POPULATION GENETIC STRUCTURE OF THE CRASHED SNOOK FISHERY (*CENTROPOMUS SPP.*)

by

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Abstract

Snook (*Centropomus spp.*) are an important game fish in the warmer coastal regions of the West Atlantic and East Pacific Oceans. In Texas, the snook fishery crashed >80 years ago and has exhibited weak signs of recovery. Here, we investigated the patterns of population genetic structure of snook in Texas with 16S mtDNA and 9 microsatellite markers. Three species of snook were identified: *C. undecimalis*, *C. poeyi*, and *C. parallelus.* The mtDNA of Texan *C. parallelus* form a monophyletic lineage that is 0.8% divergent from *C. parallelus* in Florida and Costa Rica. *Centropomus poeyi* and *C. parallelus* exhibited a pattern consistent with rampant hybridization and introgression and were so intertwined that we analyzed them as a single stock. Both *C. undecimali*s and *C. poeyi - C. parallelus* exhibited elevated homozygosity consistent with inbreeding. Mild chaotic genetic patchiness in *C. undecimalis* and *C. poeyi - C. parallelus* was mostly explained by elevated levels of kinship that are also associated with inbreeding. There was one instance where a sample of juveniles shared higher kinship coefficients with adults from another location than each other, suggesting that the juveniles originated near the sample of adults, in Texas. Overall, these results suggest that the recovery of the Texas snook populations has been slow due to low amounts of migration from other locations in the Gulf of Mexico.

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Acknowledgments

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This thesis is dedicated to first generation Americans and indigenous people of the Americas who face unimaginable hardships but continue to hold their head up high with pride as they pursue higher education despite the challenge. *Ma ximopillan cualli ohtli* (May you have a smooth journey in the Nahuatl language).

Disclaimer

This project is a collaboration between the Texas Parks and Wildlife Department (TPWD) and TAMU-CC. Here, we incorporate 16S mitochondrial DNA work produced by Joel Anderson from TPWD and from Chris Chapa's Master thesis at TAMU - Galveston. The conclusions drawn from our nuclear DNA work would not have been possible without them.

Introduction

Sustainable fisheries are fundamental for food security (Kent, 1997) and a stable economy (Bailey, 1988). When fishery resources are not managed properly, harvest can be unsustainable and, consequently, lead to reduced population sizes (Jackson et al., 2001; Myers and Worm, 2003; Pauly et al., 1998). Once abundance is dramatically reduced and scarce, historical harvest data can provide a meaningful historical perspective of past abundance that can be used to set management goals (Jackson, et al., 2001).

All snook species (*Centropomus* spp., Family Centropomidae), are popular game fish that support recreational fisheries within their ranges and are an important food resource in Central and South America. Snook are protandrous euryhaline fish distributed along the West Atlantic Ocean, Gulf of Mexico and East Pacific Ocean, where they inhabit tropical and subtropical estuarine systems. Their range is limited to warmer water temperatures, and they are susceptible to mass fish kills from winter freezes (Howells et al. 1990). In the Atlantic Ocean, although snook stay in warmer waters, snook have been found as far north as North Carolina (Martin and Shipp, 1971; Merriner et al., 1970), with a sighting in New York (Schaeffer, 1972), and as far south as Brazil, with an occurrence in northern Argentina (Scenna et al., 2006) in 1996. In the Western Gulf of Mexico, the northern limit of snook is debated, but it is generally agreed to be limited to Texas (Colley, 1974; Martin and King, 1991; Matlock and Osburn, 1987; Pope et al., 2006).

Common snook (*C. undecimallis*), the largest and most-studied of the species have high fidelity to spawning sites (Adams et al., 2009). They tolerate a wide span of

salinities and migrate between rivers and open estuary systems (Blewett et al., 2009). Along the Gulf Coast of Florida, snook are known to be mostly sedentary and associated with nearshore islands where they spawn (Taylor et al., 1998). The larvae migrate into brackish and freshwater rivers where they develop into juveniles, moving back towards their spawning grounds as they mature. As juveniles, snook will seek shelter from larger predators in seagrass beds within estuarine habitats for the first 2 to 5 months of their lives (Gilmore et al., 1983). In Texas, Huber et al. (2014) found young *C. undecimalis* (3 years and younger) were among the most numerous fish inhabiting the lower Rio Grande; however, the adult spawning grounds are unknown. Adults snook species are associated with structures such as pier pilings, oyster reefs, and pot-holes within seagrass beds and are known to be ambush predators (pers. obs.). Unlike Florida, the Texas coast consists of long barrier islands that create large bay systems along the Gulf of Mexico, and adult habitat utilization of snook is not well understood. In addition, Texas is home to the largest hypersaline lagoon in the world Laguna Madre (Tunnell and Judd, 2002). Since the locations where spawning snook aggregate can be characterized as habitats having high salinity (~35.2 ppt) (Lowerre-Barbieri et al., 2003), it seems likely, however, that spawning would be confined within the barrier islands where bays, such as Laguna Madre, can provide these high salinities, thus reducing the potential for larval dispersal among bay systems.

Commercial harvest data indicates that the Texas snook fishery peaked in 1928 with 104,451 kg harvested, followed by an almost continuous decrease in landings (Figure 1). Commercial landings in South Texas remained low until 1961, after which no further commercial landings were reported (Matlock and Osburn, 1987). In 1987, the

commercial fishery was closed and harvest was restricted to recreational fishing with a daily bag limit of 3 snook between the size 20 - 28 in (51 - 71 cm). In 1995 the daily bag limit was reduced to one snook per day, and the slot size was narrowed to 24 - 28 in (61 - 71 cm). In 1975, the Texas Parks and Wildlife Department (TPWD) began conducting a fishery-independent gillnet survey, which uses 7.6 - 15.2cm stretch mesh in four 45.7m sections that are fished overnight for 10-week periods during the spring and fall (Martizez-Andrade and Fisher, 2010). Although few snook have been recorded, there has been an increase in their occurrences from 1975 to 2014 (Figure 2), suggesting that the snook fishery has been slowly recuperating over the 28 years since the closure of the commercial harvest. However, because of the low abundance of snook in South Texas after the fishery crashed, little information is available concerning habitat use and stock structure, causing managers to take a cautious approach in regulating these species. Their inconspicuous nature also makes them difficult and labor intensive to sample for studies, which has resulted in limited information on their basic life history and ecology in Texas[1].

