhat surprising considering the
589 strong tidal flux through these channels (Smith 1994; Smith and Lee 2003), however, weakly-
590 swimming larvae caught in the tidal flow may struggle to settle in areas close to the channels before
591 being transported offshore or into the Bay. Furthermore, larval exchange could be limited by spatially
592 and temporally variable inflow and outflow through the channels (Smith 1994; Yeung et al 2001; Lee
593 and Smith 2002). That being said, despite moderate statistical support for a barrier, the F_{ST} value
594 across the barrier was only 0.033, showing low genetic differentiation. Furthermore, they did not form
595 separate populations in Geneland and FLOCK analyses. This indicates that although genetic
596 differentiation is higher than would be expected due to distance alone, it is only a weak barrier to gene
597 flow. More substantial population structure was found in the seagrass *Syringodium filiforme* between
598 the Bay and Atlantic sides of the Keys in the same area (Bijak et al 2018). This is likely due to
599 vegetative propagation of *S. filiforme* through the sediments compared to larval propagation of *S.*
600 *vesparium* through the water column.
601
602 The Barrier analysis also showed a putative barrier occurring between the Middle Keys and Lower
603 Keys sites. However, this had low bootstrap support, and is likely to be an artefact of the isolation by
604 distance pattern in the area, rather than a physical or oceanographic barrier to dispersal (Meirmans
605 2012).
606
607 Other patterns of population structure within Florida did not correlate with known physical or
608 oceanographic features. For example, sponges near Waltz Key (a semi-isolated lagoon) were
609 genetically different than those at many other sites in the Lower Keys, but not those in the Middle
610 Keys. Although counterintuitive, this is not uncommon. Unexpected patterns of fine-scale genetic
611 structure have also been observed in other sponges found along the Florida Keys reef tract (DeBiasse
612 et al 2010; Chaves-Fonnegra et al 2015). Furthermore, a dispersal model based on water circulation
613 patterns and larval characteristics did not accurately predict genetic connectivity patterns for *A.*
614 *cervicornis* across the Florida Reef Tract, with the genetic data revealing more complex connections
615 than the model predicted (Drury et al 2018). Such patterns of chaotic genetic patchiness in the marine

environment can be caused by 'sweepstakes reproductive success', the random survival of certain larval cohorts due to oceanographic conditions (Hedgecock 1982; Hedgecock 1994; Hedgecock and Pudovkin 2011). These effects can be found in species with high fecundity and high larval mortality. Alternatively, variable current regimes, as found in the Florida Keys (Lee et al., 1992), can result in temporally variable dispersal pathways. Both of these situations could lead to spatially heterogeneous genetic structure through genetic drift.

Sponge restoration implications

Our results have important implications for sponge restoration practice. Genetic diversity in *S. vesparium* is naturally high and, in addition, clonality is low, with only two identical genotypes found in our dataset. To maintain these genetic diversity levels, restoration should be carried out though the selection of genetically-diverse donor sponges. Donor sponges should not be extensively fragmented to produce a number of genetically identical transplants in a single location; instead, minimal fragmentation of many individuals and transplantation of whole sponges should be used. By maintaining high genetic diversity, restored populations can uphold evolutionary potential and resilience against future stressors, as well as avoid the negative fitness consequences of inbreeding. As our results indicate an absence of population clusters within the Keys, strong local adaptation does not appear to be present. This indicates that outbreeding depression is not a concern, and sourcing donor sponges does not have to be restricted to certain sites or environmental conditions.

Our findings highlight the importance of restoration work in Florida. Connectivity on the regional scale was low in our study, suggesting that immigration and gene flow into Florida may be limited. Populations in Cuba or the Gulf of Mexico may be more connected to Florida that the sites sampled here. However, patterns observed in this study suggest that migration is likely to be limited due to the oceanographic distances and limited pelagic larval duration. Active management on the local scale is therefore likely to be of vital importance to ensure that population numbers are maintained.

643 We show that connectivity is present over the range of the Keys, and we did not observe genetically
644 isolated sites that would need to be prioritised for restoration action. However, our results also imply
645 that connectivity in Florida is unpredictable, as we observed unexplained fine-scale structure.
646 Furthermore, isolation by distance suggests that dispersal is distance-limited. These results show that
647 natural repopulation of barren areas may be slow, especially if healthy populations are moderately
648 distant. This may be compounded by the loss of acoustic larval recruitment cues in the area, itself
649 caused by loss of sponge-associated endosymbionts (Butler et al 2016). Given the crucial role of this
650 important keystone species, sponge restoration is an important strategy in facilitating a more rapid
651 return to ecosystem function following mass mortality events. However, this approach must be
652 coupled with thorough investigation into the causes of the ongoing mass mortalities and ecosystem
653 instability in the Florida Keys, and the implementation of measures to mitigate these issues.
654 Furthermore, genetic diversity and its distribution among sites should be monitored regularly to
655 ensure that genetic variation is maintained throughout the restoration program. This can now be
656 accomplished relatively quickly using the molecular tools described in this study.
657
658
659
660
661
662
663
664
665
666
667
668
669

670 **References**

671 Alberto F (2009) MsatAllele_1.0: An R package to visualize the binning of microsatellite alleles. J
672 Hered 100:394–7. doi: 10.1093/jhered/esn110

673 Baums IB, Johnson ME, Devlin-Durante MK, Miller MW (2010) Host population genetic structure
674 and zooxanthellae diversity of two reef-building coral species along the Florida Reef Tract and
675 wider Caribbean. Coral Reefs 29:835–842. doi: 10.1007/s00338-010-0645-y

676 Bautista-Guerrero E, Carballo JL, Maldonado M (2014) Abundance and reproductive patterns of the
677 excavating sponge *Cliona vermifera*: A threat to Pacific coral reefs? Coral Reefs 33:259–266.
678 doi: 10.1007/s00338-013-1094-1

679 Bell JJ, Smith D, Hannan D, et al (2014) Resilience to disturbance despite limited dispersal and self-
680 recruitment in tropical barrel sponges: implications for conservation and management. PLoS
681 One 9:e91635. doi: 10.1371/journal.pone.0091635

682 Bellinger MR, Johnson JA, Toepfer J, Dunn P (2003) Loss of genetic variation in Greater Prairie
683 Chickens following a population bottleneck in Wisconsin, U.S.A. Conserv Biol 17:717–724.
684 doi: 10.1046/j.1523-1739.2003.01581.x

685 Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under
686 dependency. Ann Stat 29:1165–1188. doi: 10.1214/aos/1013699998

687 Berry DL, Goleski JA, Koch F, et al (2015) Shifts in cyanobacterial strain dominance during the onset
688 of harmful algal blooms in Florida Bay, USA. Microb Ecol 70:361–371. doi: 10.1007/s00248-
689 014-0564-5

690 Bijak AL, Dijk K jent van, Waycott M (2018) Population structure and gene flow of the tropical
691 seagrass, *Syringodium filiforme*, in the Florida Keys and subtropical Atlantic region. PLoS One
692 13:1–18. doi: 10.1371/journal.pone.0203644

693 Blacket M, Robin C, Good R, et al (2012) Universal primers for fluorescent labelling of PCR
694 fragments- an efficient and cost-effective approach to genotyping by fluorescence. Mol Ecol
695 Resour 12:456–63. doi: 10.1111/j.1755-0998.2011.03104.x

696 Brazeau DA, Sammarco PW, Gleason DF (2005) A multi-locus genetic assignment technique to
697 assess sources of *Agaricia agaricites* larvae on coral reefs. Mar Biol 147:1141–1148. doi:

10.1007/s00227-005-0022-5

Bristol RM, Tucker R, Dawson DA, et al (2013) Comparison of historical bottleneck effects and genetic consequences of re-introduction in a critically endangered island passerine. Mol Ecol 22:4644–4662. doi: 10.1111/mec.12429

Brown RR, Davis CS, Leys SP (2017) Clones or clans: the genetic structure of a deep-sea sponge, *Aphrocallistes vastus*, in unique sponge reefs of British Columbia, Canada. Mol Ecol 26:1045–1059. doi: 10.1111/mec.13982

Butler J, Stanley JA, Butler MJ (2016) Underwater soundscapes in near-shore tropical habitats and the effects of environmental degradation and habitat restoration. J Exp Mar Bio Ecol 479:89–96. doi: 10.1016/j.jembe.2016.03.006

Butler M, Paris C, Goldstein J, et al (2011) Behavior constrains the dispersal of long-lived spiny lobster larvae. Mar Ecol Prog Ser 422:223–237. doi: 10.3354/meps08878

Butler MJ, Dolan TW (2017) Potential impacts of everglades restoration on lobster and hard bottom communities in the Florida Keys, FL (USA). Estuaries and Coasts 40:1523–1539. doi: 10.1007/s12237-017-0256-8

Butler MJ, Hunt JH, Herrnkind WF, et al (1995) Cascading disturbances in Florida Bay, USA: cyanobacteria blooms, sponge mortality, and implications for juvenile spiny lobsters *Panulirus argus*. Mar Ecol Prog Ser 129:119–125. doi: 10.3354/meps129119

Butler MJ, Weisz JB, Butler J (2018) The effects of water quality on back-reef sponge survival and distribution in the Florida Keys, Florida (USA). J Exp Mar Bio Ecol 503:92–99. doi: 10.1016/j.jembe.2018.03.001

Chandler LM, Walters LJ, Sharp WC, Hoffman EA (2017) Genetic structure of natural and broodstock populations of the long-spined sea urchin, *Diadema antillarum*, throughout the Florida Keys. Bull Mar Sci 93:1–9. doi: 10.5343/bms.216.1101

