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Genetic structure among spawning aggregations of the gulf coney *Hyporthodus acanthistius*

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ABSTRACT: Many large groupers form spawning aggregations, returning to the same spawning sites in consecutive spawning seasons. Connectivity between spawning aggregations is thus assured by larval dispersal. This study looks into the genetic structure and gene flow among spawning aggregations of a large grouper, the gulf coney *Hyporthodus acanthistius*, in the northern Gulf of California. First, using the mitochondrial control region and 11 microsatellites, we calculated F_{ST} metrics and conducted a Bayesian clustering analysis to determine structure among 5 spawning aggregations. Shallow genetic structure was found, separating the southernmost spawning aggregate from the remainder. Second, we used the results from the structure analysis and local water circulation patterns to delineate 3 distinct models of gene flow. The best-supported model, in which the southernmost spawning aggregate formed one group and all other spawning aggregates were nested into a second group, was the one that was consistent with water circulation during the species' spawning season. Larval retention within a seasonal anticyclonic gyre that formed during the gulf coney's spawning season may be responsible for the patterns found. This study highlights the importance of local oceanographic conditions in dictating the structure among spawning aggregations even at small geographic scales and contributes to informed management plans for this overexploited grouper.

KEY WORDS: Grouper · Dispersal · Connectivity · Sea of Cortez · Oceanography · Eddies · Retention · Migration models · Rooster hind · *Epinephelus*

INTRODUCTION

Knowledge of how genetic variation is partitioned in the ocean is fundamental for understanding the ecology, conservation and management of marine resources (Mora & Sale 2002, Gell & Roberts 2003, Cowen et al. 2007, Francis et al. 2007, Planes et al. 2009). One of the strongest drivers of genetic structure is connectivity, i.e. the demographic linking of local populations via the dispersal of larvae, juveniles or adults (Sale et al. 2005), which influences almost all ecological and evolutionary processes in metapopulations (Hanski & Gaggiotti 2004). Genetic connectivity has been shown across a range of geographical scales among different marine taxa, ranging from virtually panmictic throughout considerably large geographic ranges (Bowen et al. 2001, Lessios et al. 2003, Klanten et al. 2007, Beldade et al. 2009, Leray

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et al. 2010) to clearly structured populations at very small scales (Sotka et al. 2004, Bernardi 2005, Barber et al. 2006, Gerlach et al. 2007, Beldade et al. 2012).

Many fish form spawning aggregations (i.e. groups of conspecific fish that gather for the purpose of spawning, with densities or numbers significantly higher than those found in the area of aggregation during non-reproductive periods; Domeier & Colin 1997), including groupers, snappers, jacks, surgeonfishes, damselfishes and parrotfishes (Sala et al. 2003, Erisman et al. 2007, Gladstone 2007, Sadovy de Mitcheson et al. 2008, Gerhardinger et al. 2009). Some groupers return to the same spawning sites in consecutive spawning seasons (Sala et al. 2001, Starr et al. 2007), in some cases covering large distances to do so (Bolden 2000). If adult spawning aggregation site fidelity is indeed ubiquitous among large groupers, then the dispersal of the pelagic larval stages that are subjected to transport by ocean currents should be the main driver of genetic connectivity. Two elements underline the importance of oceanographic characteristics to the dispersal of spawning aggregation offspring. First, the specific location of spawning aggregations appears to maximize the rapid advection of eggs and larvae away from the reef environment (e.g. Choat 2012, Colin 2012a). Second, knowledge of the onset of sensorial and swimming abilities of pelagic larvae, which in the case of groupers is still largely unknown, is essential to understand how larval abilities might steer the dispersal process (e.g. Colin 2012b, Hamner & Largier 2012). Larval abundance and even the magnitude of recruitment events appear to be correlated with oceanographic and climatic parameters, such as temperature, salinity and depth (but see e.g. Aburto-Oropeza et al. 2010, Marancik et al. 2012).

