Vol. 610: 83–97, 2019
https://doi.org/10.3354/meps12842

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 1

Population genetics and biophysical modeling inform metapopulation connectivity of the Caribbean king crab *Maguimithrax spinosissimus*

J. Antonio Baeza[1,2,3,*], Daniel Holstein[4], Rodolfo Umaña-Castro[5], Luis M. Mejía-Ortíz[6]

[1]Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA
[2]Smithsonian Marine Station at Fort Pierce, Fort Pierce, FL 34949, USA
[3]Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile
[4]Department of Oceanography and Coastal Sciences, College of the Coast and Environment, Louisiana State University, Baton Rouge, LA 70803, USA
[5]Laboratorio de Análisis Genómico (LAGEN), Universidad Nacional, Heredia, Costa Rica
[6]Laboratorio de Bioespeleología y Carcinología, División de Desarrollo Sustentable, Universidad de Quintana Roo, Quintana Roo, Mexico

ABSTRACT: Marine organisms with a short pelagic larval duration (PLD) are assumed to display significant population structure given low long-distance dispersal ability. For the wider Caribbean, theoretical and empirical considerations suggest that species with short PLDs inhabiting each of the following areas should be genetically distinct: Costa Rica (CR), Mexico (MX), and Florida Keys (FL-K), USA. This study tested the hypothesis of significant genetic differentiation in *Maguimithrax spinosissimus* populations across the wider Caribbean using a combination of biophysical modeling and population genetics. Biophysical modeling predicted dissimilar connectivity patterns among CR, MX, and FL-K depending upon assumed PLD. Eight days of dispersal only provided rare connections from MX to FL-K, and low likelihood multi-generational connections between CR and FL-K. In turn, 12 d of dispersal was sufficient to connect MX to FL-K through direct and indirect routes in biophysical models, but CR and FL-K remained connected only through multiple generational steps. After 16 d of dispersal, direct connections between CR and FL-K may occur, and by 20 d of dispersal, connections are likely between all sampled patches. Population genetic analyses based on partial sequences of the mtDNA 12S, 16S, and COI genes denoted the existence of a single, relatively high-frequency haplotype shared among all 3 populations, which suggests a longer PLD than predicted by laboratory study. In turn, an analysis of molecular variance and pairwise F_{ST} values demonstrated significant genetic differentiation among the studied populations. Altogether, the above information suggests low to moderate connectivity among populations in *M. spinosissimus*. Subpopulation split predictions suggest multi-generational or stepping-stone connectivity between CR and both MX and FL-K, and provides a framework for understanding the structure of *M. spinosissimus* metapopulations throughout the region. Overall, this study agrees with the notion of significant population structure in marine species with lower dispersal ability.

KEY WORDS: Crab · Demographic history · Coalescence · Phylogeny

1. INTRODUCTION

Exploring genetic structure and biophysical connectivity among conspecific populations is an important exercise for understanding the processes governing species distribution (Sotka et al. 2004), for the efficient management of exploited species (Palumbi 2004, Marko & Hart 2011, Kough et al. 2013), for guiding

*Corresponding author: jbaezam@clemson.edu

the establishment of sound conservation strategies (Goerlitz et al. 2003, Palumbi 2004, Baums 2008, Carpenter et al. 2011), and ultimately, for shedding light on evolutionary processes (Avise 2000, Weersing & Toonen 2009). In marine systems, organisms were long thought to exhibit high population connectivity, high gene flow, and subsequent low population genetic structure (Avise 2000, Cowen et al. 2000). This assumption was based on the perception that geographical barriers are less obvious in marine, as opposed to terrestrial, environments (Cowen et al. 2000). Thus, oceanic currents were expected to favor rather than constrain population inter-connectivity, and thus promote genetic homogenization over large geographical scales (e.g. over ≥1000s of km). Further, many marine organisms disperse during extended larval periods (compared to terrestrial species) and occupy wide geographical ranges; thus, marine species were expected to have considerable long-distance dispersal ability, also favoring genetic homogenization across large spatial scales (Avise 2000).

Active research on this topic and the subsequent body of literature accumulated over more than 50 yr, however, have provided limited support for the view of high levels of contemporary gene flow and low genetic differentiation among populations of marine organisms (Avise 2000, Cowen et al. 2000, 2006, Hellberg 2009). For instance, some studies have reported remarkable levels of genetic differentiation among populations in species with considerable dispersal ability (fish: Magoulas et al. 2006, Taylor & Hellberg 2003; invertebrates: Baums et al. 2005, Arnaud-Haond et al. 2008), although little to no genetic population differentiation occurs in some other species with large geographic ranges and extended larval periods (fishes: Zardoya et al. 2004, Costagliola et al. 2004; invertebrates: Naro-Maciel et al. 2011, McMillen-Jackson & Bert 2004), suggesting population genetic structure is determined by a range of biophysical mechanisms.

In marine organisms, conditions that potentially drive connectivity among populations can be divided into intrinsic and extrinsic factors. Extrinsic conditions include, among others, geographic distance among populations (i.e. isolation by distance: Planes & Fauvelot 2002), local adaptation processes (Riginos & Nachman 2001), and oceanographic phenomena that can favor (or reduce) gene flow among populations (Cowen et al. 2000, 2006, White et al. 2010). Intrinsic conditions include biological traits such as mode of development (direct vs. planktonic), duration of the pelagic larval period in species with indirect development (Teske et al. 2007), larval behaviors (Cowen et al. 2000, 2006), reproductive season (Watson et al. 2011), and generation times (Rolán-Alvarez et al. 1995), among others. Among the above, the mode of larval development and the duration of the pelagic larval period appear to be of utmost importance in driving population connectivity (Teske et al. 2007, Pelc et al. 2010). For instance, species with rapid larval development have been shown to exhibit greater genetic structure than that observed in similar species with extended larval periods and putatively high potential for dispersion (Collin 2001, Rocha et al. 2002, Modica et al. 2017). However, the effect of type of development has not been observed in other species (see Marko & Hart 2011 and references therein). Overall, the relative importance of the intrinsic and extrinsic conditions described above in shaping marine phylogeographic patterns remains poorly understood (Avise 2000, Snyder et al. 2014).

In this study, we were interested in exploring population differentiation and connectivity in a species with low dispersal potential but a large geographic range (i.e. ≥1000s of km). For this purpose, the Caribbean king crab *Maguimithrax spinosissimus* was selected as a model organism given its wide pan-Caribbean geographic distribution (Provenzano & Brownell 1977, Williams 1984, J. A. Baeza pers. obs.). We explored how the interaction of oceanographic distance and rapid larval development affected population structure and connectivity in this species. *M. spinosissimus* mostly dwells among coral and coral rocks in the shallow subtidal throughout its range, but is also present in man-made structures such as rock jetties (Provenzano & Brownell 1977, Williams 1984, Baeza et al. 2012, 2015). The dispersal potential of this crab is relatively low given its rapid and abbreviated lecitotrophic larval development that includes only 2 zoeal stages and 1 post-larval megalopae (i.e. in the laboratory, development from hatching to first crab instar has been shown to last 5–6 d without food; Provenzano & Brownell 1977). Also, adults and juveniles of this species do not seek refuge in structures that are prone to dislodge from the marine bottom and drift away (Baeza et al. 2015). Furthermore, no indication of long-distance migration to breeding grounds has been reported for this species (Hazlett & Rittschof 1975). Considering the above, reduced gene flow was expected in *M. spinosissimus* across the greater Caribbean region. The interaction of limited dispersal potential and both antecedent and contemporary oceanographic processes are expected to result in reduced gene flow and genetic differentiation in this crab, at least among distantly located populations (e.g. northwest-

ern Atlantic Ocean versus central Caribbean Sea versus south Caribbean Sea) (Avise 2000, Cowen et al. 2000, 2006).