Genetic approaches can help fishery managers make informed decisions for effectively regulating fisheries that are difficult to distinguish morphologically, such as centropomids. As genetic methods become less expensive, their use has increased significantly (von der Heyden et al., 2014). Genetic markers can provide valuable information regarding marine species evolution, population structure, and connectivity. Indeed, prior to the application of genetic analysis, only common snook (*Centropomus*

[1] Vega, R., Texas Parks and Wildlife pers. comm

undecimalis) was believed to be a regular Texas resident. Using protein electrophoresis, TPWD identified small-scale fat snook (*C. parallelus*) along the southern extreme of the Texas Gulf Coast for the first time, but they were unsure if these were a few transient individuals or a resident breeding population (Martin and King, 1991). Fisheries managers have identified two other species of snook in Texas using mitochondrial DNA sequencing: the Mexican snook (*C. poeyi*) and large-scale fat snook (*C. mexicanus*, sometimes also inaccurately called Mexican snook because of the species nomenclature[2]). It should be noted, however, that mitochondria have their own genome and, in some circumstances, can be unreliable for species identification due to introgression across species boundaries (Rubinoff et al., 2006).

Nuclear microsatellite markers have proven to be extremely useful in inferring patterns of population genetic structure and can also be used to infer relationships among individuals (Selkoe and Toonen, 2006). Gold et al. (2002), for example, identified two slightly divergent king mackerel stocks resulting from reduced gene flow between the Atlantic and Gulf of Mexico coasts in the Florida peninsula. Patterns of relatedness among individuals inferred with microsatellite markers can be used to further explain patterns of genetic structure among samples. In addition, Iacchei et al. (2013) found that genetic structure in Pacific lobsters was driven by elevated levels of kinship near upwelling areas. Patterns of relatedness can also be used to link juveniles to the adult populations from which they originated (Gorospe et al., 2015; Harrison et al., 2014). In

[2] McKee, Texas A&M University, pers. comm.

fact, direct connections between parents and offspring have been discovered in reef fish using microsatellites (Christie, 2010).

Most data on the movement patterns and connectivity of *Centropomus* spp. come from tagging studies along the Gulf Coast of Florida. Adams, et al. (2009) report a short average distance (1.98 - 2.5km) between tag and recapture locations for *C. undecimalis* using both passive and acoustic tags. In fact, 88% of the snook were detected exclusively on the island where they were originally tagged for an entire year, suggesting high fidelity to spawning locations. This sort of behavior, when associated with spawning, can lead to population segregation (Adams et al., 2006; McCairns and Fox, 2004).

The population structure of snook species in Texas is unknown, and these species are currently managed as a single fishery; however, genetic tools could be used to better understand the diversity of snook and identify the number of stocks. The purpose of this study is to identify discrete stocks of snook and test for non-random patterns in the spatial distribution of genetic diversity of *Centropomus* spp. residing along the Texas Gulf Coast using mitochondrial DNA sequence and nuclear microsatellite genetic markers. Genetic patterns will be used to infer stock structure and patterns of kinship.

Materials and methods

Sample collections

Tissue samples were collected from *Centropomus* spp. captured in a variety of estuarine habitats between Aransas Pass, Texas and Rio Grande along the Texas-Mexican border. The Texas seashore has barrier islands that form several bay systems between the islands and the mainland (Figure 3). Common habitats within the bay systems include salt

marshes, mangroves, and seagrass beds. There are also several dredged ship channels with rocky reef-type habitats that connect the bay systems to the Gulf of Mexico.

Collections were conducted by the Texas Parks and Wildlife Department, fishing guides, local experienced snook anglers, and researchers (AG). The Texas Parks and Wildlife Department (TPWD), Palacios Field Station (Palacios, TX) collected 446 adult and juvenile snook from 2009 - 2010. These collections included 9 sites throughout the South Texas Coast and one site in Mexico, from North to South: Aransas Pass (J1), Redfish Bay (A1), Arroyo Colorado (J2), Laguna Vista (J3), South Bay (A3), Brazos-Santiago Pass (A4), San Martin (A5), Rio Grande Mouth (J4), and Rio Grande Slough (J5) (Figure 3). All adults were collected via hook-and-line, and juveniles were collected by seining. From July - October in 2012 and 2013, 111 adult snook fin clips were sampled haphazardly via hook-and-line using artificial lures with the assistance of local anglers from Packery Channel (A2), South Bay (A3), and Brazos-Santiago Pass (A4). Juveniles were not sampled during the spring months of 2012 and 2013 due to abnormally low precipitation causing water levels to be too low for sampling.

DNA extraction

The Texas Parks and Wildlife extracted genomic DNA from a single eyeball using the DNeasy Blood and Tissue Kit (Qiagen, USA) and the PUREGENE DNA Isolation Kit (Gentra Systems Inc., Minneapolis, MN, USA) following the manufacturer's' protocol with final rehydration volumes of 75 - 100µL. Genomic DNA from samples collected by Texas A&M University - Corpus Christi were extracted from fin clips using the DNeasy Blood and Tissue Kit (Qiagen, USA) following the standard protocol with the following

modifications: sample were incubated at 56ºC for 25 minutes with 100µL AE buffer twice in the final steps of elution. Isolated DNA samples were stored at -80ºC.

Microsatellite genotyping

DNA was amplified as nine loci using primer sets developed by (Seyoum et al., 2005) Polymerase chain reaction (PCR) was conducted in a solution composed of water (3µL), GoTaq Green Master Mix from Promega (5µL), 10µM forward primer (0.25µL), 10uM reverse primer (0.50µL), 10µM fluor dye (0.25µL), and 1.5µL of 50ng/µL genomic DNA. During PCR amplification, samples were denatured for 2 minutes at 95ºC; then subjected to 30 cycles of denaturing at 95ºC for 30 seconds, annealing at the temperatures listed in Table 1 for 30 seconds, and extension at 72ºC for 30 seconds; eight cycles of 95ºC for 15 seconds, 53ºC for 30 seconds, and 72ºC for 30 seconds; and a final extension of 72ºC for 10 minutes. Note that the additional 8 cycles in the PCR protocol targets the M13 fluorescent dyes to maximize signal strength (Schuelke, 2000). To verify if microsatellite amplification was successful, we visualized the PCR products using gel electrophoresis in 1X tris-acetate-EDTA (TAE) with a 1.2% agarose gel stained with GelStar (Lonza Rockland, Inc). Fragment length analysis was conducted by the Genomics Core Lab at Texas A&M University - Corpus Christi with an ABI 3730. Allele lengths were scored using the program GeneMapper (v. 5.0, Applied Bio-systems, Inc.) and binned using Tandem (Matschiner and Salzburger, 2009) at the default settings.