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–31. doi: 10.1093/molbev/msl191

Charlesworth D, Charlesworth D, Willis JH, Willis JH (2009) The genetics of inbreeding depression. Nat Rev Genet 10:783–96. doi: 10.1038/nrg2664

726 Chaves-Fonnegra A, Feldheim KA, Secord J, Lopez J V (2015) Population structure and dispersal of
727 the coral-excavating sponge *Cliona delitrix*. Mol Ecol 24:1447–66. doi: 10.1111/mec.13134
728 Chiappone M, Sullivan KM (1994) Ecological structure and dynamics of nearshore hard-bottom
729 communities in the Florida Keys. Bull Mar Sci 54:747–756.
730 Chybicki IJ, Burczyk J (2009) Simultaneous estimation of null alleles and inbreeding coefficients. J
731 Hered 100:106–13. doi: 10.1093/jhered/esn088
732 Coelho MAG, Lasker HR (2016) Larval behavior and settlement dynamics of a ubiquitous Caribbean
733 octocoral and its implications for dispersal. Mar Ecol Prog Ser 561:109–121. doi:
734 10.3354/meps11941
735 Colella MA, Ruzicka RR, Kidney JA, et al (2012) Cold-water event of January 2010 results in
736 catastrophic benthic mortality on patch reefs in the Florida Keys. Coral Reefs 31:621–632. doi:
737 10.1007/s00338-012-0880-5
738 Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent
739 population bottlenecks from allele frequency data. Genetics 144:2001–2014. doi: Article
740 Cowen R, Sponaugle S (2009) Larval dispersal and marine population connectivity. Ann Rev Mar Sci
741 1:443–466. doi: 10.1146/annurev.marine.010908.163757
742 Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science
743 311:522–7. doi: 10.1126/science.1122039
744 Culley TM, Stamper TI, Stokes RL, et al (2013) An efficient technique for primer development and
745 application that integrates fluorescent labeling and multiplex PCR. Appl Plant Sci 1:1300027.
746 doi: 10.3732/apps.1300027
747 Dailianis T, Tsigenopoulos CS, Dounas C, Voultsiadou E (2011) Genetic diversity of the imperilled
748 bath sponge *Spongia officinalis* Linnaeus, 1759 across the Mediterranean Sea: patterns of
749 population differentiation and implications for taxonomy and conservation. Mol Ecol 20:3757–
750 72. doi: 10.1111/j.1365-294X.2011.05222.x
751 de Bakker DM, Meesters EHWG, van Bleijswijk JDL, et al (2016) Population genetic structure,
752 abundance, and health status of two dominant benthic species in the Saba Bank National Park,
753 Caribbean Netherlands: *Montastraea cavernosa* and *Xestospongia muta*. PLoS One

754 11:e0155969. doi: 10.1371/journal.pone.0155969
755 DeBiasse MB, Richards VP, Shivji MS (2010) Genetic assessment of connectivity in the common
756 reef sponge, *Callyspongia vaginalis* (Demospongiae: Haplosclerida) reveals high population
757 structure along the Florida reef tract. Coral Reefs 29:47–55. doi: 10.1007/s00338-009-0554-0
758 DeBiasse MB, Richards VP, Shivji MS, Hellberg ME (2016) Shared phylogeographical breaks in a
759 Caribbean coral reef sponge and its invertebrate commensals. J Biogeogr 43:2136–2146. doi:
760 10.1111/jbi.12785
761 Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the EM
762 algorithm. J R Stat Soc Ser B 39:1–38. doi: 10.1111/j.2517-6161.1977.tb01600.x
763 Dray S, Dufour AB, Chessel D (2007) The ade4 Package - II: Two-table and K-table methods. R
764 News 7:47–52.
765 Drury C, Paris CB, Kourafalou VH, Lirman D (2018) Dispersal capacity and genetic relatedness in
766 *Acropora cervicornis* on the Florida Reef Tract. Coral Reefs 37:585–596. doi: 10.1007/s00338-
767 018-1683-0
768 Drury C, Schopmeyer S, Goergen E, et al (2017) Genomic patterns in *Acropora cervicornis* show
769 extensive population structure and variable genetic diversity. Ecol Evol. doi: 10.1002/ece3.3184
770 Duchesne P, Turgeon J (2012) FLOCK provides reliable solutions to the "number of populations"
771 problem. J Hered 103:734–743. doi: 10.1093/jhered/ess038
772 Duran S, Pascual M, Estoup A, Turon X (2004) Strong population structure in the marine sponge
773 *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Mol Ecol 13:511–522.
774 doi: 10.1046/j.1365-294X.2004.02080.x
775 Ehlers A, Worm B, Reusch TBH (2008) Importance of genetic diversity in eelgrass *Zostera marina*
776 for its resilience to global warming. Mar Ecol Prog Ser 355:1–7. doi: 10.3354/meps07369
777 Ereskovsky A V. (2018) Sponge Reproduction. In: Skinner MK (ed) Encycl. Reprod., 2nd edn. pp
778 485–490
779 Evans SM, Vergés A, Poore AGB (2017) Genotypic diversity and short-term response to shading
780 stress in a threatened seagrass: Does low diversity mean low resilience? Front Plant Sci 8:1417.
781 doi: 10.3389/fpls.2017.01417

782 Fiore CL, Freeman CJ, Kujawinski EB (2017) Sponge exhalent seawater contains a unique chemical
783 profile of dissolved organic matter. PeerJ 5:e2870. doi: 10.7717/peerj.2870
784 Fourqurean JW, Robblee MB (1999) Florida Bay: A history of recent ecological changes. Estuaries
785 22:345–357.
786 Frankham R (2005) Genetics and extinction. Biol Conserv 126:131–140. doi:
787 10.1016/j.biocon.2005.05.002
788 Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large and
789 consistent benefits of gene flow. Mol Ecol 24:2610–2618. doi: 10.1111/mec.13139
790 Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on
791 contemporary adaptation. Funct Ecol 21:434–443. doi: 10.1111/j.1365-2435.2006.01228.x
792 Garza JC, Williamson EG (2001) Detection of reduction in population size using data from
793 microsatellite loci. Mol Ecol 10:305–18.
794 Giles EC, Saenz-Agudelo P, Hussey NE, et al (2015) Exploring seascape genetics and kinship in the
795 reef sponge *Stylissa carteri* in the Red Sea. Ecol Evol 5:2487–502. doi: 10.1002/ece3.1511
796 González-Rivero M, Ereskovsky A V., Schönberg CHL, et al (2013) Life-history traits of a common
797 Caribbean coral-excavating sponge, *Cliona tenuis* (Porifera: Hadromerida). J Nat Hist 47:2815–
798 2834. doi: 10.1080/00222933.2013.802042
799 Griffiths SM, Fox G, Briggs PJ, et al (2016) A Galaxy-based bioinformatics pipeline for optimised,
800 streamlined microsatellite development from Illumina next-generation sequencing data. Conserv
801 Genet Resour 8:481–486. doi: 10.1007/s12686-016-0570-7
802 Guardiola M, Frotscher J, Uriz J (2012) Genetic structure and differentiation at a short-time scale of
803 the introduced calcarean sponge *Paraleucilla magna* to the western Mediterranean.
804 Hydrobiologia 687:71–84. doi: 10.1007/s10750-011-0948-1
805 Guardiola M, Frotscher J, Uriz M-J (2016) High genetic diversity, phenotypic plasticity, and invasive
806 potential of a recently introduced calcareous sponge, fast spreading across the Atlanto-
807 Mediterranean basin. Mar Biol 163:123. doi: 10.1007/s00227-016-2862-6
808 Guillot G, Santos F, Estoup A (2008) Analysing georeferenced population genetics data with
809 Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface.

Bioinformatics 24:1406–1407. doi: 10.1093/bioinformatics/btn136

Guillot Gi, Mortier F, Estoup A (2005) Geneland: a computer package for landscape genetics. Mol Ecol Notes 5:712–715. doi: 10.1111/j.1471-8286.2005.01031.x

Hedgecock D (1994) Does variance in reproductive success limit effective population sizes of marine organisms? Genet. Evol. Aquat. Org. pp 122–134

Hedgecock D (1982) Genetic consequences of larval retention: theoretical and methodological aspects. Estuar. Comp. Elsevier, pp 553–568

Hedgecock D, Pudovkin AI (2011) Sweepstakes reproductive success in highly fecund marine fish and shellfish: A review and commentary. Bull Mar Sci 87:971–1002. doi: 10.5343/bms.2010.1051

Herrnkind WF, Butler IV MJ, Hunt JH, Childress M (1997) Role of physical refugia: implications from a mass sponge die-off in a lobster nursery in Florida. Mar Freshw Res 48:759. doi: 10.1071/MF97193

Hicke JA, Allen CD, Desai AR, et al (2012) Effects of biotic disturbances on forest carbon cycling in the United States and Canada. Glob Chang Biol 18:7–34. doi: 10.1111/j.1365-2486.2011.02543.x

Hoer DR, Tommerdahl JP, Lindquist NL, Martens CS (2018) Dissolved inorganic nitrogen fluxes from common Florida Bay (U.S.A.) sponges. Limnol Oceanogr. doi: 10.1002/lno.10960

Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proc Natl Acad Sci 101:8998–9002. doi: 10.1073/pnas.0402642101

Jombart T (2008) Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405. doi: 10.1093/bioinformatics/btn129

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94. doi: 10.1186/1471-2156-11-94