The greater Caribbean, including the North Atlantic (e.g. Florida Keys), represents a basin with a complex geographical history and highly diverse flow regimes, with areas that either constrain or favor genetic admixture, as suggested by high-resolution biophysical models (Cowen et al. 2000, 2006, Holstein et al. 2014) and empirical studies (Avise 2000, Taylor & Hellberg 2003, Wise et al. 2004, Duran & Rützler 2006, Soltis et al. 2006, Eytan & Hellberg 2010, Piñeros & Gutiérrez-Rodríguez 2017, Truelove et al. 2017). For species with a larval period much greater than *M. spinosissimus*, both biophysical models (Cowen et al. 2006, Kough et al. 2013) and empirical genetic studies (Salas et al. 2010) predict genetic dissimilarity between northern (Florida Keys, Florida, USA), central (Mexico) and southern (Costa Rica) Caribbean populations. This is largely due to the existence of the semi-permanent Panama–Colombia Gyre off the coast of Costa Rica, Panama, and Colombia that encourages larval retention in the southwestern Caribbean, and thus prevents larval export from southern to northern Caribbean populations (Cowen et al. 2000, 2006). Genetic dissimilarity between Mexican and other populations in the Caribbean have been reported before in several species of vertebrates and invertebrates (Taylor & Hellberg 2003, Duran & Rützler 2006, Eytan & Hellberg 2010). In general, taking into account theoretical predictions arising from biophysical modeling and empirical studies, genetic differentiation is expected at least between southernmost and northernmost populations of *M. spinosissimus* in the wider Caribbean (see also Hurtado-Alarcón et al. 2018).

The aim of this study was to synthesize biophysical estimates of connectivity and estimates of genetic distinctiveness (or the lack thereof) in the Caribbean king crab *M. spinosissimus* across the greater Caribbean region. These goals will help to improve our understanding of population connectivity in a widely distributed marine organism with low to moderate dispersal potential. Limited biophysical modeling of connectivity has been developed for Caribbean marine species with low dispersal capability (an exception being the coral *Porites astreoides*, for which biophysical models predicted highly fragmented populations with potentially multiple Caribbean metapopulations; Holstein et al. 2014, see also Baums et al. 2006). Thus, our first goal was to develop a biophysical model describing the dispersal of *M. spinosissimus* throughout the Caribbean region, and specifically from 3 geographically distinct sampling populations, using a range of pelagic larval durations (PLDs) to capture variation in dispersal potential. Connectivity matrices were then split into subpopulations (Jacobi et al. 2012), which were analyzed for quality and compared to genetic dissimilarity among populations in this species. We conducted population-level genetic comparisons using partial sequences of COI, 16S, and 12S mitochondrial DNA. These fragments are well suited for population genetic studies (see Hellberg 2009, Baeza & Fuentes 2013).

2. MATERIAL AND METHODS

2.1. Biophysical modeling

Lagrangian particle tracking of *Maguimithrax spinosissimus* larvae was performed in surface currents taken offline from the Global HYCOM circulation model (1/12° resolution; www.hycom.org) to evaluate the potential of population connectivity in the Caribbean Sea. The Connectivity Modeling System (Paris et al. 2013) was used to track virtual *M. spinosissimus* larvae released from 3202 coral reef localities throughout the Caribbean (3202 polygons, 8 × 8 km sensu Holstein et al. 2014), including 3 from which *M. spinosissimus* genetic samples were obtained (Florida Keys [FL-K], Mexico [MX], and Costa Rica [CR]; see below). Simulations were run for 8, 12, 16, and 20 d of dispersal to account for potential variation and for unknowns in the PLD of *M. spinosissimus*. This was accomplished by running a single simulation for 20 d, and sub-sampling the model output.

Virtual larvae released from all localities were tracked following monthly spawns over 92 mo of hydrographic forcing and a 20 d larval duration (January 2009–August 2016). A total of 100 virtual larvae were released from each location each month, resulting in 29 458 400 tracked larvae. This number of particles was chosen to saturate potential connections in the full 20 d dispersal (Fig. A1 in the Appendix). Because spawning phenology is relatively undocumented, monthly spawns were meant to capture variability in dispersal potential. Virtual larvae were subject to horizontal diffusion, with the coefficient 7.154 $m^2 s^{-2}$, estimated based on the resolution of the oceanographic model and according to estimations developed in Matsuzaki & Fujita (2017). This value is likely higher than actual diffusion for these larvae, and was used to saturate potential connections in the model. Because little is known about *M. spinosis-*

simus larval behaviors, such as vertical swimming or buoyancy traits, virtual larvae were treated as passive. Virtual larvae were competent to settle after 6 d (Provenzano & Brownell 1977) and remained competent throughout their larval duration (8, 12, 16, and 20 d). After a complete dispersal simulation, each time a particle encountered coral reef habitat throughout its trajectory, that event was recorded. These events were accumulated into a connectivity matrix (M) that described the number of connections recorded between every set of habitat patches. M was then normalized to matrix C, such that $C_{i,j}$ described the probability of a particle release at patch i arriving at patch j (sensu Paris et al. 2013).

These normalized connectivity matrices were used to predict potential *M. spinosissimus* genetic subpopulations (sensu Jacobi et al. 2012) to be contrasted again with empirical population-level genetic comparisons. The subpopulation split algorithm identifies potential subpopulations using a minimization procedure that splits the connectivity matrix recursively to minimize connectivity between those subpopulations (also see Chollett et al. 2017). This procedure produces many potential 'splits' with differing numbers of subpopulations, and varying mean connectivity (Q), with lower Q splits having better defined subpopulations with lower connectivity between them, and higher Q splits having poorly defined, 'leaky' subpopulations. We first extracted subpopulations with the lowest Q scores for any given number of predicted subpopulations, and sub-selected one split with the lowest number of predicted subpopulations for each simulation using the following rules: (1) connectivity between subpopulations containing the sampling sites in MX and FL-K must have greater connectivity than subpopulations containing the sampling sites in MX and CR; and (2) subpopulations containing sampling sites in MX and FL-K must not be identical (MX and FL-K must be in separate predicted subpopulations).

2.2. Collection of *M. spinosissimus*

A total of 78 *M. spinosissimus* were collected from 3 different localities in the greater western Caribbean; Long Key, Florida (north Caribbean), Cozumel, Mexico (central Caribbean), and Cahuita, Costa Rica (south Caribbean) during 2015. The rationale for choosing and sampling the 3 localities was to have a simple experimental design (taking into account logistic, monetary, and time limitations) with which to test for population dissimilarity among locations within the greater Caribbean. The 3 chosen localities are expected to have varying but predictable genetic differentiation based on our biophysical model. In all localities, crabs (between 17 and 31 specimens locality^{-1}; see 'Results') were collected from crevices or among rocks in coral reefs. In Florida, crabs were collected near the Tennessee Reef Lighthouse, approximately 4.8 km off Long Key (24.8190° N, 80.8140° W). In Cozumel, crabs were collected 3 km northeast of Punta Molas Lighthouse (20° 37′ 1.21″ N, 86° 44′ 22.47″ W). Lastly, in Costa Rica, crabs were collected 1 km off Punta Uva (9.6528° N, 82.6904° W). Crabs were either preserved in the field in 95% alcohol immediately after collection or transported alive to the laboratory for later preservation. The species identity of each collected crab was confirmed using Rathbun (1925), Williams (1984), and Wagner (1990).