16S mtDNA PCR Reactions and Sequencing

16S mtDNA reactions and sequences were conducted by Texas Parks and Wildlife Department at the Perry R. Bass Marine Fisheries Research Station in Palacios, TX. We

used 16S mtDNA primers designed by Palumbi (1991) using similar protocols developed by Tringali et al. (1999b) and amplified DNA under a modified touchdown protocol by using Ready-To-Go PCR beads (GE Healthcare, Piscataway, New Jersey, USA). The primer sequences used for 16s PCR were:

16Sar (5'-CGCCTCTTTATCAAAAAC-3') and

16Sbr (5'- CCGGTCTGAACTCAGATCACG-3')

The PCR reaction master mix consisted of 1 µL of template DNA (50 ng/µL), one Ready-To-Go bead, 0.4 µM forward primer (12µL) and 0.4 µM reverse primer(12µL) for a total of 25µL. The touchdown PCR protocol utilized for all reactions consisted of the following: an initial denaturing period of 2 minutes at 95ºC, 10 cycles of initial amplification (95ºC for 30 seconds; 55ºC for 30 seconds, lowering 1ºC each cycle; and 72 ºC for 1 minute), 20 cycles of primary product amplification (95ºC for 30 seconds; 55ºC for 30 seconds; and 72ºC for 1 minute, adding 3 seconds of extension per cycle), and a final extension period of 7 minutes at 72ºC.

After amplification, PCR products were purified using ExoSAP-IT (USB, Cleveland, Ohio, USA) to free DNA of excess primers and nucleotides. Following the manufacturer's recommendations, 5µL of PCR product were mixed with 2µL of enzyme and placed on a thermocycler for 15 minutes at 37ºC, followed by an inactivation step for 30 minutes at 80ºC.

The sequencing reaction followed the protocol of Anderson and Karel (2009) using 10µL volumes of Genomelab Quick Start Master Mix DTCS (Beckman Coulter Inc., Fullerton, California, USA). Primers for sequencing were the same as those used in PCR.

Cycle sequencing parameters were: 30 cycles of denaturing at 96ºC for 20 seconds, annealing at 50ºC for 20 seconds, and extension at 60ºC for 4 minutes. Sequencing reactions were precipitated by adding 1/20 volume of a cocktail containing 2µL sodium acetate (3M), 2µL EDTA (100mM) and 1µL glycogen, followed by 2 volumes of 95% ethanol, and centrifuged at 3,700 rpm for 30 minutes to form pellets. The resulting pellets were then rinsed twice with a 70% ethanol, dried, and rehydrated by using a formamide sample loading solution (Beckman Coulter, Fullerton, California, USA). Finally, the sequences were separated and analyzed on a Beckman CEQ8000 capillary sequencer (Beckman Coulter, Fullerton, California, USA) using default sequencing module parameters.

Species identification

Morphological features can be unreliable in distinguishing among snook species, because many traits overlap substantially across several snook species (Chapa, 2012; Rivas, 1986). Juveniles are especially difficult to identify due to damage of lateral line scales, which is key for identification (Rivas, 1986), during sampling and transport. Consequently, 16S mtDNA sequences were utilized to identify *Centropomus* spp. samples. Mitochondrial DNA identity was confirmed by constructing a maximum likelihood phylogeny and identifying well-supported monophyletic lineages containing positively identified specimens by Tringali, et al. (1999b) and de Oliveira et al. (2014). All published 16S mtDNA sequences from centropomidae species in GenBank (listed in Table 2) were aligned with with the unique 16S haplotypes sampled in the present study using Geneious v. 7.1.4 (Kearse et al., 2012) and then checked by eye for alignment errors. The sequences were aligned and trimmed to 528bp. A maximum likelihood

phylogenetic reconstruction tree was conducted with the RAxML web server at http://embnet.vital-it.ch/raxml-bb/ (Stamatakis et al., 2008) using all aligned sequences and the outgroup: nile perch (*Lates niloticus*). RAxML output file was imported to FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk) to visualize. A rooted haplotype network was also created using the program Network v. 4.6.1.1. (fluxus-engineering.com).

In addition to using 16S mtDNA sequences to distinguish between the species of snook, an assignment test was performed with the microsatellite genotype data using the program STRUCTURE 2.3.4 (Prichard et al., 2000). Thirty replicated runs of Markov Chain Monte Carlo (MCMC) simulations using the admixture model (K = 2, 3, and 4; 200,000 burn-in steps; 1,000,000 MCMC iterations) were conducted. Concordance in the mtDNA identity and the groups of genetic similarity based upon microsatellite markers was used to determine the true species affiliation. Where mtDNA and nDNA assignments differed, nDNA was given more weight in determining species affiliation.

Genetic Analysis

Data was imported into MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al., 2004) to check for null alleles and scoring errors due to stutter or allelic drop-out. Observed heterozygosity (H_o) and total heterozygosity (H_t), as well as allelic richness (A), and effective number of alleles (A_e) for each species group were then calculated using GenoDive 2.0 b14 (Meirmans and Van Tienderen, 2004). A nested analysis of molecular variance (AMOVA; (Excoffier et al., 1992) was conducted using GenoDive to determine whether genetic structure among sampling locations could be explained by differences in geography (North vs. South), habitat (estuarine vs. ocean), or life stage (adult vs.

juvenile). Standard *F*-statistics were calculated, where microsatellite alleles were classified, in a binary system, as identical or different to make a genetic distance matrix. Ten-thousand permutations were conducted to generate the null distributions and test whether the observed *F*-statistics were significantly different than zero. Pairwise F_{ST} values for all pairs of locations and their respective *P*-values were calculated in GenoDive using 100,000 permutations. An isolation by distance (IBD; (Wright, 1943) analysis was conducted with a Mantel test (100,000 permutations) to determine if there was a relationship between genetic distance and geographic distance. Because land serves as a barrier to the dispersal of most marine species, the minimum travel distance by water between sites was utilized in the IBD analysis, as suggested by Bird et al. (2007).

Kinship

Pairwise kinship coefficients (Loiselle et al., 1995) were calculated for all individuals within each species group using GenoDive v. 2.0 b14 (Meirmans and Van Tienderen, 2004). Kinship was estimated as the relative probability of identity by descent with respect to the allele frequencies of the full dataset, which provide an index of relative relatedness between pairs of individuals. Coancestry coefficients were exported in the form of a matrix and binned according to parameters set by (Iacchei, et al. (2013)) $1.0 > k > 0.375$; $0.375 > k > 0.1875$; $0.1875 > k > 0.09375$; and $0.09375 > k > 0.047$. A heat map was created in order to visualize patterns of kinship within and among sites. Statistical significance of the level of kinship within each sample was determined by permuting the individuals among locations (10,000 permutations) to generate a null distribution against which the observed levels of kinship were compared.