Jost L (2008) GST and its relatives do not measure differentiation. Mol Ecol 17:4015–4026. doi: 10.1111/j.1365-294X.2008.03887.x

Kearney KA, Butler M, Glazer R, et al (2015) Quantifying Florida Bay habitat suitability for fishes

838 and invertebrates under climate change scenarios. Environ Manage 55:836–856. doi:
839 10.1007/s00267-014-0336-5
840 Keenan K, Mcginnity P, Cross TF, et al (2013) DiveRsity: An R package for the estimation and
841 exploration of population genetics parameters and their associated errors. Methods Ecol Evol
842 4:782–788. doi: 10.1111/2041-210X.12067
843 Kough AS, Paris CB (2015) The influence of spawning periodicity on population connectivity. Coral
844 Reefs 34:753–757. doi: 10.1007/s00338-015-1311-1
845 Kough AS, Paris CB, Behringer DC, Butler IV MJ (2015) Modelling the spread and connectivity of
846 waterbourne marine pathogens: the case of PaV1 in the Caribbean. ICES J Mar Sci 72:i139–
847 i146. doi: 10.1093/icesjms/fsu209
848 Kourafalou VH, Kang H (2012) Florida Current meandering and evolution of cyclonic eddies along
849 the Florida Keys Reef Tract: Are they interconnected? J Geophys Res Ocean 117:n/a-n/a. doi:
850 10.1029/2011JC007383
851 Lauzon-Guay AJ-S, Scheibling RE (2007) Importance of spatial population characteristics on the
852 fertilization rates of sea urchins. Biol Bull 212:195–205.
853 Lee TN, Rooth C, Williams E, et al (1992) Influence of Florida Current, gyres and wind-driven
854 circulation on transport of larvae and recruitment in the Florida Keys coral reefs. Cont Shelf Res
855 12:971–1002. doi: 10.1016/0278-4343(92)90055-O
856 Lee TN, Smith N (2002) Volume transport variability through the Florida Keys tidal channels. Cont
857 Shelf Res 22:1361–1377. doi: 10.1016/S0278-4343(02)00003-1
858 Lee TN, Williams E (1999) Mean distribution and seasonal variability of coastal currents and
859 temperature in the Florida Keys with implications for larval recruitment. Bull Mar Sci 64:35–56.
860 Levitan DR (1991) Influence of body size and population density on fertilization success and
861 reproductive output in a free-spawning invertebrate. Biol Bull 181:261–268. doi:
862 10.2307/1542097
863 López-Legentil S, Pawlik JR (2009) Genetic structure of the Caribbean giant barrel sponge
864 *Xestospongia muta* using the I3-M11 partition of COI. Coral Reefs 28:157–165. doi:
865 10.1007/s00338-008-0430-3

866 Maldonado M (2006) The ecology of the sponge larva. Can J Zool 84:175–194. doi: 10.1139/z05-177
867 Maldonado M, Riesgo A (2008) Reproduction in Porifera: a synoptic overview. Treballs la SCB
868 59:29–49.
869 Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic)
870 variation: how barriers can be detected by using Monmonier's algorithm. Hum Biol 76:173–190.
871 Mariani S, Piscitelli MP, Uriz MJ (2001) Temporal and spatial co-occurrence in spawning and larval
872 release of *Cliona viridis* (Porifera: Hadromerida). J Mar Biol Assoc United Kingdom 81:565–
873 567. doi: 10.1017/S0025315401004246
874 Mariani S, Uriz MJ, Turon X (2000) Larval bloom of the oviparous sponge *Cliona viridis*: Coupling
875 of larval abundance and adult distribution. Mar Biol 137:783–790. doi: 10.1007/s002270000400
876 Meirmans PG (2012) The trouble with isolation by distance. Mol Ecol 21:2839–2846. doi:
877 10.1111/j.1365-294X.2012.05578.x
878 Meirmans PG, Van Tiendener PH (2004) Genotype and Genodive: two programs for the analysis of
879 genetic diversity of asexual organisms. Mol Ecol Notes 4:792–794. doi: 10.1111/j.1471-
880 8286.2004.00770.x
881 Monmonier MS (1973) Maximum-difference barriers: an alternative numerical regionalization
882 method. Geogr Anal 5:245–261. doi: 10.1111/j.1538-4632.1973.tb01011.x
883 Muhling BA, Smith RH, Vásquez-Yeomans L, et al (2013) Larval fish assemblages and mesoscale
884 oceanographic structure along the Mesoamerican Barrier Reef System. Fish Oceanogr 22:409–
885 428. doi: 10.1111/fog.12031
886 Narum SR (2006) Beyond Bonferroni: Less conservative analyses for conservation genetics. Conserv
887 Genet 7:783–787. doi: 10.1007/s10592-005-9056-y
888 Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press.
889 Padua A, Cunha HA, Klautau M (2017) Gene flow and differentiation in a native calcareous sponge
890 (Porifera) with unknown dispersal phase. Mar Biodivers 1–11. doi: 10.1007/s12526-017-0742-z
891 Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time
892 estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol

13:55–65.

Pante E, Simon-Bouhet B (2013) marmap: A package for importing, plotting and analyzing bathymetric and topographic data in R. PLoS One 8:e73051. doi: 10.1371/journal.pone.0073051

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research- an update. Bioinformatics 28:2537–2539. doi: 10.1093/bioinformatics/bts460

Pérez-Portela R, Noyer C, Becerro MA (2015) Genetic structure and diversity of the endangered bath sponge *Spongia lamella*. Aquat Conserv Mar Freshw Ecosyst 25:365–379. doi: 10.1002/aqc.2423

Peterson B, Chester C, Jochem F, Fourqurean JW (2006) Potential role of sponge communities in controlling phytoplankton blooms in Florida Bay. Mar Ecol Prog Ser 328:93–103.

Piry S, Alapetite A, Cornuet J-M, et al (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. J Hered 95:536–539. doi: 10.1093/JHERED/ESH074

Piscitelli M, Corriero G, Gaino E, Uriz MJ (2011) Reproductive cycles of the sympatric excavating sponges *Cliona celata* and *Cliona viridis* in the Mediterranean Sea. Invertebr Biol 130:1–10. doi: 10.1111/j.1744-7410.2010.00216.x

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci 94:9197–9201. doi: 10.1073/pnas.94.17.9197

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. J Hered 86:248–249.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17:230–237. doi: 10.1046/j.1523-1739.2003.01236.x

Richards VP, Bernard AM, Feldheim KA, Shivji MS (2016) Patterns of population structure and dispersal in the long-lived "redwood" of the coral reef, the giant barrel sponge (*Xestospongia muta*). Coral Reefs 35:1097–1107. doi: 10.1007/s00338-016-1435-y

Riesgo A, Pérez-Portela R, Pita L, et al (2016) Population structure and connectivity in the Mediterranean sponge *Ircinia fasciculata* are affected by mass mortalities and hybridization. Heredity (Edinb) 117:427–439. doi: 10.1038/hdy.2016.41

921 Riesgo A, Taboada S, Pérez-Portela R, et al (2019) Genetic diversity, connectivity and gene flow
922 along the distribution of the emblematic Atlanto-Mediterranean sponge *Petrosia ficiformis*
923 (Haplosclerida, Demospongiae). BMC Evol Biol 19:1–18. doi: 10.1186/s12862-018-1343-6
924 Rosell D, Uriz MJ (2002) Excavating and endolithic sponge species (Porifera) from the
925 Mediterranean: Species descriptions and identification key. Org Divers Evol 2:55–86. doi:
926 10.1078/1439-6092-00033
927 Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. Mol
928 Ecol Notes 4:137–138. doi: 10.1046/j.1471-8286.2003.00566.x
929 Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S (2011) Connectivity dominates larval
930 replenishment in a coastal reef fish metapopulation. Proc R Soc B Biol Sci 278:2954–61. doi:
931 10.1098/rspb.2010.2780
932 Sbordoni V, De Matthaeis E, Cobolli Sbordoni M, et al (1986) Bottleneck effects and the depression
933 of genetic variability in hatchery stocks of *Penaeus japonicus* (Crustacea, Decapoda).
934 Aquaculture 57:239–251. doi: 10.1016/0044-8486(86)90202-4
935 Schönberg CHL (2002) *Pione lampa*, a bioeroding sponge in a worm reef. Hydrobiologia 482:49–68.
936 doi: 10.1023/A:1021258901538
937 Selkoe K, Toonen R (2011) Marine connectivity: a new look at pelagic larval duration and genetic
938 metrics of dispersal. Mar Ecol Prog Ser 436:291–305. doi: 10.3354/meps09238
939 Smith NP (1994) Long-term Gulf to Atlantic transport through tidal channels in the Florida Keys.
940 Bull Mar Sci 54:602–609.
941 Smith NP, Lee TN (2003) Volume transport through tidal channels in the Middle Florida Keys. J
942 Coast Res 19:254–260.
943 Sorte CJB, Davidson VE, Franklin MC, et al (2017) Long-term declines in an intertidal foundation
944 species parallel shifts in community composition. Glob Chang Biol 23:341–352. doi:
945 10.1111/gcb.13425
946 Sponaugle S, Lee T, Kourafalou V, Pinkard D (2005) Florida Current frontal eddies and the
947 settlement of coral reef fishes. Limnol Oceanogr 50:1033–1048. doi: 10.4319/lo.2005.50.4.1033
948 Stevely JM, Sweat DE, Bert TM, et al (2010) Sponge mortality at Marathon and Long Key, Florida :

patterns of species response and population recovery. Proc 63rd Gulf Caribb Fish Inst 63:384–400.