2.3. DNA extraction and sequence alignment

Total genomic DNA was extracted from each crab, usually from claws, pereopods, or from abdominal muscle tissue using the Omega BIO-TEK® E.Z.N.A.® Blood & Tissue DNA Kit following the manufacturer's protocol. Polymerase chain reaction (PCR) was used to amplify target regions of 3 mitochondrial genes: 16S (557 bp region excluding primers), 12S (400 bp) and COI (658 bp). For amplification of the 16S gene segment, we used the primers 16L2 (5′-TGC CTG TTT ATC AAA AAC AT-3′) and 1472 (5′-AGA TAG AAA CCA ACC TGG-3′) (Schubart et al. 2000). For amplification of the 12S gene segment, we used the primers 12Sf (5′-GAA ACC AGG ATT AGA TAC CC-3′) and 12S1R (5′-AGC GAC GGG CGA TAT GTA C-3′) (Mokady et al. 1994 modified from Kocher et al. 1989). For amplification of the COI gene segment, we used a modified version of the Folmer's primers HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAR AAY CA-3′) and LCO1490 (5′-GGT CAA CAA ATC ATA AAG AYA TYG G-3′) (Folmer et al. 1994; modified by Chris Meyer at the Laboratory of Analytical Biology, National Museum of Natural History, Smithsonian Institution). Standard PCR 25 µl reactions (17.5 µl of GoTaq® Green Master Mix [Promega®], 2.5 µl each of the 2 primers [10 mM], and 2.5 µl DNA template) were performed on a C1000 Touch™ Thermal Cycler (BIORAD®) under the following conditions: initial denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 1 min, followed by chain extension at 72°C for 10 min. PCR products were purified with ExoSapIT (a mixture of exonuclease

and shrimp alkali phosphatase; Amersham Pharmacia) and then sent for sequencing with the ABI Big Dye Terminator Mix (Applied Biosystems) to the Clemson University Genomics Institute (CUGI), which is equipped with an ABI Prism 3730xl genetic analyzer (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands and a consensus sequence for the 2 strands was obtained using the software Sequencer 5.4.1 (Gene Codes). The final set of consensus sequences was aligned using multiple sequence comparison by log-expectation in MUSCLE (Edgar 2004), as implemented in MEGA6 (Tamura et al. 2013). All sequences obtained during this study were deposited in GenBank (accession numbers: MK454549–MK454626, MK454993–MK455070, and MK454628–MK454705).

The COI aligned sequences were well defined and chromatograms had no double peaks, ambiguous positions, or indels. Nonetheless, we tested for the occurrence of stop codons that could denote the presence of nuclear copies of mitochondrial-derived genes (numts) or COI pseudogenes (Zhang & Hewitt 1996, Song et al. 2008, Moulton et al. 2010). Numts are copies of mitochondrial genes moved to the nuclear genome that become nonfunctional and noncoding. Consequently, these numts can confuse phylogenetic and phylogeographic analyses (Song et al. 2008, Moulton et al. 2010, Baeza & Fuentes 2013). To check for the presence of numts, we followed Song et al. (2008) and did a basic local alignment search (BLAST) of all COI sequences in the National Center for Biotechnology Information (NCBI) against the database nucleotide collection (nr/nt) and optimized for highly similar sequences to include only haplotypes that showed E-values ≥1.0×10^{-45} and similarity ≥90% with spider crabs. All retrieved sequences were of majoid crabs, most commonly of the genus *Maguimithrax* (= *Damithrax*), followed by representatives from the genera *Mithrax* and *Mithraculus*. After this, the COI haplotypes were translated using the invertebrate mitochondrial code in MEGA6 (Tamura et al. 2013) to verify the protein coding frameshifts and stop codons for each of the 6 putative reading frames in dnaSP (Librado & Rozas 2009).

2.4. Phylogeographic and population genetic analyses

The software POPART (http://popart.otago.ac.nz) was used to estimate haplotype networks for all 3 gene fragments combined. This software implements, among others, the statistical parsimony procedure described in Crandall (1994) and Templeton et al. (1992); the procedure calculates an unrooted tree and provides a 95% plausible set of the relationships among haplotypes.

The software Arlequin v.3.5.1.3 (Excoffier et al. 2005) was used to assess diversity at each sampling locale. The standard diversity indices herein calculated for each locality were number of haplotypes, haplotypic diversity (Nei 1987), and nucleotide diversity site^{-1} (Tajima 1983, Nei 1987).

To test for genetic variance within and among populations, an analysis of molecular variance (AMOVA; Excoffier et al. 1992) was conducted in the same software using uncorrected haplotype pairwise differences as a measure of divergence. To evaluate differentiation between locations (Slatkin 1993), population pairwise F_{ST} values were calculated using the observed number of unweighted haplotype pairwise differences and the number of haplotypes. Significance of the different F_{ST} values was determined through 10 000 permutations. We predicted significantly different F_{ST} values among all of the different studied populations.

3. RESULTS

3.1. Biophysical modeling and subpopulation split predictions

Modeling 8 d of dispersal was barely sufficient to connect the sampled population in Cozumel (Mexico) to the sampled population in Florida (USA) directly, and no intermediate multi-generational connections were made based on sub-population split modeling (Figs. 1 & 2). This suggests highly isolated sub-populations, connected directly only very rarely. Modeling 12 d of dispersal, however, was sufficient to directly connect these 2 sampled populations both directly and through high probability multi-generational pathways as predicted with subpopulation split modeling, which suggests a potential semipermeable oceanic barrier to *Maguimithrax spinosissimus* larval connectivity between Mexico and Florida, should *M. spinosissimus* larval duration be fewer than 12 d *in situ* (Figs. 1 & 2).

Larvae dispersing from MX experience strong flow velocities that vary considerably in time, resulting in a diffuse probability density surface (Fig. 1). Virtual larvae released from CR remained entrained in coastal flow fields, and did not appear to disperse long distances, with no direct connections between

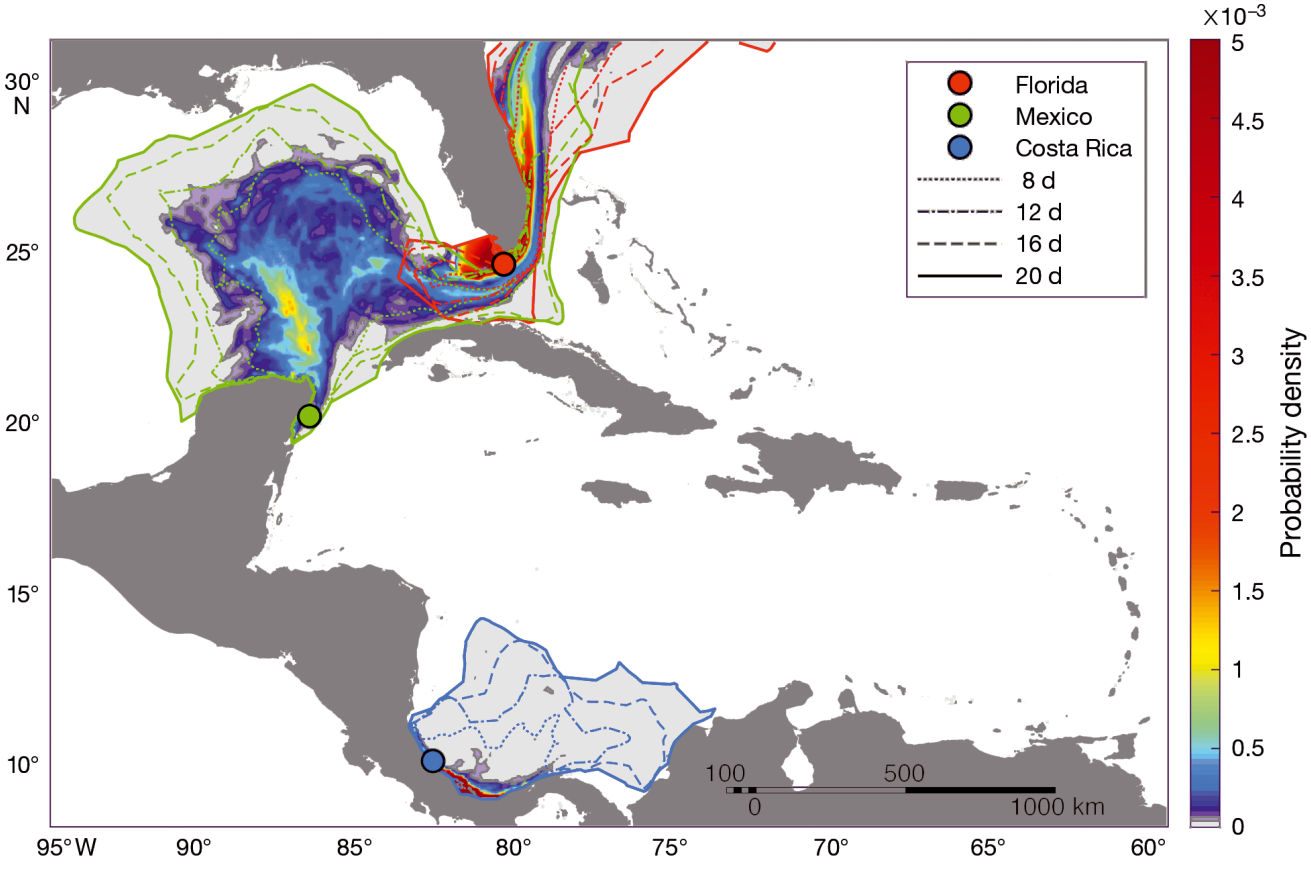

Fig. 1. Probability density surfaces for *Maguimithrax spinosissimus* virtual larvae released from the genetic sampling locations with competency periods of 6–8, 6–12, 6–16, and 6–20 d, bounded by nonconvex hulls

sampled populations. Subpopulation split modeling did predict the potential for direct connections between CR and MX sub-populations, and also with FL-K subpopulations, depending on larval duration. The biophysical model did not predict the potential for direct connectivity between sampled CR populations and populations in MX or FL-K.