Results

Phylogenetic Species identification

Mitochondrial 16S DNA (496-502 bp) was sequenced for 539 individuals from 18 locations and used to construct a maximum likelihood phylogenetic tree (Figure 4). The positively-identified species in GenBank form well-supported branches and clades (bootstrap values > 50), indicating the 16S can be used to assign mtDNA identity at the species level. Two hundred six samples from this study, represented by 14 haplotypes (Figure 5A), and 6 *C. undecimalis* haplotypes from GenBank formed a monophyletic clade with high bootstrap support (97) and shallow branch depths, indicating that all of these samples have the mtDNA of *C. undecimalis*. Three samples from this study, represented by 3 haplotypes, differing by 1 bp, and 1 *C. poeyi* haplotype from GenBank also form a monophyletic clade with strong bootstrap support (100), indicating the species affiliation of the mtDNA is with *C. poeyi*. Three hundred thirty samples from this study, represented by 7 haplotypes (Figure 5B) and 1 *C. parallelus* haplotype from GenBank form a monophyletic clade with high bootstrap support (86). However, the samples from this study form a monophyletic clade with substantial bootstrap support (64) and exhibit 0.8% sequence divergence from the *C. parallelus* mtDNA sequence in GenBank that represents samples from Florida, Costa Rica, and Puerto Rico (Tringali et al., 1999). This level of sequence divergence is similar to that observed between the species *C. mexicanus* (GenBank Accession # U85014; Tringali et al., 1999a and *C. parallelus* (1.0%) using the same genetic marker.

Two of the species in this study, *C. undecimalis* and *C. poeyi* appear to be sister taxa, with 5.2% 16S sequence divergence. The third species, *C. parallelus*, is more distantly related to *C. undecimalis* and *C. poeyi* (~9.8% sequence divergence).

Using STRUCTURE to analyze the microsatellite nDNA markers, 3 genetic groups of snook were identified (Figure 6B): I (green), II (red), and III (blue). With greater than 99% probability, 184 samples were assigned to group I (green), 0 were assigned to group II (red), and 7 were assigned to group III (blue). The remaining 308 samples could not be assigned to any of the groups with 99% probability. Although two individuals assigned to >98% probability to group II and 94-93% probability to group II, respectively, only 5 out of 9 loci amplified. If the threshold probability of assignment is relaxed to 50%, then 184 samples were assigned to group I, 46 samples were assigned to group II, and 211 samples were assigned to group III. The majority of samples can be unambiguously assigned as either belonging to group I or not belonging to group I. However, the line separating individuals that assign to either group II or III is less distinct, with a smooth gradient in assignment probability that can be visualized in Figure 6B.

Samples identified to species using the phylogenetic technique, based on mtDNA, were identified in the nuclear microsatellite STRUCTURE analysis to assess concordance between the mtDNA and nDNA sample classifications (*C. undecimalis*, *C. poeyi*, and *C. parallelus* versus groups I, II, and III). With reproductive incompatibility and complete lineage sorting, the expectation is that the mtDNA and nDNA groupings should match, and there was a general pattern of concordance (Figures 7). For samples identified as *C. undecimalis* with mtDNA, 74% assigned to nDNA group I (green) with >99% probability

and 97.8% assigned with >50% probability (Figure 7A). While not shown, 1% of samples with >99% assignment to group I had the mitochondria of *C. parallelus*. All three individuals with the mtDNA of *C. poeyi* were assigned to group II (red) with > 80% probability (Figure 7B). Two percent of individuals with the mtDNA of *C. parallelus* were assigned to group III (blue) with > 99% probability and 80% were assigned with >50% probability (Figure 7C). There were, however, 19% of individuals with the mtDNA of *C. parallelus* that assigned to group III with <50% probability (Figure 7C). The majority of these individuals were assigned to group II with >50% probability (not shown). There was no detectable relationship between STRUCTURE assignments and the geographic location of the sample (Figure 8).

For the purposes of conducting population genetic analysis, it was determined that microsatellite nDNA group I, *C. undecimalis*, was sufficiently distinct to be separated from groups II and III as an independently evolving lineage. Individuals assigned to microsatellite groups II and III, however, appear to be exchanging genes despite their affiliation with 2 deeply divergent species, *C. poeyi* and *C. parallelus*, within the *Centropomus* genus (see Discussion for a complete justification). Therefore, groups II and III are analyzed as a single stock.

nDNA Diversity

The genotypes of 536 individuals collected from nine locations were scored for nine nuclear microsatellite loci (Table 3). There was no evidence of scoring errors due to stutter or allelic drop-out; however, all loci exhibited an excess of homozygotes, which could be the result of null alleles (Callen et al., 1993; Falush et al., 2007). When

accounting for the effect of null alleles on the *F*-statistics using FreeNA (Chapuis and Estoup, 2007), the *F*-statistics were not affected by the null alleles; consequently, the following results were calculated from the data without alteration to account for null alleles.

Centropomus undecimalis had a mean allelic richness of 18.222 (± 2.808 Standard Error), mean effective number of alleles equal to 5.47 (± 0.77 SE), mean observed heterozygosity of 0.675 (± 0.033 SE), and mean expected heterozygosity of 0.824 (± 0.031 SE) for the microsatellite loci (Table 4). The *C. parallelus* - *C. poeyi* group had slightly lower values than *C. undecimalis* for the mean allelic richness (A = 15.111 ± 1.419 SE), mean effective number of alleles (A_e = 3.873 ± 0.56 SE), mean observed heterozygosity (H_o = 0.611 ± 0.061 SE), and mean expected heterozygosity (H_e = 0.753 ± 0.032 SE).

nDNA Genetic Structure

Neither life stage (juvenile vs. adult), nor habitat (estuarine vs. ocean), nor geography (North vs. South) explained the genetic structure observed in *C. undecimalis* (F_{CT} = -0.001-0.005, P = 0.055 - 0.336, Table 5). In *C. parallelus* - *C. poeyi*, however, geographic location (North vs. South) explained some of the genetic structure observed (F_{CT} = 0.002, P = 0.019, Table 6). However, neither life stage nor habitat explained the genetic structure observed in *C. parallelus* - *C. poeyi* (F_{CT} < -0.001, P = 0.135 - 0.338, Table 6). There is genetic structure among sampling locations of *C. undecimalis* (F_{SC} = 0.005 and P = 0.01 - 0.018). There was genetic structure detected among sampling locations in *C. parallelus* - *C. poeyi*, with F_{SC} = 0.007 - 0.009 and P <0.00001; but the

structure among sampling locations was only evident in locus CUN17 (data not shown). Due to the lower amount of genetic diversity in *C. parallelus - C. poeyi*, these results should be interpreted as indicating there is more genetic structure among samples of *C. parallelus - C. poeyi* than *C. undecimalis* (Bird et al., 2011; Meirmans, 2006). The majority of genetic structure, however, was found among individuals in both species, with the estimates of the inbreeding coefficients (F_{IS}) ranging from 0.114 to 0.170. There were appreciably higher levels of F_{IS} in *C. undecimalis* than *C. parallelus - C. poeyi*. When comparing the genetic variation among sampling sites for *C. undecimalis* and *C. paralellus - C. poeyi*, there were significant differences among sites (F_{ST} = 0.006 - 0.008, $P < 0.001$, Table 5 and 6).