Sweeney BW, Bott TL, Jackson JK, et al (2004) Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proc Natl Acad Sci 101:14132–14137. doi: 10.1073/pnas.0405895101

Szpiech ZA, Jakobsson M, Rosenberg NA (2008) ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics 24:2498–2504. doi: 10.1093/bioinformatics/btn478

Taboada S, Riesgo A, Wiklund H, et al (2018) Implications of population connectivity studies for the design of marine protected areas in the deep sea: An example of a demosponge from the Clarion-Clipperton Zone. Mol Ecol. doi: 10.1111/mec.14888

Tellier M-AS, Bertelsen R (2008) Monitoring the flora and fauna of the nearshore hardbottom habitats of the Florida Keys. F2196-05-08-F Final Report. Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, Marathon, FL.

Thomson JA, Burkholder DA, Heithaus MR, et al (2015) Extreme temperatures, foundation species, and abrupt ecosystem change: an example from an iconic seagrass ecosystem. Glob Chang Biol 21:1463–1474. doi: 10.1111/gcb.12694

Trakhtenbrotl A, Nathan R, Perry G, Richardson DM (2005) The importance of long-distance dispersal in biodiversity conservation. Divers Distrib 11:173–181.

Truelove NK, Kough AS, Behringer DC, et al (2017) Biophysical connectivity explains population genetic structure in a highly dispersive marine species. Coral Reefs 36:233–244. doi: 10.1007/s00338-016-1516-y

Valentine MM, Butler MJ (2019) Sponges structure water-column characteristics in shallow tropical coastal ecosystems. Mar Ecol Prog Ser 608:133–147. doi: 10.3354/meps12758

van der Meer MH, Horne JB, Gardner MG, et al (2013) Limited contemporary gene flow and high self-replenishment drives peripheral isolation in an endemic coral reef fish. Ecol Evol 3:n/a-n/a. doi: 10.1002/ece3.584

Wall CC, Rodgers BS, Gobler CJ, Peterson BJ (2012) Responses of loggerhead sponges *Spechiospongia vesparium* during harmful cyanobacterial blooms in a sub-tropical lagoon. Mar Ecol Prog Ser 451:31–43. doi: 10.3354/meps09537

Warburton FE (1966) The behaviour of sponge larvae. Ecology 47:672–674.

Whitlock MC (2000) Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles and sexual selection. Evolution (N Y) 54:1855. doi: 10.1554/0014-3820(2000)054[1855:FONAAT]2.0.CO;2

Wright S (1943) Isolation by Distance. Genetics 28:114–38.

Wright S (1949) The genetical structure of populations. Ann Eugen 15:323–354. doi: 10.1111/j.1469-1809.1949.tb02451.x

Wulff J (2006) Rapid diversity and abundance decline in a Caribbean coral reef sponge community. Biol Conserv 127:167–176. doi: 10.1016/j.biocon.2005.08.007

Xavier JR, Soest RWM Van, Breeuwer JAJ, Menken SBJ (2010) Phylogeography, genetic diversity and structure of the poecilosclerid sponge *Phorbas fictitius* at oceanic islands. Contrib to Zool 79:119–129.

Yeung C, Jones DL, Criales MM, et al (2001) Influence of coastal eddies and counter-currents on the influx of spiny lobster, *Panulirus argus*, postlarvae into Florida Bay. Mar Freshw Res 52:1217–1232.

Data accessibility

Microsatellite sequences are logged in NCBI GenBank (accession numbers KX758633- KX758644), and raw Illumina sequences of *Spheciospongia vesparium* genomic DNA are deposited in the NCBI Sequence Read Archive (accession reference SRP158118).

1004

1005 **Figure legends**

1006 **Fig. 1** *Spheciospongia vesparium* sampling sites. Inset map shows sampling sites in Greater
1007 Caribbean (BZ: Caye Caulker, Belize; BH: Abaco, Bahamas; BAR: Codrington Lagoon, Barbuda).
1008 Main map shows Florida Keys sampling sites (PK: Pigeon Key; SCB: Snake Creek Basin; SB:
1009 Steamboat Channel; CKA: Craig Key (Atlantic); FK: Fiesta Key; LKB: Long Key (Bay-side); LKA:
1010 Long Key (Atlantic); GKB: Grassy Key Bank; BK: Bamboo Key; KC: Kemp Channel; LC: Little
1011 Crane Key; WK: Waltz Key; BC: Boca Chica Channel; LP: Lakes Passage). Lower Keys = dark
1012 purple; Middle Keys = medium pink; Upper Keys = light pink. Basemaps: Natural Earth, ESRI.

1013

1014 **Fig. 2** Average allelic richness and private allelic richness per site (rarefied to maximum sample size
1015 g=10). Error bars +/- 1 SE

1016

1017 **Fig. 3** Membership coefficients per individual at K=4 clusters inferred from Geneland (admixture
1018 model) for sponges collected from 14 locations in Florida and a single location each in Barbuda,
1019 Bahamas, and Belize. Individual sponges are each represented by a single bar; colours indicate cluster
1020 identity, and height of the bar shows estimated proportion of membership to cluster

1021

1022 **Fig. 4** Discriminant analysis of principle components (DAPC) for *S*. *vesparium* sampling sites over
1023 a) all sites; b) Florida sites. Points represent individual sponges, sampling sites are coded by colour,
1024 and inertia ellipses summarise the point cloud for each site. Insets are scree plots showing the
1025 proportion of principle components retained in the analysis and the proportion of variance they
1026 represent (shaded portion).

1027

1028 **Fig. 5** Principle coordinates analysis (PCoA) using null allele corrected pairwise F_{ST} values a) among
1029 all sampling sites, and b) among Florida sites (Upper Keys = light pink, Middle Keys = medium pink,
1030 Lower Keys = dark purple). BZ: Caye Caulker, Belize; BH: Abaco, Bahamas; BAR: Codrington
1031 Lagoon, Barbuda; PK: Pigeon Key; SCB: Snake Creek Basin; SB: Steamboat Channel; CKA: Craig

1032 Key (Atlantic); FK: Fiesta Key; LKB: Long Key (Bay-side); LKA: Long Key (Atlantic); GKB:
1033 Grassy Key Bank; BK: Bamboo Key; KC: Kemp Channel; LC: Little Crane Key; WK: Waltz Key;
1034 BC: Boca Chica Channel; LP: Lakes Passage.
1035
1036 **Fig. 6** a) Inferred barriers to gene flow among *Spheciospongia vesparium* sites in the Florida Keys,
1037 using Monmonier's (1973) algorithm as implemented in Barrier v2.1 software. Green circles represent
1038 spatial projection of sites, blue lines show Voronoï polygons, and red lines show inferred barriers.
1039 Grey numbers show bootstrap score (out of 100). PK: Pigeon Key; SCB: Snake Creek Basin; SB:
1040 Steamboat Channel; CKA: Craig Key (Atlantic); FK: Fiesta Key; LKB: Long Key (Bay-side); LKA:
1041 Long Key (Atlantic); GKB: Grassy Key Bank; BK: Bamboo Key; KC: Kemp Channel; LC: Little
1042 Crane Key; WK: Waltz Key; BC: Boca Chica Channel; LP: Lakes Passage.
1043 b) Satellite map of sites at Long Key (Bay-side) (LKB), Long Key (Atlantic) (LKA), Fiesta Key (FK)
1044 and Craig Key (Atlantic) (CKA), with red line to show separation of sites by inferred barrier.
1045 Basemap: Bing.
1046
1047 **Fig. 7** Genetic isolation by distance for *Spheciospongia vesparium* using pairwise calculations of
1048 linearised F_{ST} (F_{ST}/[1-F_{ST}]) and the logarithm of oceanographic distance. Regression line with shaded
1049 95% confidence intervals
1050

Tables

Table 1: Sampling details for *Spheciospongia vesparium*

Location	Site	ID	Latitude, Longitude	Date	n
Florida Keys, USA:					
Upper Keys	Pigeon Key	PK	25.0594, -80.4979	7/14	18
	Snake Creek Basin	SCB	24.9831, -80.5602	7/14	17
	Steamboat Channel*	SB	24.9559, -80.6492	7/14	19
Middle Keys	Craig Key (Atlantic)	CKA	24.8350, -80.7599	6/16	10
	Fiesta Key*	FK	24.8430, -80.7890	7/14	18
	Long Key (Bay-side)*	LKB	24.8143, -80.8307	7/14	18
	Long Key (Atlantic)	LKA	24.8021, -80.8435	6/16	17
	Grassy Key Bank*	GKB	24.7917, -80.9598	7/14	17
	Bamboo Key*	BK	24.7442, -80.9950	7/14	19
Lower Keys	Kemp Channel	KC	24.6768, -81.4757	7/14	20
	Little Crane Key	LC	24.7840, -81.5120	7/14	20
	Waltz Key	WK	24.6510, -81.6521	7/14	17
	Boca Chica Channel	BC	24.6049, -81.7150	7/14	19
	Lakes Passage	LP	24.5694, -81.8757	7/14	32
Abaco, Bahamas	Mermaids Reef	BH	26.5537, -77.0527	7/15	12
Barbuda, Antigua and Barbuda	Codrington Lagoon, Barbuda	BAR	17.6547, -61.8527	5/15	20
Caye Caulker, Belize	Caye Caulker	BZ	17.7422, -88.0354	5/13	10

n = number of samples successfully genotyped and used in analysis. * indicates areas that have been affected by cyanobacterial blooms.