The northern Gulf of California (NGC) is home to several fishes that aggregate to spawn and is part of one of the most productive marine ecosystems in the world, contributing most of Mexico's fishery resources (Arvizu-Martínez 1987, Lluch-Cota et al. 2007, Erisman et al. 2012). The NGC covers a relatively small area extending from the Colorado delta in the north to Bahia de Los Angeles and Isla Tiburon in the south (Fig. 1). In this region, in-depth knowledge of water circulation patterns and other geomorphological characteristics (Fig. 1) provide a unique opportunity to describe genetic structure and test models of gene flow in locally occurring species. In the NGC, the main oceanographic features comprise intense tidal mixing (Argote et al. 1995) and a seasonally reversing gyre, anticyclonic in summer (June to September) (Fig. 1B) and cyclonic in winter (Fig. 1C) (Lavín et al. 1997, Marinone et al. 2008); strong coastal currents along the eastern Sonora coastline (Peguero-Icaza et al. 2011); and small residual currents and small eddies in the upper gulf (Marinone et al. 2011). These characteristics are likely to influence the transport of larvae in the NGC (Marinone et al. 2004, Cudney-Bueno et al. 2009). Both local water circulation and bottom geomorphologic characteristics may have important implications for the formation of spawning aggregations as well as for the fate of eggs or larvae released there (Cherubin et al. 2011, Karnauskas et al. 2011). In the NGC, there are 2 deep basins, the Delfin Basin (800 m) and the Wagner Basin (200 m), and several sills (Lavín et al. 1997) whose putative part in limiting dispersal of larvae or concentrating prey for early larval stages remains unclear (e.g. Karnauskas et al. 2011).

The gulf coney *Hyporthodus acanthistius* (formerly *Epinephelus acanthistius*; Craig & Hastings 2007) is a

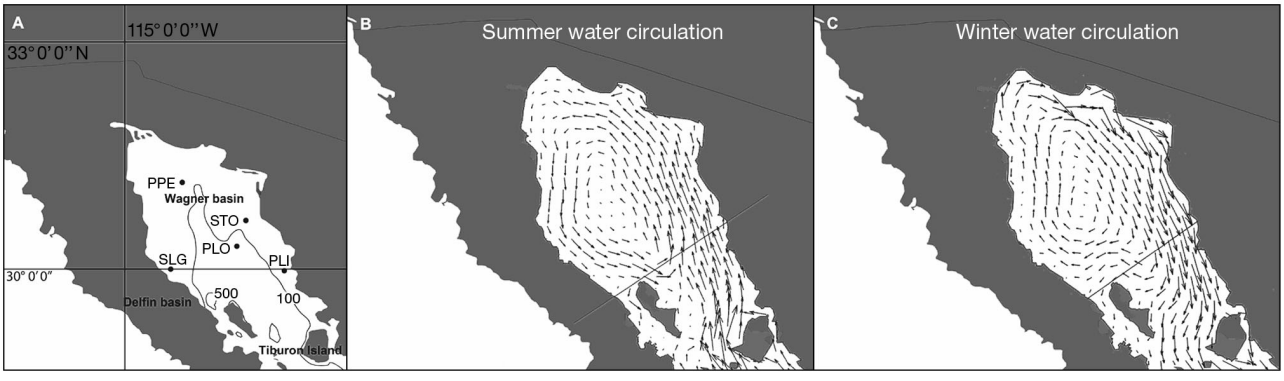

Fig. 1. Northern Gulf of California (NGC) including (A) bathymetry (depth in meters) and named sampled spawning aggregations of the gulf coney *Hyporthodus acanthistius* (PLI, Puerto Libertad; PLO, Puerto Lobos; STO, Santo Tomas; PPE, Puerto Peñasco; and SLG, San Luiz Gonzaga); (B) ocean circulation in the summer (only the month of July is represented); and (C) in the winter (only the month of January is represented). Ocean circulation reproduced from Marinone (2003) by permission of the American Geophysical Union