Pairwise F_{ST} comparisons among sampling locations exhibited a pattern somewhat consistent with chaotic genetic patchiness (Johnson and Black, 1982; Johnson and Black, 1984) Table 7). For *C. undecimalis*, site J3 (juvenile site #3: Laguna Vista) was significantly differentiated from 2 other samples: sites A3 (adult site #3, South Bay) and J4 (juvenile site #4, Rio Grande Mouth); whereas for *C. parallelus - C. poeyi*, sites J2 (juvenile site #2: Arroyo Colorado), A2 (adult site #2: Packery Channel), A3 (adult site #3: South Bay), A4 (adult site #4: Brazos-Santiago Pass), and A5 (adult site #5: San Martin) were differentiated from other sites. There was no pattern of isolation by distance for either *C. undecimalis* ($r^2 = 0.15$, $P = 0.0704$, Figure 9) or *C. poeyi - C. parallelus* ($r^2 = 0.001$, $P = 0.41$; Figure 10).

Kinship

Kinship coefficients used to determine patterns of relatedness within and among samples of juveniles and adults indicated significantly elevated kinship patterns. For *C. undecimalis,* kinship coefficients are non-random and greater than expected within sample site J3 (juvenile site #3, Laguna Vista Ditch; Figure 11). For *C. parallelus - C. poeyi*, there is a cluster of higher kinship coefficients within site A5 (adult site#5, San Martin), between site A5 and a subsample of site J2 (juvenile site#2, Arroyo Colorado; Figure 12).

Discussion

Phylogenetic Relationships

The extensive sampling and genetic analysis conducted here indicate that there are at least three species of *Centropomus* in Texas: *C. undecimalis, C.parallelus,* and *C. poeyi.* Consistent with Tringali, et al. (1999a) and the expectations underlying DNA barcoding (Hajibabaei et al., 2007), phylogenetic analysis confirms that each species designation is associated with an independently evolving lineage of mtDNA.

Unexpectedly, the *C. parallelus* mtDNA haplotypes from Texas form an independently evolving lineage that is 0.8% divergent from the *C. parallelus* haplotype that was sampled in Florida (n = 2), and Costa Rica (n = 1). While more sampling outside of Texas is required to make a firm conclusion, this pattern is consistent with a contemporary lack of dispersal between Texas and both Florida and Central America. Given that the *C. mexicanus* and *C. parallelus* lineages are only slightly more divergent (1%) than the two *C. parallelus* lineages, the West-Northwest Gulf of Mexico's *C.*

parallelus likely represent an independent fishery stock and possibly a cryptic species (De Queiroz, 1998; 2007).

Hybridization, Admixture and Introgression

It is difficult to morphologically distinguish among snook species, as evidenced by several changes in the nomenclature of *C. undecimalis* and *C. parallelus* between 1792 and 1906 (Rivas, 1986). With the mtDNA sequencing and nDNA microsatellite genotyping data collected here, it is clear that some of the difficulty in determining species identity morphologically is due to gene flow among the species.

Most prominently, the nuclear microsatellite loci exhibit a striking pattern that is consistent with rampant hybridization and genetic introgression between *C. poeyi* and *C. parallelus* (groups II & III, respectively, Figure 6B). While the species have not completely merged, it is probably most beneficial to manage these fish as a single stock in Texas. There are several observations that lead to a conclusion of genetic admixture, as opposed to incomplete lineage sorting. Critically, mtDNA sequences indicate the C. poeyi and C. parallelus are distantly related (16S divergence = 9.8%), non-sibling species. Using sister taxa that reside in the Pacific and Atlantic Oceans, such as *C. medius* and *C. pectinatus*, as a benchmark, it is clear that the most recent common ancestor of *C. poeyi and C. parallelus* existed well before the Isthmus rose 3 million years ago (Knowlton and Weigt, 1998; Leigh et al., 2014). Further, all of the extant 16S lineages that are 9.8% divergent are completely resolved (Figure 4), indicating that a sufficient amount of time has passed for new haplotypes to evolve and shared haplotypes among independent lineages to go extinct. Thus, the data indicate that following speciation,

reproductive incompatibility did not fully develop between *C. poeyi and C. parallelus*, allowing for viable hybrids with appreciable fitness. This process of hybridization and introgression would lead to multilocus genotypes that are difficult to reliably assign to one species or another, as is observed here between *C. poeyi* and *C. parallelus* (Figure 6B).

There is a conspicuous paucity of *C. poeyi* mtDNA in the samples (n=3) collected here despite 46 samples assigning to C. poeyi with >50% probability based upon nuclear microsatellite markers. It is possible that in this region fish with the mitochondria of *C. parallelus* are more fit than those with the mitochondria of *C. poeyi*.

There was also some evidence of introgression between *C. undecimalis and C. parallelus*. Again, *C. undecimalis and C. parallelus* have deeply divergent 16S lineages (9.8%) and more than enough time has passed for shared mtDNA haplotypes to become extinct (Figure 5). Yet, there are 3 individuals that have the mtDNA of *C. parallelus* and the nDNA of *C. undecimalis* and 2 individuals that have the mtDNA of *C. undecimalis* and the nDNA of *C. parallelus*. This pattern is consistent with hybridization followed by low fitness of hybrids and backcrossing (Bird, et al., 2011), thereby limiting the extent of admixture between the populations and sharply delineating the stocks.

Utility of Genetic Markers for Snook Identification

Based on the historical morphological description of snook in Texas, it was formerly assumed all snook species in Texas were *C. undecimalis*[3]. The most reliable morphological indicator of species here was length, where all fish longer than 63 cm (n =

[3] Vega, R., Texas Parks and Wildlife pers. comm.

5) were *C. undecimalis*. Genetic information was necessary to reliably decipher the species in most cases where only a fin clip was available. Barcoding with mtDNA was 74% accurate for identifying *C. undecimalis*, 2% accurate in identifying the *C. poeyi - C. parallelus* group, and 2% accurate in identifying *C. parallelus*. In order to decipher among the identities of individuals within the *C. poeyi - C. parallelus* group, however, several nuclear loci are necessary and a substantial proportion of fish in this group are genetically intermediate.