Table 2: Average genetic diversity and inbreeding coefficients over all loci per site

Site	rAR *(± SE)*	rPR *(± SE)*	H_O	H_S	Avg Fi	95% HPDI
PK	3.408 *(±0.421)*	0.054 *(±0.029)*	0.397	0.569	0.0995	0 – 0.2662
SCB	3.698 *(±0.378)*	0.115 *(±0.073)*	0.368	0.595	**0.1875**	0 – 0.3516
SB	3.857 *(±0.379)*	0.030 *(±0.011)*	0.427	0.655	**0.1089**	0 – 0.2566
CKA	3.844 *(±0.349)*	0.112 *(±0.061)*	0.251	0.650	**0.3433**	0 – 0.6069
FK	3.699 *(±0.411)*	0.158 *(±0.070)*	0.386	0.611	0.0499	0 – 0.1574
LKB	4.399 *(±0.360)*	0.211 *(±0.063)*	0.339	0.711	**0.1698**	0 – 0.3972
LKA	3.742 *(±0.211)*	0.077 *(±0.034)*	0.365	0.638	**0.1509**	0 – 0.2773
GKB	3.638 *(±0.267)*	0.065 *(±0.033)*	0.394	0.636	0.0546	0 – 0.1623
BK	3.429 *(±0.315)*	0.022 *(±0.013)*	0.383	0.612	**0.1608**	0 – 0.3016
KC	3.862 *(±0.297)*	0.134 *(±0.086)*	0.379	0.659	0.0785	0 – 0.2180
LC	3.823 *(±0.304)*	0.020 *(±0.008)*	0.418	0.645	0.0403	0 – 0.1213
WK	3.819 *(±0.256)*	0.139 *(±0.050)*	0.356	0.662	**0.2187**	0 – 0.4180
BC	3.883 *(±0.376)*	0.100 *(±0.045)*	0.417	0.644	**0.1138**	0 – 0.2655
LP	3.806 (±0.315)	0.122 (±0.063)	0.412	0.635	**0.0622**	0 – 0.1760
BH	4.375 *(±0.340)*	0.819 *(±0.253)*	0.504	0.735	0.0361	0 – 0.1191
BAR	3.940 *(±0.331)*	0.956 *(±0.351)*	0.404	0.653	**0.0816**	0 – 0.1700
BZ	4.390 *(±0.414)*	0.929 *(±0.310)*	0.481	0.713	0.0367	0 – 0.1277
FL	4.080 *(±0.332)*	1.095 *(±0.133)*	0.383	0.655	**0.0547***	0.0025 – 0.1002
BH	-	1.538 *(±0.324)*	-	-	-	-
BAR	-	1.344 *(±0.392)*	-	-	**-**	-
BZ	-	1.568 *(±0.386)*	-	-	-	-

rAR *(± SE)*: Average rarefied allelic richness (± Standard error); rPR *(± SE)*: Average rarefied private allelic richness (± Standard error); H_O: Observed heterozygosity; H_S: Nei's gene diversity/ expected heterozygosity; AvgFi: Null allele corrected inbreeding coefficient (values in bold denote sites where the 'nfb' [null alleles, inbreeding and genotyping error] model has the lowest DIC, values not in bold denote where the 'nb' [null alleles and genotyping] model has the lowest DIC, * denotes significance; 95% HDPI: Posterior 95% probability intervals. Analyses repeated for all Florida sites grouped together ('FL'); here, rarefied private allelic richness was recalculated for all sites, as this is a relative measure.

Table 3: Pairwise F_{ST} (below diagonal, shaded) and D (above diagonal, not shaded) between pairs of sites for *Spheciospongia vesparium*. Significant ($p < 0.05$ following Benjamini –Yekutieli correction) F_{ST} pairwise comparisons in bold

	PK	SCB	SB	CKA	FK	LKB	GKB	LKA	BK	KC	LC	WK	BC	LP	BH	BAR	BZ
PK	--	0.027	0.028	0.166	0.089	0.031	0.074	0.091	0.055	0.141	0.133	0.147	0.078	0.138	0.613	0.593	0.311
SCB	0.019	--	0.029	0.205	0.058	0.01	0.071	0.077	0.035	0.098	0.081	0.149	0.048	0.1	0.564	0.577	0.28
SB	0.016	0.017	--	0.076	0.023	-0.016	0.042	0.029	-0.002	0.081	0.055	0.094	0.003	0.059	0.557	0.56	0.276
CKA	**0.1**	**0.116**	0.041	--	0.055	0.082	0.039	-0.035	0.145	0.045	0.085	0.062	0.083	0.037	0.616	0.62	0.27
FK	**0.057**	0.037	0.013	0.035	--	0.006	0.003	0.038	0.054	0.031	0.02	0.118	0.016	0.025	0.62	0.606	0.257
LKB	0.017	0.005	-0.007	0.038	0.003	--	0.004	0.041	0.027	0.012	0.004	0.071	-0.002	0.052	0.544	0.476	0.172
GKB	**0.046**	**0.043**	0.022	0.023	0.002	0.002	--	0.024	0.082	0.024	0.013	0.033	0.044	0.025	0.626	0.607	0.259
LKA	**0.055**	**0.047**	0.015	-0.019	0.022	0.02	0.013	--	0.081	0.01	0.038	0.056	0.041	0.03	0.564	0.597	0.234
BK	0.036	0.022	-0.001	**0.082**	0.033	0.014	**0.047**	**0.046**	--	0.113	0.087	0.093	0.018	0.094	0.592	0.523	0.273
KC	**0.08**	**0.056**	**0.041**	0.025	0.017	0.006	0.012	0.005	**0.06**	--	0.008	0.074	0.049	0.024	0.547	0.537	0.198
LC	**0.078**	**0.048**	0.029	**0.047**	0.012	0.003	0.007	0.021	**0.049**	0.004	--	0.08	0.035	0.012	0.6	0.553	0.176
WK	**0.085**	**0.081**	**0.046**	0.032	**0.062**	0.031	0.017	0.029	**0.051**	**0.036**	**0.041**	--	0.047	0.064	0.668	0.648	0.31
BC	**0.047**	0.028	0.002	0.046	0.01	-0.001	0.024	0.022	0.01	0.026	0.019	0.024	--	0.038	0.58	0.544	0.246
LP	**0.081**	**0.059**	**0.032**	0.024	0.015	**0.027**	0.014	0.017	**0.053**	0.013	0.007	**0.034**	0.021	--	0.575	0.565	0.245
BH	**0.249**	**0.226**	**0.197**	**0.213**	**0.234**	**0.171**	**0.224**	**0.205**	**0.225**	**0.193**	**0.215**	**0.221**	**0.208**	**0.215**	--	0.407	0.527
BAR	**0.273**	**0.259**	**0.23**	**0.252**	**0.261**	**0.184**	**0.251**	**0.247**	**0.233**	**0.221**	**0.232**	**0.253**	**0.229**	**0.24**	**0.154**	--	0.425
BZ	**0.154**	**0.134**	**0.116**	**0.115**	**0.119**	**0.064**	**0.114**	**0.103**	**0.126**	**0.086**	**0.08**	**0.124**	**0.106**	**0.112**	**0.168**	**0.169**	--

Table 4: Hierarchical analysis of molecular variance (AMOVA) within and among *Spheciospongia vesparium* individuals, sites and groups: 1) All sites, Florida sites grouped together; 2) Only Florida and the Bahamas sites, grouping according to Florida-Bahamas inferred barrier from 'Barrier' analysis; 3) Only Fiesta Key, Long Key (Bay-side), Long Key (Atlantic) and Craig Key (Atlantic) sites; grouping according to Middle Keys Bay-side and Atlantic sites inferred barrier from 'Barrier' analysis.

Source of variation	**Sum of squared deviations**	**Variance components**	**% variance**	**F-value**	**Std. Dev.**
1) All sites					
Within individuals	577.500	2.361	48.5	0.515	0.053
Among individuals, within sites	1185.082	1.504	30.9	0.389	0.070
Among sites in Florida	113.578	0.116	2.4	0.029	0.005
Among Florida, Bahamas, Belize and Barbuda	138.482	0.886	18.2	0.182	0.041
Florida and Bahamas (Barrier 1)					
Within individuals	510.000	2.333	47.0	0.530	0.053
Among individuals, within sites	1064.428	1.519	30.6	0.394	0.072
Among sites in Florida	113.568	0.116	2.3	0.029	0.005
Between Florida and Bahamas	46.429	0.998	20.1	0.201	0.050
Atlantic and Bay-side Middle Keys (Barrier 2)					
Within individuals	104.00	2.060	51.5	0.485	0.075
Among individuals, within sites	263.863	1.834	45.9	0.471	0.080
Between sites, within Atlantic/Bay-side grouping	10.611	-0.025	-0.6 (0)	-0.007	0.009
Between Atlantic and Bay-side	11.114	0.130	3.3	0.033	0.011