tropical and subtropical large grouper that occurs from southern California to Peru (Heemstra & Randall 1993), including the Gulf of California (or Sea of Cortez) (Cudney-Bueno & Turk-Boyer 1998, Aburto-Oropeza et al. 2008). It is found at depths greater than ~45 m usually in silty areas adjacent to rocky reefs (Thomson et al. 2000), and spawns in aggregations on muddy bottoms during the spring and summer months (Cudney-Bueno & Turk-Boyer 1998). During the spawning period, artisanal fishermen heavily target this species (Aburto-Oropeza et al. 2008). Indeed, the high commercial value and temporal and spatial predictability of their mass gatherings make groupers a prime target for fisheries. Despite its present 'Least Concern' conservation status (IUCN 2012), the abundance of the gulf coney in the NGC has been rapidly declining over the past 2 decades (Aburto-Oropeza et al. 2008). Elsewhere, there are many examples of collapsed grouper spawning aggregations because of overfishing such as the Nassau grouper *E. striatus* (e.g. Sala et al. 2001, Aguilar-Perera 2006) and the gulf grouper *Mycteroperca jordani* (Sáenz-Arroyo et al. 2005). Given the threat of overfishing to fish that form spawning aggregations (Sadovy de Mitcheson et al. 2013), it is imperative to provide connectivity data to devise informed management plans.

In this study, we integrate molecular evidence from highly variable molecular markers (control region and 11 microsatellites) to assess genetic structure and connectivity among spawning aggregations of the gulf coney in the NGC. Oceanographic and geomorphological regional characteristics are used to delineate particular models of gene flow across the spawning aggregation network. This study provides essential information for the management and recovery of this threatened fishery in the NGC.

MATERIALS AND METHODS

Sampling and DNA extraction

Fin clips of *Hyporthodus acanthistius* were collected in 2003 aboard fishing boats that operated at 5 spawning locations in the NGC: Puerto Libertad, Puerto Lobos, Santo Tomás, Puerto Peñasco, and San Luiz Gonzaga (Fig. 1). Immediately after collection, fin clips were placed in 95% ethanol and stored at ambient temperature in the field and then at 4°C in the lab. Total genomic DNA was extracted from 20 mg of fin tissue by Proteinase K digestion in lysis buffer (10 mM Tris, 400 mM NaCl, 2 mM EDTA, 1% sodium dodecyl sulfate) overnight at 55°C. This was followed by purification using phenol/chloroform extractions and alcohol precipitation (Sambrook et al. 1989).

mtDNA and microsatellites

We amplified the 5′ end of the hyper-variable portion of the mitochondrial control region using the universal primers CR-A and CR-E (Lee et al. 1995). Each 100 µl reaction contained 10 to 100 ng of DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 units of *Taq* DNA polymerase (Perkin-Elmer), 150 mM of each dNTP, and 0.3 mM of each primer and was amplified with a cycling profile of 45 s at 94°C, 1 min at 52°C and 1 min at 72°C for 35 cycles. After purification of amplified DNA genes following the manufacturer's protocol (ABI, Perkin-Elmer), we sequenced on an ABI 3100 automated sequencer (Applied Biosystems).

All individuals were genotyped for 13 microsatellites following protocols described in Molecular Ecology Resources Primer Development Consortium et al. (2009). Each individual was genotyped using GENEMAPPER 3.7 (Applied Biosystems). To estimate potential genotyping errors, we re-amplified and rescored 21 randomly picked samples and evaluated concordance between the first and second score. Overall, the genotyping error rate was less than 2%, which is reasonable for population differentiation studies based on allele frequencies (Bonin et al. 2004), and less than 2% of data were missing for any given locus. Data were scanned for null alleles and stuttering using MICROCHECKER 2.2.3 (van Oosterhout et al. 2004) and for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium after 10 000 permutations using ARLEQUIN 3.5 (Excoffier & Lischer 2010). Two microsatellites, EAC_A08 and EAC_B08, were dropped from the analysis because of the putative presence of null alleles.