Subpopulation split predictions ranged from as few as 3 to as many as 128 Caribbean subpopulations of *M. spinosissimus* (Fig. 3). Of these splits, 'best' splits have the lowest *Q* for that number of predicted subpopulations. Thus, best splits have the least 'leaky' subpopulations (Jacobi et al. 2012). Of the best splits, only those in which connectivity was predicted to be highest between subpopulations that contained the Mexican and Floridian sampled populations were retained, and for which those sampled locations fell into separate subpopulations. The most parsimonious split (with fewest predicted subpopulations) for each larval duration was retained (Fig. 4).

The subpopulation split prediction after 8 d of dispersal predicts ~38 Caribbean subpopulations, that the MX and FL-K subpopulations are connected directly only directionally (MX–FL-K), and that connectivity between CR and FL-K subpopulations must occur over multiple generations. In the CR–FL-K direction, there is a minimum of 2 steps, and connectivity is through the MX subpopulation. In the FL-K–CR direction, connectivity occurs over at least 7 multi-generational steps, through northern Cuba, the Turks and Caicos, the Bahamas, Hispaniola, and Jamaica (Fig. 2). This subpopulation split has the lowest *Q* among subpopulations (Fig. 4). At 12 d of dispersal, the subpopulation split algorithm predicts ~26 subpopulations, with high bi-directional direct and indirect connectivity between the MX and FL-K subpopulations. Notably, the least resistance path to connect the MX and FL-K subpopulations in this split occurs over multiple generations, and occurs through coral reefs in the Gulf of Mexico. CR and FL-K reefs are not directly connected, but bidirectional connectivity exists between CR and MX subpopulations, enhancing the potential for multiple generational connectivity among all 3 sampled subpopulations. Modeling 16 d of dispersal further enhances connectivity among sampled subpopula-

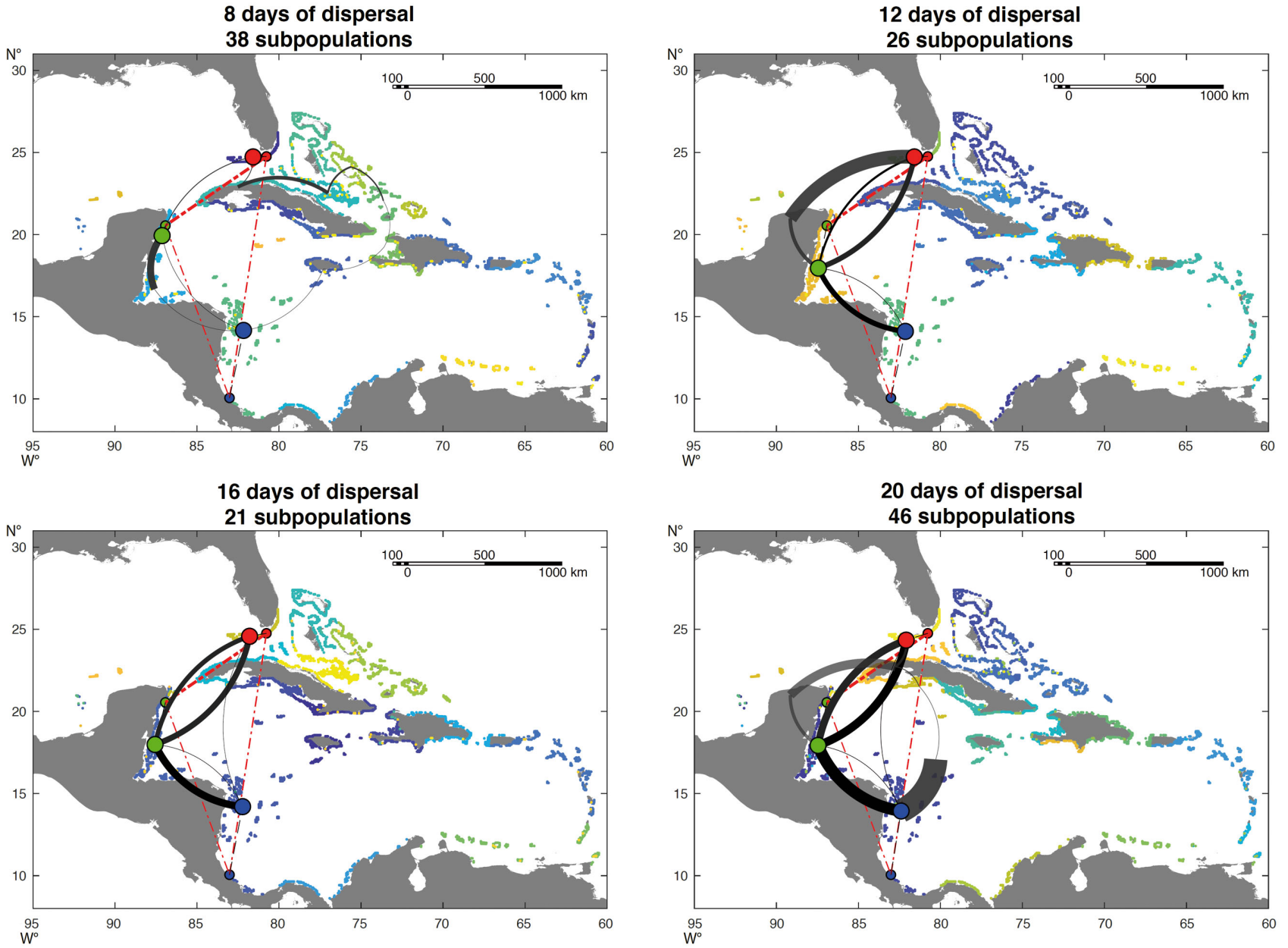

Fig. 2. Geographic representations of retained subpopulation splits based on 8, 12, 16, and 20 d of *Maguimithrax spinosissimus* larval dispersal and connectivity. Coral reef habitats are represented as points, colored according to their assigned subpopulation. Genetic sampling sites are represented by smaller colored circles; red: Florida (FL-K); green: Mexico (MX); blue: Costa Rica (CR). These sampling sites are shown connected by black dashed lines to similarly colored, but larger, circles representing the Euclidean spatial mean, or centroid, of their assigned subpopulation in each split. They are further connected to each other with red dot-dashed lines that represent the genetic results, and are weighted inversely with F_{ST}. Centroids for FL-K, MX, and CR subpopulations are shown connected by black arcs along both direct (black) and least-cost (gray) paths. These arcs are weighted by connection probability, and are directional in the clockwise direction