Factors affecting the presence of snook in Texas

If *C. undecimalis* was, indeed, the only species historically inhabiting Texas waters, and there are now three species, a few hypotheses can be postulated to explain this. First, *C. parallelus* and *C. poeyi* may have colonized Texas following the fishery crash in the 1930s. Second, *C. parallelus-C. poeyi* may be utilizing new habitat, such as ship channels. Third, the warming waters of the Gulf of Mexico may be facilitating a northward expansion of additional snook species (Figueira and Booth, 2010; Roessig et al., 2004). Increases in ocean temperatures have led to a shift in historic distributions in many marine species (Perry et al., 2005). Indeed, Tolan and Fisher (2009) and Moore (1975) have noted that the increased number of snook in Texas may be linked to higher temperatures. However, given the challenge in identifying snook species morphologically, it is also possible that all three species of snook were inhabiting Texas waters for the past 400 years or longer.

Genetic structuring by life stage, habitat, or geography

In *C. undecimalis*, there was no relationship between life stage, habitat or geography, and genetic structure. For *C. poeyi - C. parallelus*, a small amount of genetic structure ($F_{ST} = 0.008$, Table 6) could be explained by the partitioning between the northern and southern samples in the vicinities of Aransas/Corpus Christi and Brownsville, respectively. This could be attributed to possible range expansion of *C. parallelus* as suggested by Martin and King (1991). As species disperse into newer areas, they can experience a reduction in population-specific F_{ST} as found by Banks et al. (2010) with sea urchins expanding their range due to climate change.

Chaotic genetic patchiness

Chaotic genetic patchiness (Johnson & Black, 1982) is the primary pattern of genetic structure evident in the data sets for both *C. undecimalis* and *C. poeyi - C. parallelus* (Tables 5-7). Chaos describes a pattern that cannot be explained, and chaotic genetic patchiness is a pattern in genetic structure that is difficult to explain. Iacchei, et al. (2013) determined that all of the chaotic genetic patchiness observed in Pacific red rock lobsters was explained by elevated levels of kinship in upwelling areas. In this study, we found elevated levels of kinship for both species in samples from the Lower Laguna Madre (Figure 3, Panel C) and within some samples from estuarine habitats. Sample J3 had elevated levels of kinship among individuals when compared to the other samples (Figure 11), and this is the only site that exhibited genetic structure (Table 7), similar to Iacchei, et al. (2013). Upon closer inspection, the fish from J3 that experienced elevated kinship were juveniles that were all collected on the same date, indicating that

young schools of *C. undecimalis* are sometimes reflective of kin relationships. Additionally, it indicates that the schools are mobile and do not remain in the same location, given that elevated kinship was not detected on any other day of sampling.

For *C. poeyi - C. parallelus*, kinship was not as closely tied to chaotic genetic patterns (Table 7, Figure 12). Samples A2 and A3 were significantly differentiated from some other samples, but exhibited the same levels of kinship as an average sample. Sample A5, on the other hand, did exhibit a non-random pattern of elevated kinship relative to the other samples and was genetically differentiated from 4 other samples. Kinship can also be used to detect similarities between samples, and samples A5 and J2 exhibited elevated kinship above the random expectation. In this case, it seems highly likely that the adults sampled in A5 are related to some of the juveniles in sample J2. One interpretation of this data is that some of the juvenile snook collected at J2 originated within Texas near site A5, which is important because there is some debate among managers whether snook in Texas originate in Mexico or Texas. The fact that A5 and J2 were collected at different times, and the high site fidelity of adult snook in Florida (Adams, et al., 2009) are consistent with a Texas origin for at least some juvenile snook.

Implications for fishery management

The main objective of this study was to describe the genetic structure of snook in Texas. Snook are currently managed as a single fishery in Texas, but there are at least 3 different species and 2 stocks in the fishery. There are low levels of chaotic genetic patchiness that are somewhat reflective of elevated kinship, which may contribute to the high levels of F_{IS}, the inbreeding coefficient. There is even some evidence that some

juvenile snook originate in Texas indicating snook are breeding within state waters. There has long been an interest in implementing a restocking program for snook in Texas

There are other questions, however, that remain to be answered. The Texan *C. parallelus* may be a cryptic species and likely is relegated to the western Gulf of Mexico, but more cosmopolitan sampling is required to demonstrate this. The population connectivity of *C. undecimalis* between Texas and other locations remains to be estimated, but some fisheries evidence suggests there may be partitioning. Specifically, the commercial snook harvest from Mexico for Veracruz, Tabasco, and Campeche are steadily increasing (>1500 tons per year) while Tamaulipas in Northern Mexico has remained/declined below 500 tons per year without signs of increase (Diario Oficial de la Federación - Mexico, 2014). If there were high levels of connectivity along the Western Gulf Coast, then the crashed fishery in Texas should have been replenished quickly by the more sustainable populations to the south. Furthermore, the role of Caribbean island populations in maintaining dispersal corridors that promote elevated genetic diversity and subsidize larval recruitment of snook remain to be evaluated. A species-range-wide perspective of the fishery will facilitate more effective management plans for sustainable harvest.

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Tables

Table 1

List of microsatellite primers used, their annealing temperatures and size ranges for this study. Primers were developed by Seyoum, et al. (2005).

Primer	Annealing temperature	Allele size range	
		C. undecimalis	*C. parallelus* & *C. poeyi*
Cun 4A	61ºC	228 - 242	214 - 244
Cun 9	60ºC	236 - 306	226 - 280
Cun 14	61ºC	173 - 223	173 - 219
Cun 16	63ºC	136 - 160	122 - 174
Cun 17	58.3ºC	234 - 228	216 - 256
Cun18	49.4ºC	135 - 189	does not amplify
Cun19	68ºC	152 - 186	148 - 182
Cun 20	55ºC	135 - 177	125 - 169
Cun 22	61ºC	185 - 245	169 - 221

Table 2

List of GenBank accession numbers used in phylogenetic tree. Each GenBank accesssion number is listed by species and collection site as described in the reference paper.