Genetic diversity and genetic structure among spawning aggregations

Genetic diversity measures for each population including number of haplotypes, haplotype diversity and nucleotide diversity were calculated with DNAsp 5 (Librado & Rozas 2009). To assess population structure, we used 2 separate approaches. In the first, more classical approach, fixation indices (F_{ST}) relying on allele frequencies were calculated using ARLEQUIN 3.5 (Excoffier & Lischer 2010). We calcu-

lated 95% confidence intervals around the F_{ST} estimates using GDA 1.1 (Lewis & Zaykin 2001), rather than just p-values, as these may not be good indicators of differentiation between populations and are dependent on sample size and variability (Jost 2008). In the second approach, we used a Bayesian model-based clustering method using microsatellite data implemented in STRUCTURE 2.3.2 (Falush et al. 2007). STRUCTURE assumes that there are *k* number of groups within which samples have compatible multilocus genotypes. Convergence of parameters (α, *F* and likelihood) in preliminary runs was used to determine the burn-in (500 000) and length (1 000 000) of each run. For our analysis, we used an admixture model, which allows individuals to have mixed ancestry, and added sampling location as a weak prior (Falush et al. 2007). Then we performed 10 replicate runs for each cluster *k* varying between 1 and 6. To determine the correct number of clusters in the sample, we followed Evanno et al. (2005), who proposed the use of an ad hoc statistic Δ*k* based on the rate of change in the log probability of data between successive *k* values. STRUCTURE HARVESTER was used to calculate Evanno's Δ*k* and illustrate the differences in likelihood and Δ*k* for each *k* (Earl & VonHoldt 2012). A Q plot was chosen to illustrate differences between populations, where each single vertical line (representing 1 individual) is partitioned into *k*-colored segments that represent that individual's estimated membership fraction in each of the *k*-inferred clusters (Pritchard et al. 2000).

Direction and magnitude of gene flow among spawning aggregations

To determine the pervasive migration pattern in the study area, we used MIGRATE-N 3.2.16 (Beerli & Palczewski 2010) to contrast 3 migration models. In all 3 models, spawning aggregates were nested according to the genetic structure suggested by the F_{ST} and structure analysis. Based on well-described local oceanographic circulation, we defined the direction of gene flow for each model as follows: (1) an unrestricted full migration model, (2) a model considering 2 population sizes and unidirectional northward gene flow and (3) a model considering 2 population sizes and unidirectional southward gene flow. Model 2 was delineated taking into account that *Hyporthodus acanthistius* spawns in the spring-summer, during which time anticyclonic circulation forces the water to flow northward on the eastern side of the NGC (see Fig. 1B) (Marinone 2012). Model 3 depicts the autumn-winter cyclonic water circulation pattern, which forces the water to flow southward (see Fig. 1C) (Marinone 2012).

MIGRATE-N provides the ratio of the marginal likelihoods (Bayes factors) of each model, which can subsequently be compared to select the most supported model (Beerli & Palczewski 2010). The best-supported model will have the highest log Bayes factors. This approach is particularly suited to our data because local hydrodynamics allow for a clear expectation of unidirectional gene flow in the study area and the nesting of aggregates reduces the number of parameters to be estimated from the data, thus increasing the power of the approach. A random subset of 30 samples from each of the 2 populations identified previously was used to compare the models. The mitochondrial locus was not used to test the models of gene flow because of its limited capability, as it is a single locus, for distinguishing the models.

A series of preliminary runs using Model 1, the unrestricted model, were used to determine convergence of posterior probabilities for each of the parameters. Running conditions chosen included 1 000 000 recorded steps, 10 long chains and 15 heated chains, a static heating scheme with the inverse of the temperature regularly spaced between 0 and 1 and a tree swapping interval of one; finally, the upper prior boundary for northward migration was set to vary between 0 and 10 000. The natural logarithm of Bayes factors with a Bezier approximation was calculated following Beerli & Palczewski (2010) as well as each model's probability by dividing each marginal likelihood by the sum of the marginal likelihoods of both models used. The best-supported model will be the one with the highest probability (Beerli & Palczewski 2010).