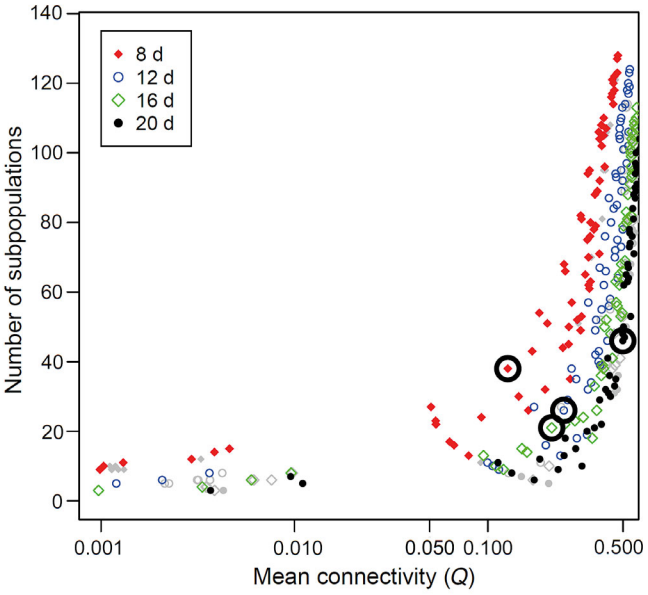

Fig. 3. Number of *Maguimithrax spinosissimus* subpopulations and subpopulation mean connectivity for predictions made using the subpopulation split algorithm (Jacobi et al. 2012) for each larval duration. Gray points are not 'best-splits' and are included for illustration. The *x*-axis is in log-scale

tions, predicts direct connectivity between CR–FL-K, and reduces the number of predicted subpopulations in the Caribbean to ~21; the lowest prediction made for this suite of simulations. By 20 d of dispersal, however, the potential for connectivity among predicted subpopulations becomes very high, and the subpopulation split algorithm predicts ~46 very leaky subpopulations, with *Q* probability over 0.50 between all Caribbean subpopulations (Fig. 4). In this split, direct and multi-generational connectivity among sampled subpopulations is high, and occurs through multiple pathways (Fig. 2). In all cases, the biophysical model predicts that connectivity is expected to be greater between Costa Rica and Mexico than between Costa Rica and Florida (Fig. 2).

In large part, increasing PLD has 2 effects on the resulting connectivity matrices (Fig. A1 in the Appendix). First, many connections are strengthened as PLD increases, which suggests that particles spend considerable time over connected patches, and the longer PLD enhances that outcome. Secondly, longer PLDs do add new long-distance connections to the connectivity matrix, but those connections tend to have low probabilities. However, the effects of increased PLD is very obvious in subpopulation splits (Figs. 2 & 4). Longer PLD 'blurs' the edges of subpopulations, making them leakier.

Some biophysical consistencies in subpopulation splits, regardless of larval duration, are important to note. The algorithm consistently predicts certain geographic subpopulations, including the Florida Keys; the north and south Bahamas; north, east, and south Cuba; Jamaica; east and west Hispaniola (roughly Haiti and the Dominican Republic); west and east Puerto Rico; the Leeward and North Windward Islands; the South Windward Islands; Venezuelan reefs; Colombian and Panamanian reefs; Costa Rican/Nicaraguan reefs; and Mexican/Belizean/Honduran reefs. The western Yucatan/Campeche may also represent separate subpopulations (Fig. 2).

3.2. Population genetic structure

The AMOVA used to test for hierarchical population structure revealed an overall mean F_{ST} value of 0.29 (Table 1). Molecular variation was greater within than among populations (71.06 and 28.94 %, respectively). However, the observed variability among populations was significant and denoted the presence of great genetic differentiation among the studied populations in the greater Caribbean ($p < 0.0001$; Table 1). Pairwise F_{ST} values were significant for all 3 comparisons of population pairs ($p < 0.0001$ in all cases) (Table 2). Pairwise F_{ST} values between CR and FL-K, and between CR and MX were almost 3 times higher than between FL and MX.

3.3. Phylogeographic distribution of alleles

Over 1570 aligned sites, a total of 33 different haplotypes were found in the 78 sampled individuals. The number of haplotypes and polymorphic sites decreased from north to south in the sampled populations (Table 3). By contrast, haplotype diversity increased from north to south. Nucleotide diversity was lower in CR compared to FL-K and MX, which exhibited similar nucleotide diversity estimates (Table 3).

The haplotype network denoted moderate to low genetic structuring considering that the 33 different haplotypes found in this study did not segregate together or form distinguishable haplotype groups according to geographical location. A single relatively high-frequency haplotype was shared among all 3 populations (Fig. 5). All populations also contained relatively large numbers of singleton haplotypes (n = 17, 8, and 7 in FL-K, MX, and CR, respectively) (Fig. 5).

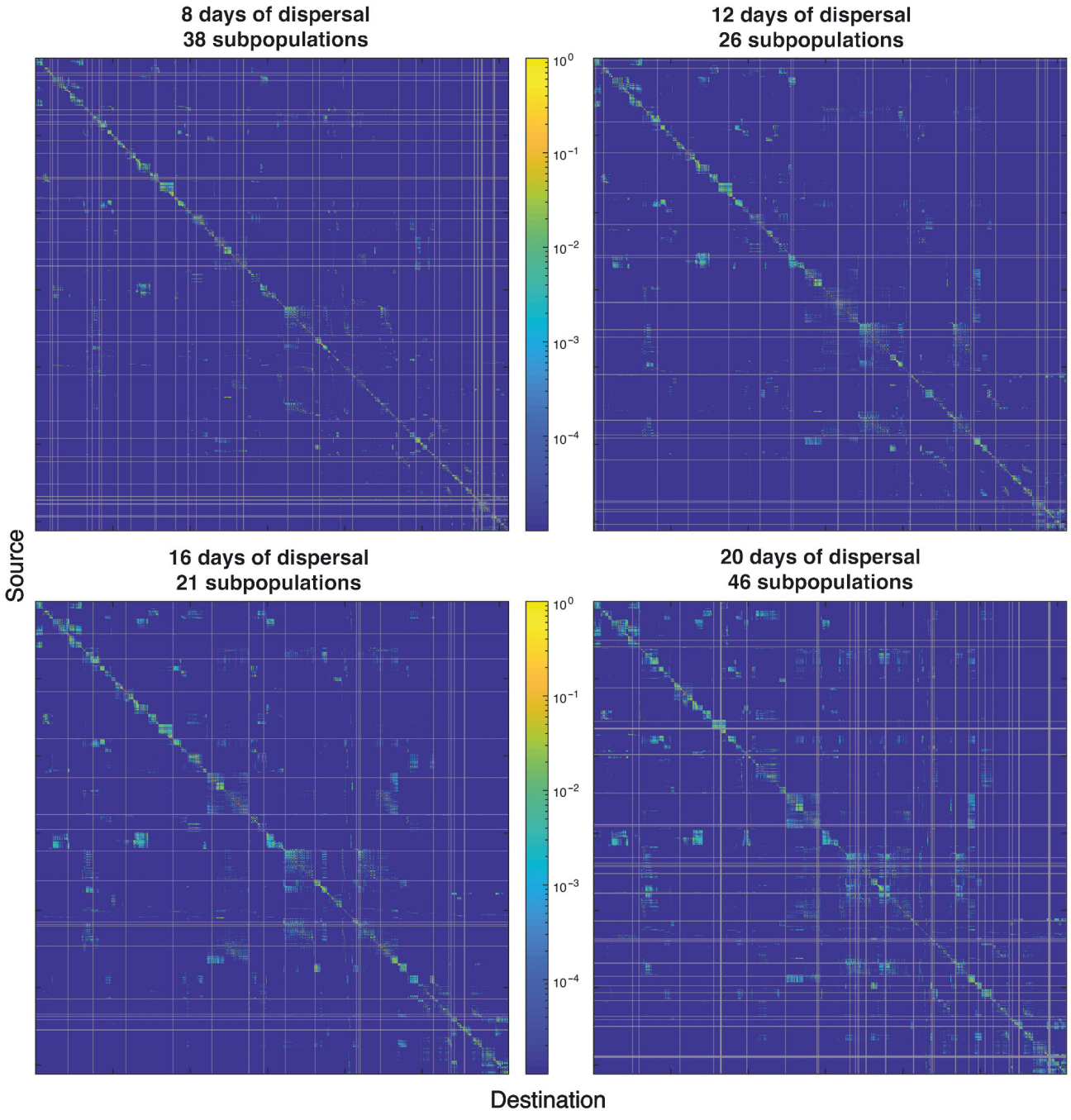

Fig. 4. Normalized connectivity matrices (C) resulting from 8, 12, 16, and 20 d of dispersal and connectivity of *Maguimithrax spinosissimus* larvae. Each matrix is rearranged by the predicted subpopulations for each dispersal scenario (see Figs. 2 & 3), and those subpopulations are separated by gray lines. These subpopulations are further arranged from west to east for some visual consistency between panels. The *y*-axes are sources, while the *x*-axes are destinations