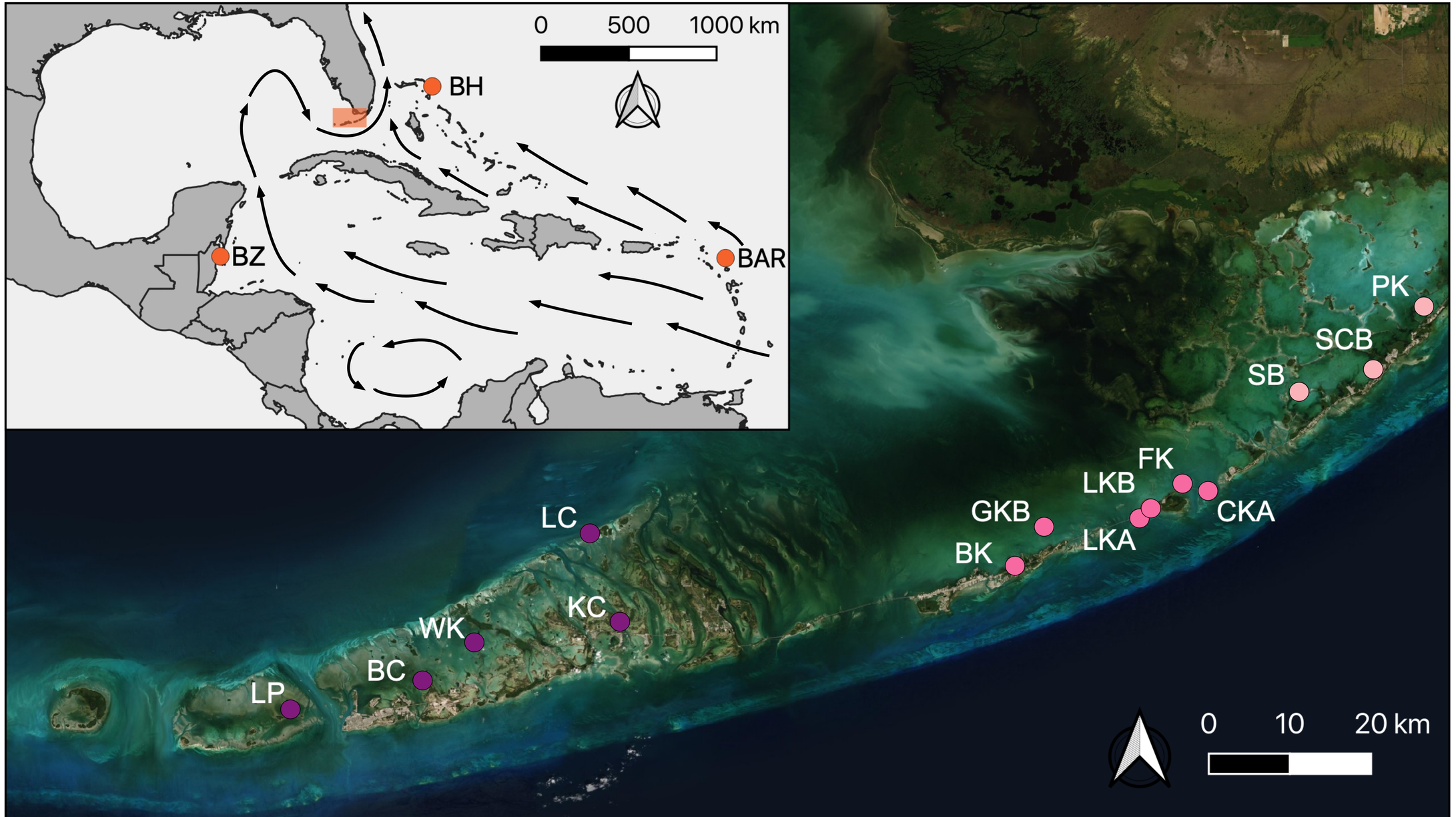


Figure 2

Click here to download Figure Figure_2.tiff

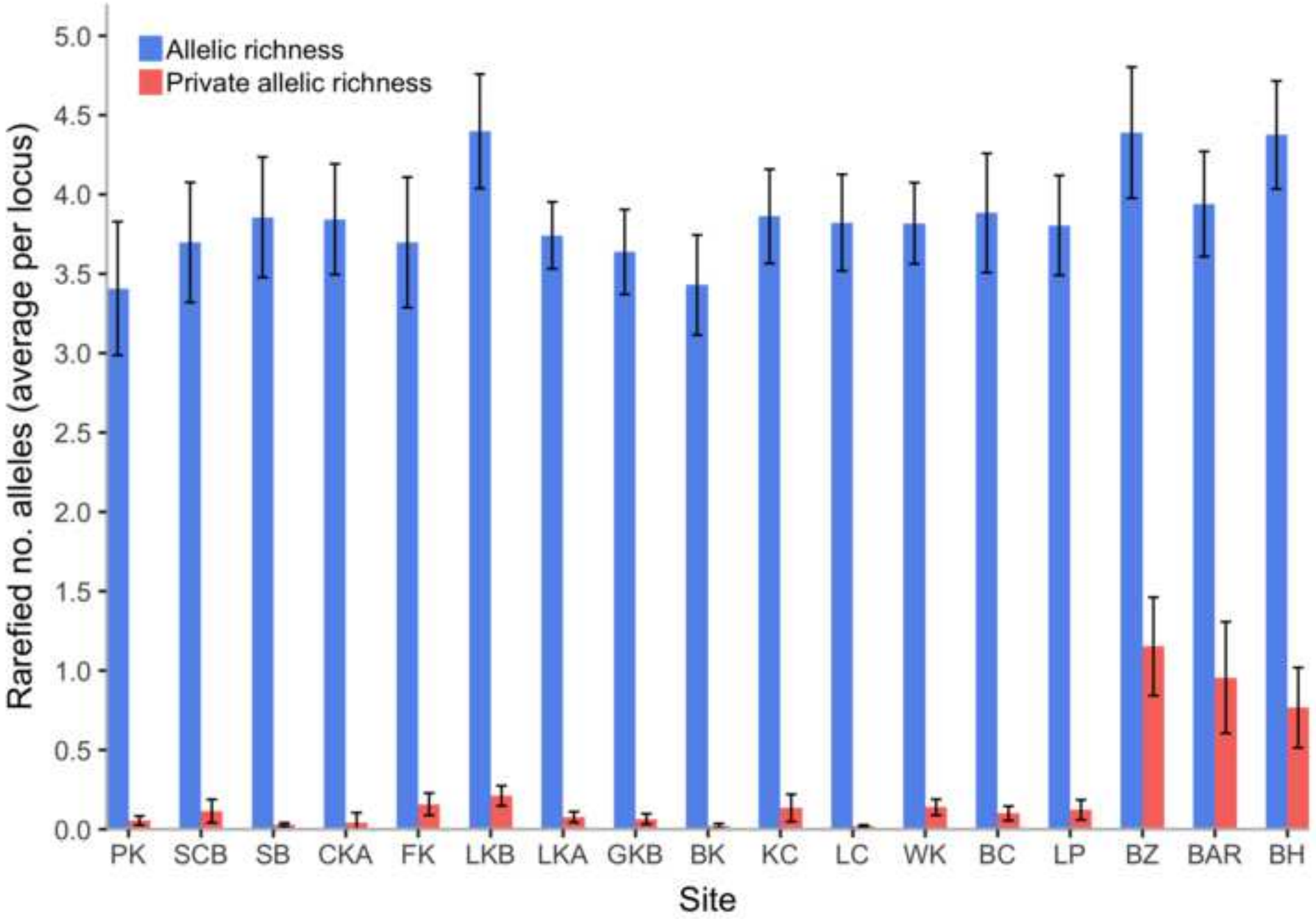


Figure 3

Click here to download Figure Figure_3.tiff

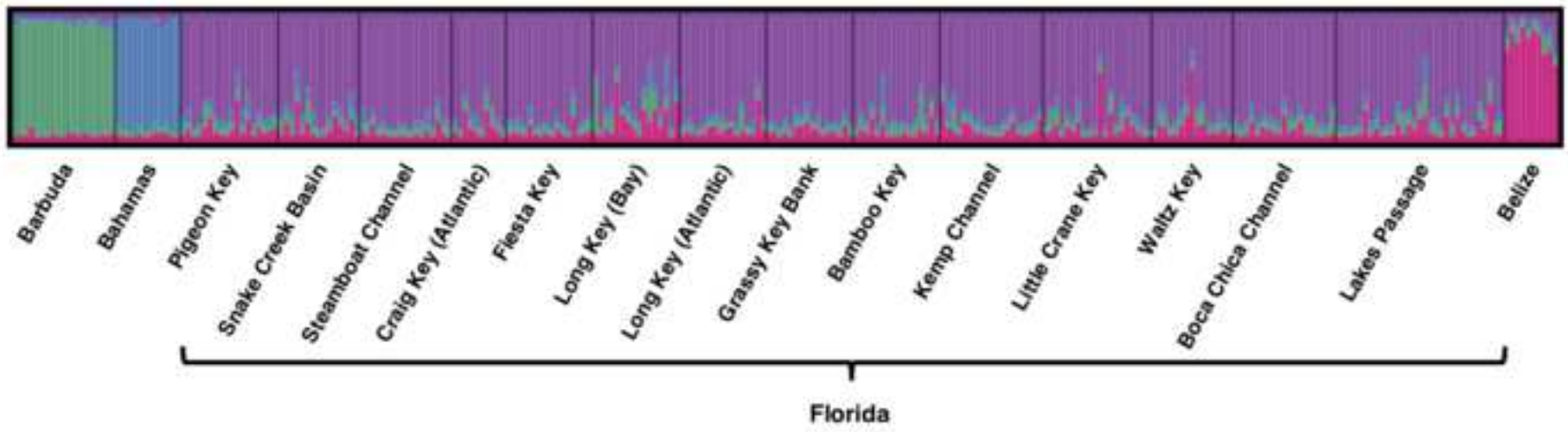
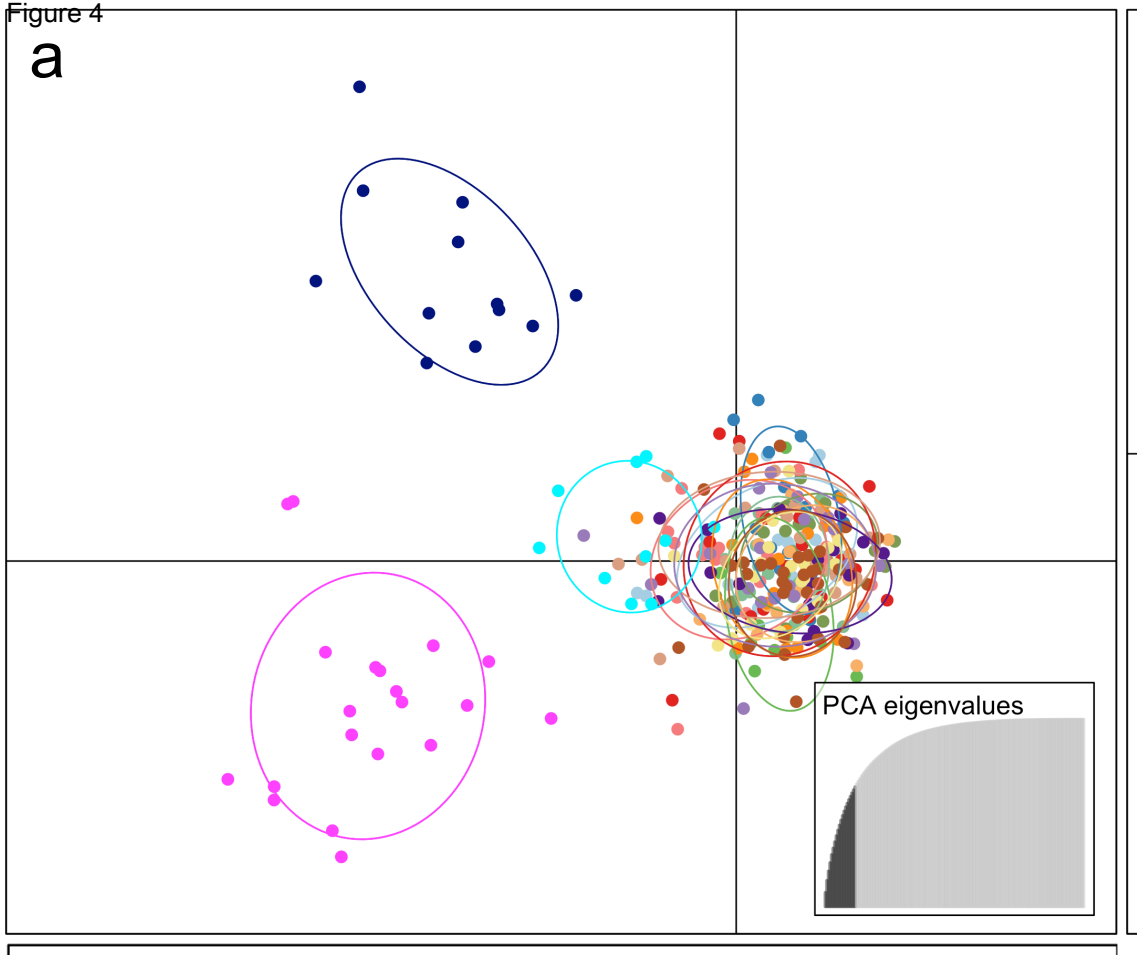
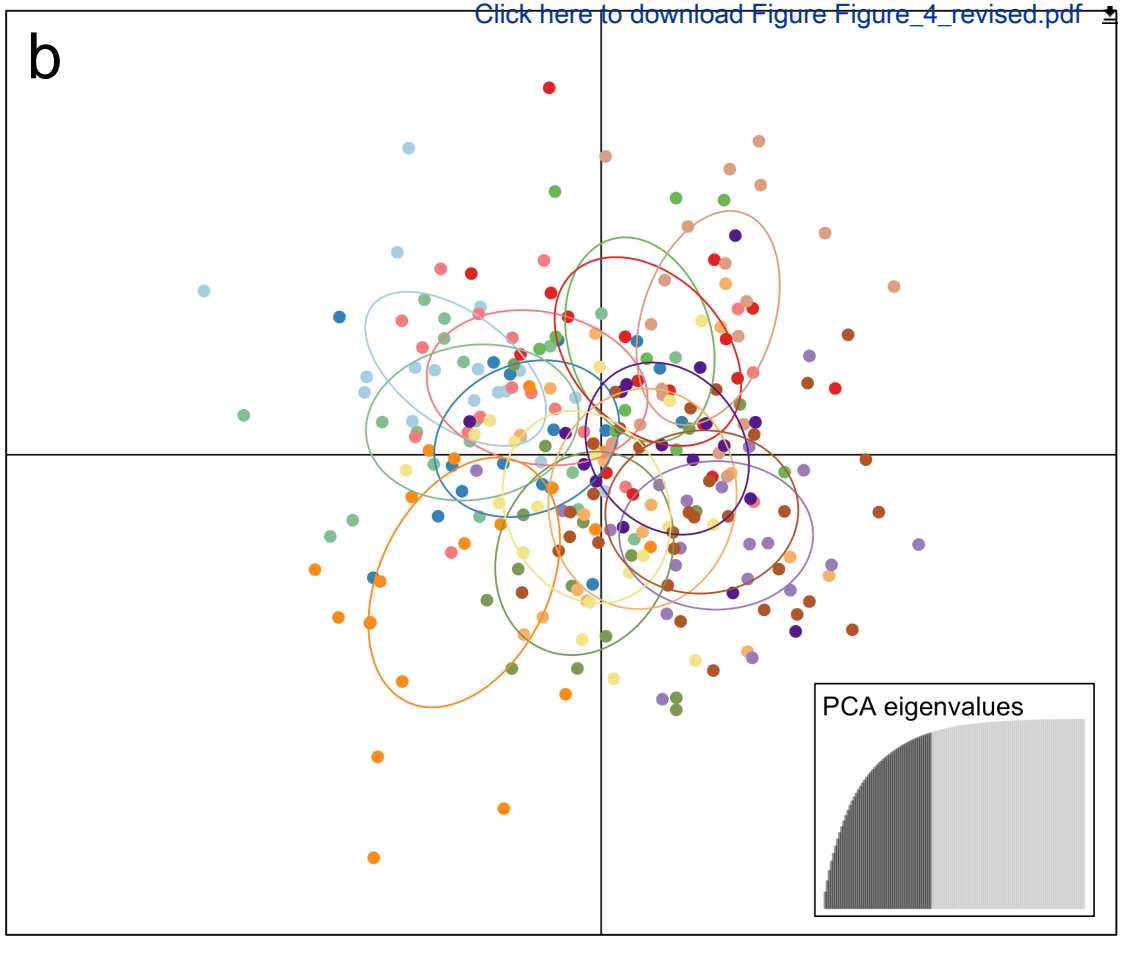


Figure 4





- Pigeon Key (PK)
- Snake Creek Basin (SCB)
- Steamboat Channel (SB)
- Craig Key (Atlantic) (CKA)
- Fiesta Key (FK)
- Long Key (Bay-side) (LKB)
- Long Key (Atlantic) (LKA)
- Grassy Key Bank (GKB)
- Bamboo Key (BK)
- Kemp Channel (KC)
- Little Crane Key (LC)
- Waltz Key (WK)
- Boca Chica Channel (BC)
- Lakes Passage (LP)
- Abaco, Bahamas (BH)
- Barbuda (BAR)
- Caye Caulker, Belize (BZ)