RESULTS

Genetic diversity

We obtained 232 sequences for a 361 bp fragment of the mitochondrial control region (Genbank KF425014 to KF425245). The sequences analyzed here had 133 polymorphic sites, 54 of which were informative. Genetic diversity was high for almost all spawning aggregations (Table 1). Genetic diversity was also calculated for the microsatellites from 246 individuals and included number of alleles, ratio of homozygotes to heterozygotes per locus, as well as HWE tests (Table S1 in the Supplement, available at www.int-res.com/articles/suppl/m499p193_supp.pdf).

Table 1. Collection sites, number of mitochondrial control region sequences used (n) and molecular diversity indices (number of haplotypes, n*h*; haplotype diversity, *h*d; and nucleotide diversity, π) for *Hyporthodus acanthistius*

Sampling site	n	n*h*	*h*d	π
Puerto Libertad	53	18	0.839	0.0061
Puerto Lobos	62	27	0.911	0.0072
Santo Tomás	21	10	0.914	0.0068
Puerto Peñasco	55	17	0.902	0.0061
San Luiz Gonzaga	41	16	0.871	0.0065
Total	232	47	0.886	0.0066

Genetic structure of spawning aggregations

Low pairwise F_{ST} values were found across both genetic markers (Table 2). In spite of the significant differences found between pairs of F_{ST} estimates derived from microsatellites, 95% confidence intervals precluded any conclusion regarding the structure between Puerto Libertad and either Santo Tomás or Puerto Peñasco population pairs (Table 2). Differentiation between the southernmost aggregation of Puerto Libertad and the remaining aggregations was identified through the Bayesian clustering method (Fig. 2). Evanno's Δ*k* based on the mean and standard deviation of likelihoods, L(*k*), for each *k* was highest for *k* = 2 (Fig. 3), confirming that *k* = 2 is the best representation of the genetic partitioning in the data. Assessment of convergence examples of skyline plots of log(α) are given in the Supplement (Fig. S1).

Gene flow between spawning aggregations

Model 2 was the most supported migration model, as demonstrated by the highest value for the natural logarithm of the Bayes' factors (Table 3). Estimates of population size and number of migrants between the defined populations as well as parameter convergence are given in the Supplement (Table S2). Our analysis aligns well with the summer anticyclonic gyre used to delineate Model 2, which is consistent with both magnitude and direction of the gulf coney's gene flow in the NGC. This period coincides with the pelagic phase of the *Hyporthodus acanthistius* larvae. The full migration model comes second to Model 2 because of the unrestricted migration between the populations to the north of Puerto Libertad. Finally, Model 3, in which gene flow follows the winter cyclonic water movement patterns described for the area, scored the lowest in explaining larval migration in the area.

Table 2. Population structure estimated by F_{ST} between *Hyporthodus acanthistius* populations calculated from the mitochondrial control region (below left) and from 11 microsatellites (above right) with 95% confidence intervals between brackets. Significant pairwise F_{ST} (at $p < 0.05$) after 10 000 permutations shown in bold. PLI, Puerto Libertad; PLO, Puerto Lobos; STO, Santo Tomás; PPE, Puerto Peñasco; SLG, San Luiz Gonzaga

	PLI	PLO	STO	PPE	SLG
PLI		**0.003** [0.000 to 0.007]	**0.003** [−0.005 to 0.009]	0.002 [0.000 to 0.018]	**0.005** [0.002 to 0.020]
PLO	**0.04254**		0 [−0.001 to 0.009]	0.001 [−0.003 to 0.003]	0.003 [−0.001 to 0.005]
STO	**0.03838**	0.00049		−0.003 [−0.005 to 0.001]	−0.005 [−0.008 to −0.002]
PPE	**0.07154**	0.00045	0.0052		−0.001 [−0.003 to 0.002]
SLG	0.00561	0.01327	0.01133	0.02289	