GenBank Accession	Common name	Species name	Collection	Author
U85007	Nile perch	*Lates niloticus*	Lake Victoria, Kenya	Tringali et al. 1999
U85008	Swordspine snook	*C. ensiferus*	Puerto Rico	Tringali et al. 1999
U85009	Humpback snook	*C. unionensis*	Panama	Tringali et al. 1999
U85010	Longspine snook	*C. armatus*	Panama	Tringali et al. 1999
U85011	Yellowfin snook	*C. robalito*	Panama	Tringali et al. 1999
U85012	Common snook	*C. undecimalis*	Florida, USA	Tringali et al. 1999
			Puerto Rico	
			Costa Rica	
			Venezuela	
			Trinidad	
U85013	White snook	*C. viridis*	Panama	Tringali et al. 1999
U85014	Mexican snook	*C. poeyi*	Alvarado, Veracruz, Mexico	Tringali et al. 1999
U85015	Black snook	*C. nigrescens*	Panama	Tringali et al. 1999
U85016	Small-scale fat snook	*C. parallelus*	Florida, USA	
			Costa Rica	
U85017	Large-scale fat snook	*C. mexicanus*	Puerto Rico	Tringali et al. 1999
U85018	Tarpon snook	*C. pectinatus*	Florida, USA	Tringali et al. 1999
			Panama	
KJ641473.1	Common snook	*C. undecimalis*	Brazil	Oliveira et al. 2014
KJ641474.1	Common snook	*C. undecimalis*	Brazil	Oliveira et al. 2014

Table 2, Cont.

GenBank Accession	Common name	Species name	Collection	Author
KJ641475.1	Common snook	*C. undecimalis*	Brazil	Oliveira et al. 2014
AF247436	Common snook	*C. undecimalis*	Florida, USA	Orrell and Carpenter 2004
HQ731428	Common snook	*C. undecimalis*		Li et al. 2011
HQ731414	Longspine snook	*C. armatus*		Li et al. 2011
HQ731415	Longspine snook	*C. armatus*		Li et al. 2012

Table 3

List of locations where samples for genotyping nDNA were collected and the sample size. Each site was inhabited by either juvenile or adult snook (life stage), could be described as either estuarine or oceanic (habitat), or located North or South (geography).

Study site	Life stage	Habitat	Geography	*C. undecimalis*	*C. parallelus* and *C. poeyi*	Total samples collected per site
Aransas Pass (J1)	Juvenile	Estuarine	North	7	32	43
Redfish Bay (A1)	Adult	Estuarine	North	8	12	20
Packery Channel (A2)	Adult	Ocean	North	0	54	54
Arroyo Colorado (J2)	Juvenile	Estuarine	South	20	81	101
Laguna Vista (J3)	Juvenile	Estuarine	South	73	68	141
South Bay (A3)	Adult	Estuarine	South	55	17	72
Brazos-Santiago Pass (A4)	Adult	Ocean	South	0	19	19
San Martin (A5)	Adult	Estuarine	South	10	15	25
Rio Grande Mouth (J4)	Juvenile	Estuarine	South	34	0	35
Rio Grande Slough (J5)	Juvenile	Ocean	South	0	26	26
Total	-			207	329	536

Table 4

Summary of genetic diversity statistics averaged across all microsatellite markers for common snook (*C. undecimalis*), and the small-scale fat snook (*C. parallelus*) and Mexican snook (*C. poeyi*) mixture. n = sample size, A = allelic richness, A_e = effective number of alleles, H_o = average observed heterozygosity across loci, H_e= expected heterozygosity across loci.

Species	Locus	n	A	A_e	H_O	H_e
C. undecimalis	Overall	184	18.222	5.47	0.675	0.824
	SE		2.808	0.77	0.033	0.031
	CUN4A		8	3.316	0.654	0.734
	CUN9		32	8.248	0.568	0.93
	CUN14		19	5.953	0.78	0.87
	CUN16		11	5.481	0.828	0.852
	CUN17		13	3.286	0.605	0.748
	CUN18		24	7.303	0.79	0.901
	CUN19		12	3.111	0.593	0.703
	CUN20		16	3.517	0.667	0.744
	CUN22		29	9.011	0.592	0.934

Species	Locus	n	A	A_e	H_O	H_e
C. parallelus, C. poeyi	Overall	334	15.111	3.873	0.611	0.753
	SE		1.419	0.56	0.061	0.032
	CUN4A		14	4.59	0.76	0.809
	CUN9		22	4.615	0.775	0.806
	CUN14		18	4.695	0.732	0.803
	CUN16		15	2.27	0.486	0.582
	CUN17		13	2.548	0.592	0.675
	CUN18		13	1.883	0.264	0.765
	CUN19		8	2.815	0.415	0.663
	CUN20		13	4.208	0.73	0.787
	CUN22		20	7.232	0.743	0.888

Table 5

Summary of analysis of molecular variance (AMOVA) indicating partitioning of genetic variation on the basis of the sample collections of common snook (*C. undecimalis*) in Texas. df = degrees of freedom and MS = mean square error.

Source of variation	df	Sum of squares	MS	Variance component	Percentage of variation	*F-statistic* (F_{CT}, F_{SC}, F_{IS})	*P*-value
Life Stage	1	6	6.4	0.01	0.1%	0.001	0.104
Location (Life Stage)	5	25	4.9	0.01	0.3%	0.003	0.056
Individual (Loc (LS))	200	867	4.3	0.63	16.9%	**0.17**	**<0.00001**
Within Individuals	207	637	3.1	3.08	82.6%		
Total	413	1535		3.72	99.9%		
Habitat	1	5	5.4	-0.003	-0.1%	-0.001	0.336
Location (Habitat)	5	26	5.3	0.02	0.5%	**0.005**	**0.010**
Individual (Loc (Hab))	200	865	4.3	0.62	16.5%	**0.166**	**<0.00001**
Within Individual	207	641	3.1	3.10	83.1%		
Total	413	1538		3.73	100.0%		
Geography	1	6	5.8	0.02	0.5%	0.005	0.055
Location (Geography)	5	27	5.4	0.02	0.5%	**0.005**	**0.018**
Individual (Loc (Geog))	200	863	4.3	0.60	16.1%	**0.163**	**<0.00001**
Within Individual	207	643	3.1	3.11	82.9%		
Total	413	1538		3.75	100.0%		
						(F_{ST}, F_{IS})	
Among Population	6	33	5.5	0.02	0.6%	**0.006**	**0.001**
Among Individual	200	864	4.3	0.62	16.5%	**0.166**	**<0.00001**
Within Individuals	207	639	3.1	3.09	82.9%		

Table 6

Summary of analysis of molecular variance (AMOVA) for the microsatellite loci of small-scale fat snook (*C. parallelus*) and Mexican snook (*C. poeyi*). df = degrees of freedom and MS = mean square error.