4. DISCUSSION

Biophysical modeling predicted dissimilar connectivity patterns depending upon the assumed PLD in *Maguimithrax spinosissimus*. Eight days of dispersal was likely not sufficient to consistently connect the sampled population in Cozumel (Mexico) to that in Florida (USA), either directly or through the multigenerational use of intermediary habitat to the degree necessary to maintain genetic exchange. This limited dispersal scenario would suggest that populations from the northern (Florida Keys) and western

Table 1. AMOVA results using pairwise differences as distance method. Significance levels established with 10 000 permutations. Each location was considered as a separate group, and significance ($p < 0.001$) is indicated in **bold**

Source of variation	df	Sum of squares	Variance components	Percentage of variation	F_{ST}
Among populations	2	19.447	**0.35136**	28.94	**0.28944**
Within populations	75	64.694	0.86259	71.06	
Total	77	84.141	1.21396		

Table 2. Pairwise genetic differentiation (F_{ST}) of populations of *Maguimithrax spinosissimus* in the greater Caribbean Sea. Significance ($p < 0.001$) is indicated in **bold**

	Costa Rica	Mexico	Florida
Costa Rica	–	–	–
Mexico	**0.35847**	–	–
Florida	**0.31928**	**0.17981**	–

(Mexico) Caribbean are isolated, connected directly only very rarely. In turn, 12 d of dispersal was sufficient to directly connect the sampled patches, and connect their predicted subpopulations bidirectionally and through multiple pathways. Importantly, the biophysical model did not predict direct connectivity between sampled populations from Costa Rica to either Floridian or Mexican sampled populations, regardless of PLD; but these connections do exist between predicted subpopulations for these patches. The Costa Rican subpopulation is directly connected to the Mexican subpopulation in 8 d of dispersal—although with low probability—and it becomes connected to the Florida subpopulation after 16 d of dispersal, although with low probability as well. Thus, although the biophysical model is unable to predict direct connections between all sampled populations, the subpopulation split algorithm allows for predictions over a more encompassing spatial, and potentially genetic, scale. Overall, our modeling results indicate major to moderate semipermeable barriers to dispersal between south, west, and north (i.e. Florida) populations in species with limited dispersal capabilities (i.e. 8–12 d of PLD).

Table 3. Standard diversity measures for populations of *Maguimithrax spinosissimus* in the greater Caribbean Sea. Shown for each population is the number of haplotypes (Nh), the number of polymorphic sites (Np), haplotype diversity (Hd), and nucleotide diversity (pi). Values in Hd and pi are presented as average ± SD

Locality	N	Nh	Np	Hd	pi
Florida	31	18	14	0.13441 ± 0.13953	0.001199 ± 0.000783
Mexico	17	10	11	0.20722 ± 0.13533	0.001459 ± 0.000942
Costa Rica	30	8	5	0.25149 ± 0.16882	0.000801 ± 0.000578

Our population genetic analyses demonstrated very great genetic differentiation among south, west, and north (i.e. Florida) populations of *M. spinosissimus*. The above was supported by the AMOVA analysis and population pairwise F_{ST} values, although we observed a single relatively high-frequency haplotype shared among all 3 sampled populations in *M. spinosissimus*. Large levels of genetic differentiation among the studied populations strongly indicates that migration is limited among these populations (Avise 2000, Balloux & Lugon-Moulin 2002). Interestingly, Costa Rica is genetically more differentiated compared to northern populations (Florida and Mexico). It needs to be highlighted that the same observed genetic pattern can be explained only by incomplete lineage sorting (Hey 2010, also see below). In general, the observed genetic structure in *M. spinosissimus* is intermediate between 2 extremes previously reported for the region. These extremes range from species organized into a single and large, highly connected, metapopulation (e.g. the spiny lobster *Panulirus argus*; Naro-Maciel et al. 2011, but see Truelove et al. 2017) to others with strong genetic differentiation even among populations separated by relatively small geographic distances (e.g. the coral *Flavia fragum*; Goodbody-Gringley et al. 2010).

Importantly, the strength of connectivity between sampled populations inferred from our empirical data was much greater than that predicted by biophysical modeling of direct patch-to-patch migration. Taking this into account, we used subpopulation split algorithms to investigate how the spatial extent of subpopulations and multi-generational connectivity may account for differences between observed and predicted connectivities. We argue that our connectivity modeling and subpopulation split predictions provide a framework for understanding how migration may occur between potential subpopulations arranged discretely in space. The subpopulation split predictions suggest a suite of potential connectivity and seascape genetic scenarios resulting in distinct, yet moderately connected Florida, Mexico, and

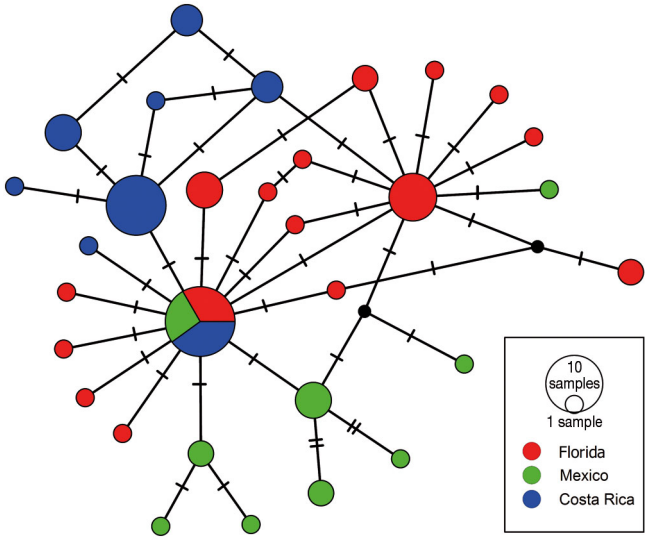

Fig. 5. Minimum parsimony haplotype network for the mitochondrial sequences of *Maguimithrax spinosissimus* in the greater Caribbean Sea. Each line separating 2 circles indicates a single substitution. Area of each circle corresponds to the number of haplotypes it represents; color represents the location where the haplotype was found

Costa Rica subpopulations. The subpopulation split predictions should be empirically tested with expanded population genetics, which would allow for iterative improvement of our understanding of *M. spinosissimus* Caribbean population connectivity. The discordance between empirical and modeling data might be explained by the following reasons, among others: (1) shortcomings in the biophysical model's parameterization, (2) shortcomings in the population genetic analyses, and/or (3) relatively high multigenerational migration potential through populations in the central Caribbean, effectively connecting Florida populations (bidirectional) to Costa Rica populations at a rate higher than would initially be expected through simple biophysical distance and major ocean currents. A final potential explanation is that Mexican and Floridian populations are moderately mixed, and should be considered one subpopulation. That the genetic distance between Costa Rica and Florida was roughly similar to the genetic distance between Costa Rica and Mexico supports the notion above.

We recognize that the biophysical model operated with several important assumptions that might limit its realism. For instance, larvae were released monthly to saturate potential connections among populations. However, *M. spinosissimus* may have restricted or heterogeneous breeding seasons, especially in peripheral areas in the Caribbean (e.g. as in *Lobatus gigas*; Boman et al. 2018), which could affect connectivity predictions in the biophysical model. Furthermore, because little is currently known about *M. spinosissimus* larval traits, such as swimming behaviors or diel migrations, particles (virtual larvae) were modeled as nearly passive (other than their settlement behaviors). Vertical swimming behaviors in reef larvae are known (i.e. in decapod crustaceans; see Cronin & Forward 1986 and references therein) to promote local retention (Paris et al. 2007). Thus, our model likely under-predicts local retention. Furthermore, little is known about the maximum *in situ* PLD for *M. spinosissimus* larvae and their ability to delay metamorphosis and settlement, as shown in other marine invertebrates, including crustaceans (Gebauer et al. 2003). Our genetic and modeling results indicate that *M. spinosissimus* may be able to remain in the dispersing plankton for a greater time than is indicated by larval rearing studies (Provenzano & Brownell 1977). The potential for larval mortality was also ignored in the current study, in order to saturate potential connections. However, larval mortality is known to be very high for most species, and has the potential to reduce connectivity (Cowen et al. 2006). Clearly, the reproductive and larval traits of this species have important implications for improving modeling inferences and effectively testing this species' metapopulation dynamics in the Caribbean.