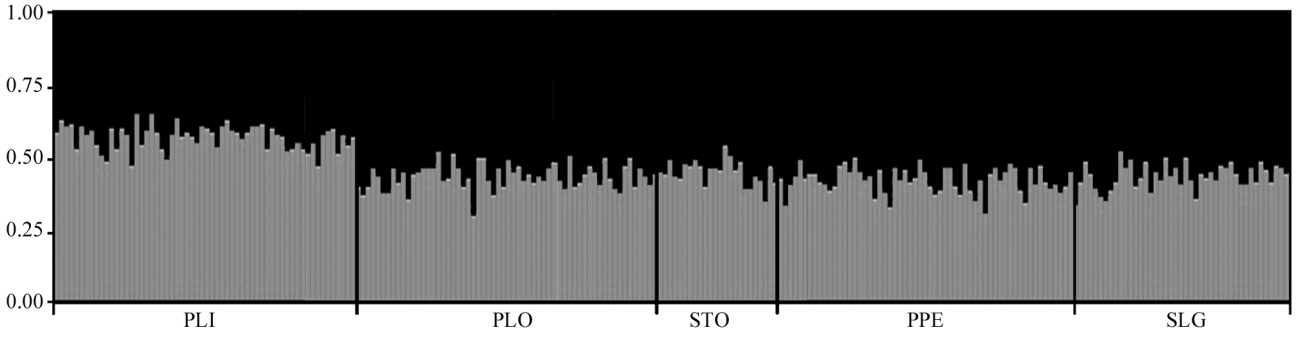

Fig. 2. Q-plot of the Bayesian population assignment test based on 11 microsatellite loci. Each vertical line represents a single *Hyporthodus acanthistius* individual; black/gray in each vertical line represent the likelihood of belonging to each of the clusters. Black vertical lines separate the spawning aggregations; population acronyms are defined in Fig. 1

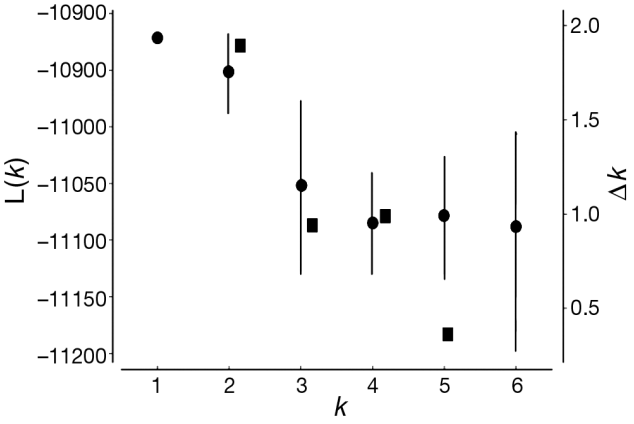

Fig. 3. Mean and standard deviation of log likelihoods, L(k) (●); and Evanno's Δk (■) for each k value estimated in 10 independent runs in STRUCTURE. The highest Δk indicates the choice of k = 2 as the one that best describes the genetic partitioning in the data

Table 3. Natural log Bayes factors (lmL) and log marginal likelihoods (lML) for each gene flow model estimated by thermodynamic integration using 11 microsatellite markers. Model details are explained in 'Materials and methods'

Model No.	Bezier lmL	Harmonic lML	Choice (Bezier)	Model probability
1	−4482.37	−4297.46	2	0.000
2	−4353.63	−4134.17	1	1.000
3	−5018.29	−4871.79	3	0.000

DISCUSSION

Genetic structure, hydrodynamics and site fidelity

In groupers, genetic differentiation of spawning aggregations has been observed but usually at large geographic scales (e.g. Rhodes et al. 2003, Rivera et al. 2004, Zatcoff et al. 2004). In this study, F_{ST} statistics based on mtDNA and microsatellites as well as Bayesian analysis were consistent in showing weak structure at a much smaller scale. In scenarios of weak genetic partitioning, F_{ST} metrics and Bayesian analysis have limited power in asserting structure (e.g. Waples & Gaggiotti 2006). In the present study, the inclusion of zero within the calculated F_{ST} 95% confidence intervals for 2 of the pairwise comparisons between Puerto Libertad and the other populations, and the limited capacity for Evanno's Δk to distinguish between a panmictic population (k = 1) and the genetic structure suggested (k = 2) as well as similar posterior probabilities for the same k values, have to be acknowledged. Nonetheless, the concordance between markers and analysis in showing that within the NGC, the southernmost spawning aggregation sampled, Puerto Libertad, was slightly distinct from all others lends support to this conclusion. Hence, we present evidence for structure between spawning aggregations at a small geographic scale. Furthermore, while unimportant over evolutionary time scales, weak genetic structure can have important implications in ecology and conservation biology (Jones & Wang 2012).