Figure 5a

Click here to download Figure Figure_5a.png

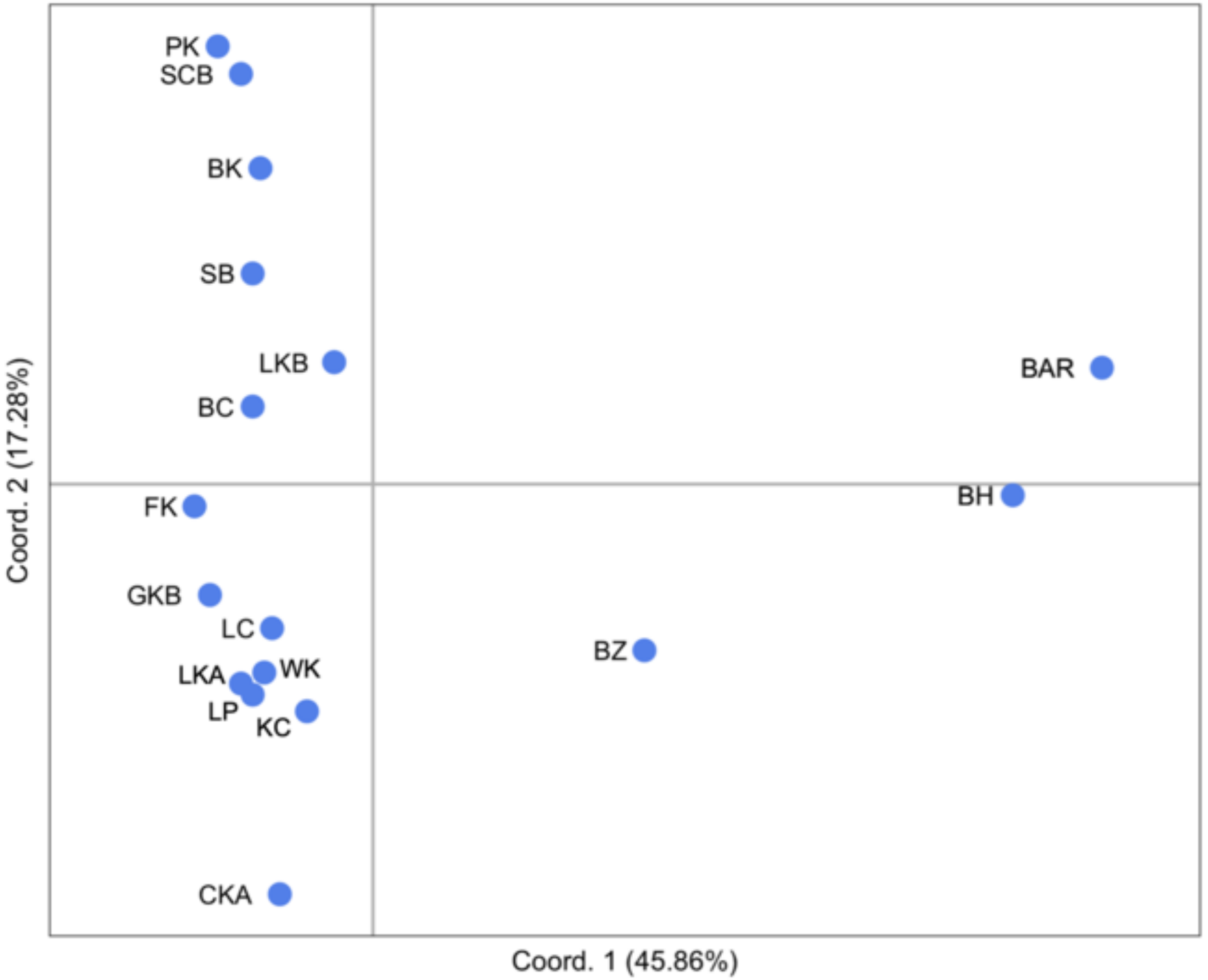


Figure 5b

Click here to download Figure Figure_5b_revised.png

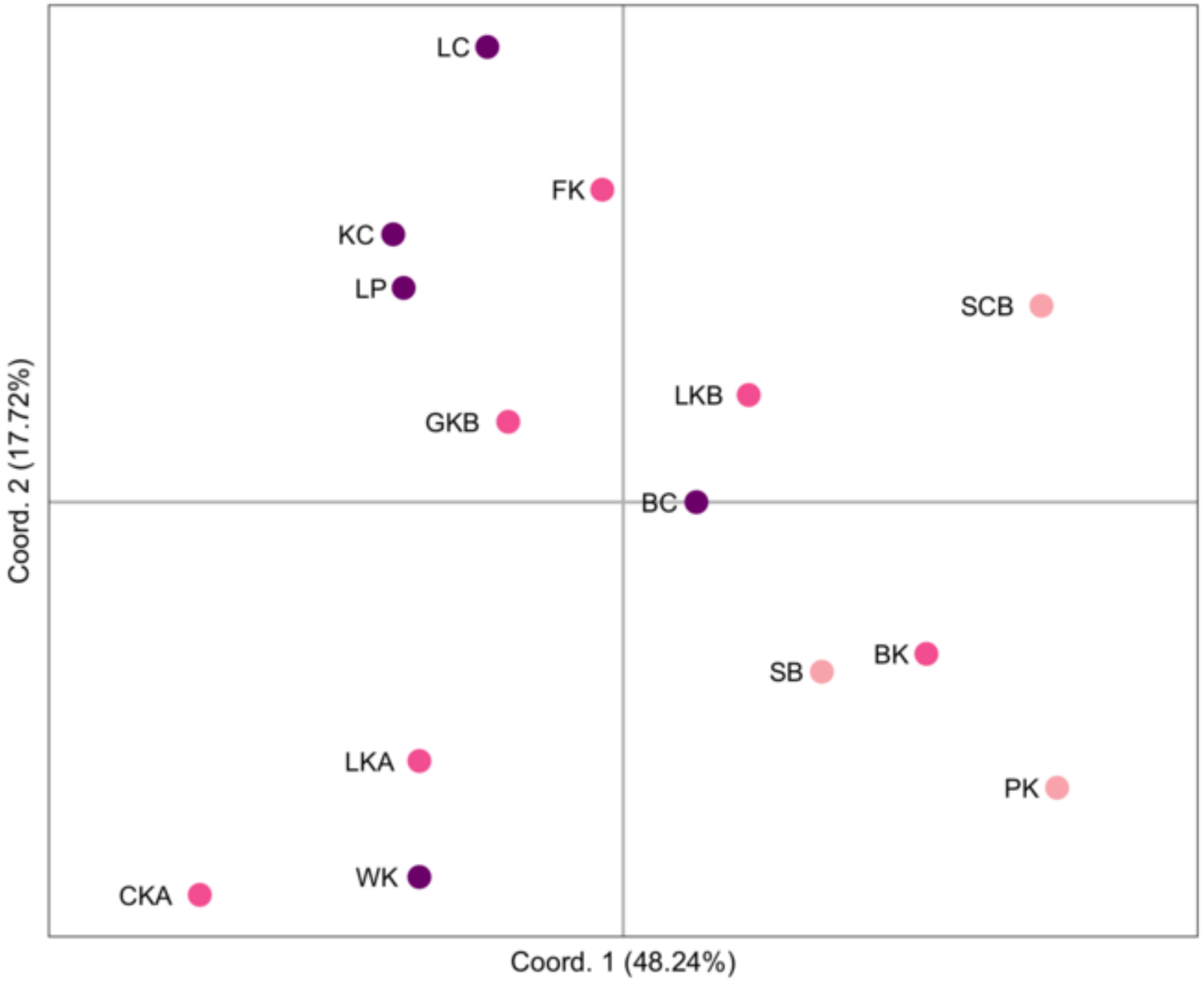


Figure 6

Click here to download Figure Figure_6_revised.pdf

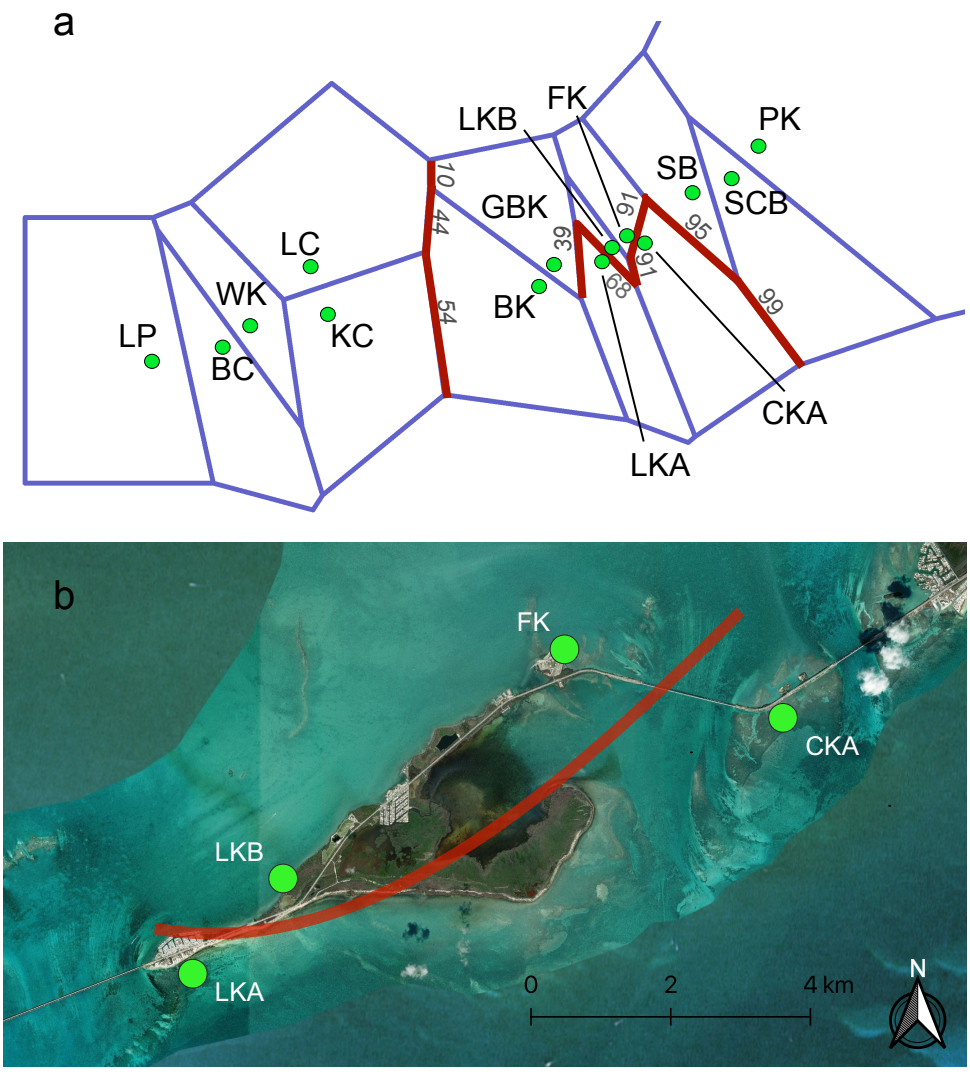


Figure 7

Click here to download Figure Figure_7_revised.png