With regards to limitations in our population genetic analyses, we are aware that the analyses we employed (e.g. AMOVA, F_{ST}) assume that mutation, drift, and migration are in an evolutionary equilibrium (Hey 2010). The above implies that these 'classical' analyses are not capable of distinguishing among different scenarios in explaining our results (Hey 2010). Indeed, 3 scenarios might explain populations sharing haplotypes but, at the same time, having statistically significant genetic distinctiveness in *M. spinosissimus*: (1) contemporary gene flow solely, (2) population divergence (with incomplete lineage sorting) from an ancestral polymorphic population solely, or (3) a combination of gene flow and divergence with incomplete lineage sorting. To distinguish among these different scenarios, coalescent-based methods and Markov chain Monte Carlo (MCMC) simulations of gene(s) genealogies can be used in the future to estimate the posterior density of parameters such as time of divergence (t; mutation scaled time since divergence), effective population sizes, and migration rates (m; mutation scaled migration rate) among the studied populations. These parameters are considered part of an 'isolation with migration' (IM) model (Hey 2010) which, in contrast to other

classical methods (e.g. AMOVA, F_{ST}), does not assume that mutation, drift, and migration are in an evolutionary equilibrium (Hey 2010). Furthermore, future studies might include adult behavior (i.e. mobility) and additional abiotic effects (i.e. seasonal changes in temperature) on larval performance to continue understanding connectivity in *M. spinosissimus*. Importantly, adults are not expected to have high migratory potential (Hazlett & Rittschof 1975, J. A. Baeza pers. obs.). In turn, warmer temperatures likely result in shorter larval durations due to more rapid development, but also in location-specific changes to hydrography (Green & Fisher 2004, O'Connor et al. 2007).

Despite the limiting assumptions of our study, the complementary insights from initial biophysical metapopulation modeling and population genetics provides an integrated partial test of metapopulation connectivity in *M. spinosissimus*. Extending this study by using more 'powerful' genetic markers (i.e. single nucleotide polymorphisms, SNPs; Benestan et al. 2015), expanding sampling localities, and integrating more specific biological traits will allow for both testing the current model and developing more refined metapopulation models for this species. Further, we have identified a potential oceanic barrier to dispersal between Mexico and Florida for organisms with limited dispersal capabilities, and identified potential multi-generational pathways through the central Caribbean that may mediate isolation by distance—and isolation associated with the Panama–Colombia Gyre—for short-distance dispersers. We argue in favor of additional studies combining modeling approaches and empirical results, as demonstrated here, to improve our understanding of connectivity in species with wide geographic distributions in the Caribbean Sea, that is known to experience major environmental problems and is in need of conservation planning.

Acknowledgements. We thank 2 anonymous reviewers for their comments that improved previous versions of this manuscript.

LITERATURE CITED

Arnaud-Haond S, Vonau V, Rouxel C, Bonhomme F, Prou J, Goyard E, Boudry P (2008) Genetic structure at different spatial scales in the pearl oyster (*Pinctada margaritifera cumingii*) in French Polynesian lagoons: beware of sampling strategy and genetic patchiness. Mar Biol 155: 147–157

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA

Baeza JA, Fuentes MS (2013) Exploring phylogenetic informativeness and nuclear copies of mitochondrial DNA (numts) in three commonly used mitochondrial genes: mitochondrial phylogeny of peppermint, cleaner and semi-terrestrial shrimps (Caridea: *Lysmata*, *Exhippolysmata*, *Merguia*). Zool J Linn Soc 168:699–722

Baeza JA, Anderson JR, Spadero J, Behringer CD (2012) Sexual dimorphism, allometry and size at first maturity of the Caribbean king crab, *Mithrax spinosissimus*, in the Florida Keys, USA. J Shellfish Res 31:909–916

Baeza JA, Simpson L, Ambrosio LJ, Guéron R, Mora N, Owen D (2015) Reproductive investment in a phyletic giant, the Caribbean king crab *Damithrax spinosissimus*: exploring egg production costs in large brooding marine invertebrates. J Shellfish Res 34:1049–1056

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11:155–165

Baums IB (2008) A restoration genetics guide for coral reef conservation. Mol Ecol 17:2796–2811

Baums IB, Miller MW, Hellberg ME (2005) Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. Mol Ecol 14:1377–1390

Baums IB, Paris CB, Chérubin LM (2006) A bio oceanographic filter to larval dispersal in a reef building coral. Limnol Oceanogr 51:1969–1981

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L (2015) RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). Mol Ecol 24:3299–3315

Boman EM, de Graaf M, Nagelkerke LAJ, Stoner AW and others (2018) Variability in size at maturity and reproductive season of queen conch *Lobatus gigas* (Gastropoda: Strombidae) in the wider Caribbean region. Fish Res 201:18–25

Carpenter KE, Barber PH, Crandall ED, Ablan-Lagman M and others (2011) Comparative phylogeography of the coral triangle and implications for marine management. J Mar Biol 2011:396982

Chollett I, Collin R, Bastidas C, Cróquer A and others (2017) Widespread local chronic stressors in Caribbean coastal habitats. PLOS ONE 12:e0188564

Collin R (2001) The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). Mol Ecol 10: 2249–2262

Costagliola D, Robertson DR, Guidetti P, Stefanni S, Wirtz P, Heiser JB, Bernardi G (2004) Evolution of coral reef fish *Thalassoma* spp. (Labridae). 2. Evolution of the eastern Atlantic species. Mar Biol 144:377–383

Cowen RK, Kamazima MML, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: Open or closed? Science 287:857–859

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522–527

Crandall KA (1994) Intraspecific cladogram estimation: accuracy at higher levels of divergence. Syst Biol 43: 222–235

Cronin TW, Forward RB (1986) Vertical migration cycles of crab larvae and their role in larval dispersal. Bull Mar Sci 39:192–201

Duran S, Rützler K (2006) Ecological speciation in a Caribbean marine sponge. Mol Phylogenet Evol 40:292–297

Edgar RC (2004) MUSCLE: multiple sequence alignment

with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Eytan RI, Hellberg ME (2010) Nuclear and mitochondrial sequence data reveal and conceal different demographic histories and population genetic processes in Caribbean reef fishes. Evolution 64:3380–3397

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Gebauer P, Paschke K, Anger K (2003) Delayed metamorphosis in decapod crustaceans: evidence and consequences. Rev Chil Hist Nat 76:169–175

Goerlitz DS, Urbán J, Rojas-Bracho L, Belson M, Schaeff CM (2003) Mitochondrial DNA variation among eastern North Pacific gray whales (*Eschrichtius robustus*) on winter breeding grounds in Baja California. Can J Zool 81:1965–1972

Goodbody-Gringley G, Vollmer SV, Woollacott RM, Giribet G (2010) Limited gene flow in the brooding coral *Favia fragum* (Esper, 1797). Mar Bio 157:2591–2602

Green BS, Fisher R (2004) Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. J Exp Mar Biol Ecol 299:115–132

Hazlett B, Rittschof D (1975) Daily movements and home range in *Mithrax spinosissimus* (Majidae, Decapoda). Mar Behav Physiol 3:101–118

Hellberg ME (2009) Gene flow and isolation among populations of marine animals. Annu Rev Ecol Evol Syst 40: 291–310

Hey J (2010) Isolation with migration models for more than two populations. Mol Biol Evol 27:905–920

Holstein DM, Paris CB, Mumby PJ (2014) Consistency and inconsistency in multispecies population network dynamics of coral reef ecosystems. Mar Ecol Prog Ser 499:1–18