Given that the NGC covers such a small geographic area, it was unexpected to observe even weak population structure among spawning aggregations that are so close geographically. The spawning aggregation at Puerto Libertad was only 40 km from the closest spawning aggregation at Puerto Lobos. Hydrodynamic features of the study area during the dispersive stage of the gulf coney's larvae are also consistent with our results, as the southernmost spawning aggregation is the only one that sits just outside of the summer anticyclonic gyre. This seasonal gyre may trap the larvae originating from all other spawning aggregations, preventing them from travelling south. Eddies may entrap fish eggs and larvae both in the open ocean (e.g. Holliday et al. 2011) and in enclosed seas (e.g. Contreras-Catala et al. 2012). Simultaneously, the northward currents described for the Puerto Libertad spawning aggregation site will transport larvae northward. The genetic differentiation of Puerto Libertad may also be reconciled with some level of site fidelity. While there are no published accounts of site fidelity or home range for *Hyporthodus acanthistius*, several grouper species such as *Epinephelus tauvina* (Kaunda-Arara & Rose 2004) have some degree of site fidelity. Other examples have come from tagging studies of *E. striatus*, in which distances traveled to spawning sites ranged from 30 km (Sala et al. 2001) to upwards of 100 km (Carter et al. 1994, Bolden 2000). Movement at even the smallest of these scales could be very relevant within the NGC. The potential homogenizing effects of dispersing larvae are best explained by the study of gene flow.

Gulf coney's gene flow in the NGC

The most supported model for the genetic exchange among spawning aggregations of gulf coney

depicts the anticyclonic summer gyre, which coincides with the pelagic stage of this species' larvae. In the NGC, using a 3-dimensional baroclinic numerical model, Marinone et al. (2008) followed particles released in this gyre and found the same south-north direction of dispersal. Calderon-Aguillera et al. (2003) showed how hydrodynamics in the NGC influenced the dispersal of blue shrimp *Litopenaeus stylirostris* larvae and more recently Cudney-Bueno et al. (2009) reported similar circulation patterns and enhanced recruitment of rock scallop *Spondyllus calcifer* larvae and black murex *Hexaplex nigritus* larvae coming from the south of the NGC and settling into marine reserves located to the north. The currents along the eastern shore of the NGC may reach speeds of up to 0.06 m s^{-1} during the summer (Cudney-Bueno et al. 2009), which, considering a planktonic larval duration of approximately 30 d for the gulf coney in the NGC (K. Rowell, Biology Department, University of Washington, pers. comm.), would easily allow them to disperse between adjoining spawning aggregates and even beyond. Within 4 wk, larvae may travel the 148 km distance that separates Puerto Libertad and Puerto Peñasco, the southernmost and northernmost spawning aggregations.

Management of spawning aggregations

Our study highlights the importance of detailing the genetic structure and gene flow between spawning aggregations even at small geographic scales where panmixia is expected. The weak genetic structure found here can have important implications in ecology and conservation biology of this species. Management programs for the gulf coney in the NGC should reinforce protection of the southernmost spawning aggregation near Puerto Libertad. Both shallow genetic structure and an anticyclonic direction of gene flow suggest that Puerto Libertad is a source population, primarily exporting larvae north into the NGC. Oceanographic data suggest that Puerto Libertad may also play an important role as a gateway for larvae and gene flow originating from the middle gulf (e.g. Danell-Jiménez et al. 2009). If this pattern proves to be true, overfishing in the middle gulf region may have dire consequences for the populations in the NGC.

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