Hurtado-Alarcón JC, Campos NH, Bermúdez A, Márquez JE (2018) Phylogeographic patterns in *Maguimithrax spinosissimus* (Decapoda: Mithracidae) from Colombian Caribbean. N Z J Mar Freshw Res 52:118–137

Jacobi MN, André C, Döös K, Jonsson PR (2012) Identification of subpopulations from connectivity matrices. Ecography 35:1004–1016

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86: 6196–6200

Kough AS, Paris CB, Butler MJ IV (2013) Larval connectivity and the international management of fisheries. PLOS ONE 8:e64970

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Magoulas A, Castilho R, Caetano S, Marcato S, Patarnello T (2006) Mitochondrial DNA reveals a mosaic pattern of phylogeographical structure in Atlantic and Mediterranean populations of anchovy (*Engraulis encrasicolus*). Mol Phylogenet Evol 39:734–746

Marko PB, Hart MW (2011) The complex analytical landscape of gene flow inference. Trends Ecol Evol 26: 448–456

Matsuzaki Y, Fujita I (2017) In situ estimates of horizontal turbulent diffusivity at the sea surface for oil transport simulation. Mar Pollut Bull 117:34–40

McMillen-Jackson AL, Bert TM (2004) Mitochondrial DNA variation and population genetic structure of the blue crab *Callinectes sapidus* in the eastern United States. Mar Biol 145:769–777

Modica MV, Russini V, Fassio G, Oliverio M (2017) Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development. Mar Environ Res 127:92–101

Mokady O, Rozenblatt S, Graur D, Loya Y (1994) Coral-host specificity of Red Sea *Lithophaga* bivalves: interspecific and intraspecific variation in 12S mitochondrial ribosomal RNA. Mol Mar Biol Biotechnol 3:158–164

Moulton MJ, Song H, Whiting WF (2010) Assessing the effects of primer specificity on eliminating numt coamplification in DNA barcoding: a case study from Orthoptera (Arthropoda: Insecta). Mol Ecol Resour 10: 615–627

Naro-Maciel E, Reid B, Holmes KE, Brumbaugh DR, Martin M, DeSalle R (2011) Mitochondrial DNA sequence variation in spiny lobsters: population expansion, panmixia, and divergence. Mar Biol 158:2027–2041

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York, NY

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. Proc Natl Acad Sci USA 104: 1266–1271

Palumbi SR (2004) Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management. Annu Rev Environ Resour 29:31–68

Paris CB, Cherubin LM, Cowen RK (2007) Surfing, spinning, or diving from reef to reef: effects on population connectivity. Mar Ecol Prog Ser 347:285–300

Paris CB, Helgers J, Van Sebille E (2013) Connectivity modeling system: a probabilistic modeling tool for the multiscale tracking of biotic and abiotic variability in the ocean. Environ Model Softw 42:47–54

Pelc RA, Warner RR, Gaines SD, Paris CB (2010) Detecting larval export from marine reserves. Proc Natl Acad Sci USA 107:18266–18271

Piñeros VJ, Gutiérrez-Rodríguez C (2017) Population genetic structure and connectivity in the widespread coral-reef fish *Abudefduf saxatilis*: the role of historic and contemporary factors. Coral Reefs 36:877–890

Planes S, Fauvelot C (2002) Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. Evolution 56:378–399

Provenzano AJ, Brownell WN (1977) Larval and early postlarval stages of the West Indian spider crab *Mithrax spinosissimus* (Lamark) (Decapoda: Majidae). Proc Biol Soc Wash 90:735–752

Rathbun MJ (1925) The spider crabs of America. Bull US Nat Mus 129:1–613

Riginos C, Nachman MW (2001) Population subdivision in marine environments: the contributions of isolation by distance, discontinuous habitat, and biogeography to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus.* Mol Ecol 10:1439–1453

Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). Mol Ecol 11:243–252

Rolán-Alvarez E, Zapata C, Alvarez G (1995) Distinct genetic subdivision in sympatric and sibling species of the genus *Littorina* (Gastropoda: Littorinidae). Heredity 74:1–9

Salas E, Molina-Ureña H, Walter RP, Heath DD (2010) Local and regional genetic connectivity in a Caribbean coral reef fish. Mar Biol 157:437–445

Schubart CD, Neigel JE, Felder DL (2000) Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. Crustac Issues 12:817–830

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279

Snyder RE, Paris CB, Vaz AC (2014) How much do marine connectivity fluctuations matter? Am Nat 184:523–530

Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS (2006) Comparative phylogeography of unglaciated eastern North America. Mol Ecol 15:4261–4293

Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proc Natl Acad Sci USA 105:13486–13491

Sotka EE, Wares JP, Barth JA, Grosberg RK, Palumbi SR (2004) Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula.* Mol Ecol 13:2143–2156

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–109

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619–633

Teske PR, Papadopoulos I, Zardi GI, McQuaid CD, Griffiths CL, Edkins MT, Barker NP (2007) Implications of life history for genetic structure and migration rates of five southern African coastal invertebrates: planktonic, abbreviated and direct development. Mar Biol 152:697–711

Truelove NK, Kough AS, Behringer DC, Paris CB, Box SJ, Preziosi RF, Butler MJ (2017) Biophysical connectivity explains population genetic structure in a highly dispersive marine species. Coral Reefs 36:233–244

Wagner HP (1990) The genera *Mithrax* Latreille, 1818 and *Mithraculus* White, 1847 (Crustacea: Brachyura: Majidae) in the western Atlantic Ocean. Zool Verh 264:1–65

Watson JR, Siegel DA, Kendall BE, Mitarai S, Rassweiller A, Gaines SD (2011) Identifying critical regions in small-world marine metapopulations. Proc Natl Acad Sci USA 108:E907–E913

Weersing K, Toonen RT (2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1–12

White C, Selkoe KA, Watson J, Siegel DA, Zacher DC, Toonen RJ (2010) Ocean currents help explain population genetic structure. Proc R Soc B 277:1685–1694

Williams AB (1984) Shrimps, lobsters and crabs of the Atlantic coast of the eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, DC

Wise J, Harasewych MG, Dillon RT (2004) Population divergence in the sinistral whelks of North America, with special reference to the east Florida ecotone. Mar Biol 145: 1167–1179

Zardoya R, Castilho R, Grande C, Favre-Krey L and others (2004) Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. Mol Ecol 13:1785–1798

Zhang DX, Hewitt GM (1996) Nuclear integrations: challenges for mitochondrial DNA markers. Trends Ecol Evol 11:247–251

Appendix

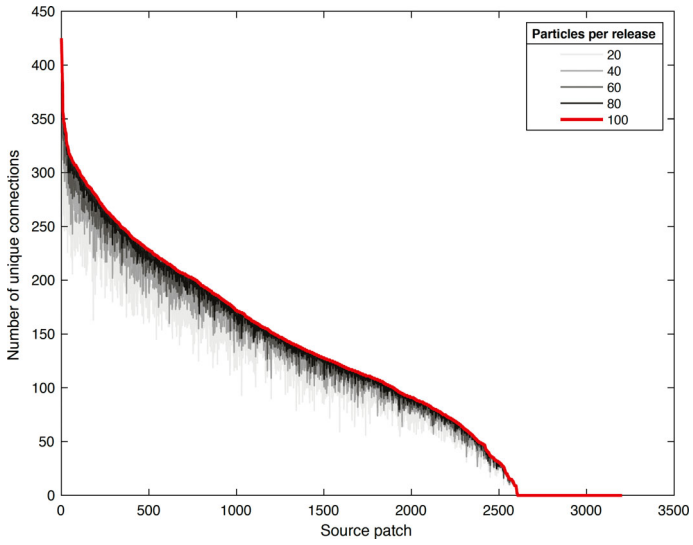

Fig. A1. Number of unique connections for each source patch of *Maguimithrax spinosissimus* using 100 particles release^{-1} (current model), as well as 80, 60, 40, and 20 particles release^{-1}. The median increase in connections when 100 particles are released per release versus 80 is 1

Editorial responsibility: Karen Miller, Hobart, Tasmania, Australia

Submitted: February 26, 2018; Accepted: December 5, 2018
Proofs received from author(s): January 29, 2019