Source of variation	df	Sum of squares	MS	Variance component	Percentage of variation	*F-statistic* (F_{CT}, F_{SC}, F_{IS})	*P*-value
Life Stage	1	4	3.9	-0.01	-0.2%	-0.002	0.338
Location (Life Stage)	7	40	5.7	0.03	0.9%	**0.009**	**<0.00001**
Individual (Loc (LS))	319	1183	3.7	0.40	12.1%	**0.121**	**<0.00001**
Within Individuals	328	953	2.9	2.90	87.3%		
Total	655	2179		3.33	100.1%		
Habitat	1	6	5.6	-0.003	-0.1%	-0.001	0.135
Location (Habitat)	7	38	5.5	0.03	0.8%	**0.008**	**<0.00001**
Individual (Loc (Hab))	319	1182	3.7	0.39	11.8%	**0.118**	**<0.00001**
Within Individual	328	958	2.9	2.92	87.5%		
Total	655	2184		3.34	100.0%		
Geography	1	8	8.1	0.01	0.2%	**0.002**	**0.019**
Location (Geography)	7	36	5.2	0.02	0.7%	**0.007**	**<0.00001**
Individual (Loc (Geog))	319	1177	3.7	0.38	11.3%	**0.114**	**<0.00001**
Within Individual	328	964	2.9	2.94	87.8%		
Total	655	2185		3.34	100.0%		
						(F_{ST}, F_{IS})	
Among Population	8	44	5.5	0.03	0.8%	**0.008**	**<0.00001**
Among Individual	319	1186	3.7	0.41	12.3%	**0.124**	**<0.00001**
Within Individuals	328	952	2.9	2.90	87.0%		

Table 7

Pairwise population structure results for 9 microsatellite loci between sites listed on Table 3. Listed are F_{ST} values (lower diagonal) and probability that $F_{ST} = 0$ (upper diagonal). Boldface indicates significance after correcting for false discovery rate (Benjamini and Hochberg, 1995; Benjamini et al., 2006).

C. undecimalis

	J1	A1	J2	J3	A3	A5	J4
J1	--	0.557	0.522	0.041	0.245	0.062	0.242
A1	0.000	--	0.609	0.104	0.606	0.616	0.178
J2	0.000	0.000	--	0.341	0.760	0.558	0.561
J3	0.019	0.012	0.001	--	**<0.001**	0.282	**<0.001**
A3	0.006	0.000	0.000	**0.010**	--	0.710	0.703
A5	0.023	0.000	0.000	0.004	0.000	--	0.784
J4	0.006	0.008	0.000	**0.013**	0.000	0.000	--

C. parallelus - C. poeyi

	J1	A1	A2	J2	J3	A3	A4	A5	J5
J1	--	0.378	0.631	0.215	0.089	0.302	0.385	0.043	0.257
A1	0.002	--	0.024	0.365	0.063	**0.005**	0.178	**0.004**	0.166
A2	0.000	0.028	--	**0.002**	**0.003**	0.673	0.089	0.015	0.166
J2	0.003	0.002	**0.031**	--	0.212	**0.005**	0.272	0.063	0.121
J3	0.013	0.025	**0.040**	0.002	--	**0.012**	0.356	0.029	0.285
A3	0.004	**0.043**	0.000	**0.043**	**0.056**	--	0.197	**<0.001**	0.209
A4	0.003	0.012	0.014	0.004	0.001	0.010	--	**0.0002**	**0.006**
A5	0.040	**0.057**	0.049	0.023	0.047	**0.060**	**0.045**	--	**<0.001**
J5	0.013	0.014	0.017	0.011	0.004	0.008	**0.020**	**0.057**	--

List of Figures

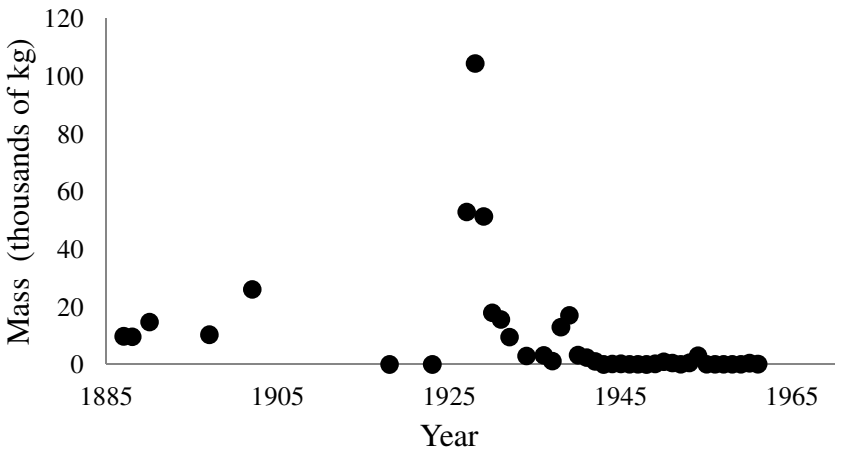

Figure 1

Reported commercial landings of *Centropomus spp.* in Texas from 1887-1961. Landing data were obtained from Matlock and Osburn (1987).

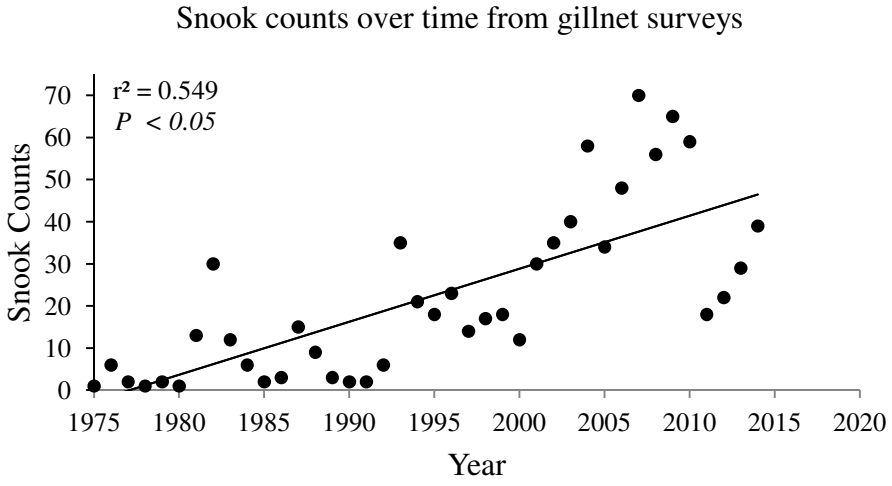

Figure 2

Texas Parks and Wildlife Department conducts bi-yearly gillnet surveys, which uses 7.6–15.2-cm stretch mesh in four 45.7-m sections that are fished for 10-week periods once in the spring and once in the fall (Martinez-Andrade and Fisher, 2012). The number of snook caught during these surveys have increased since 1975 (unpublished data).

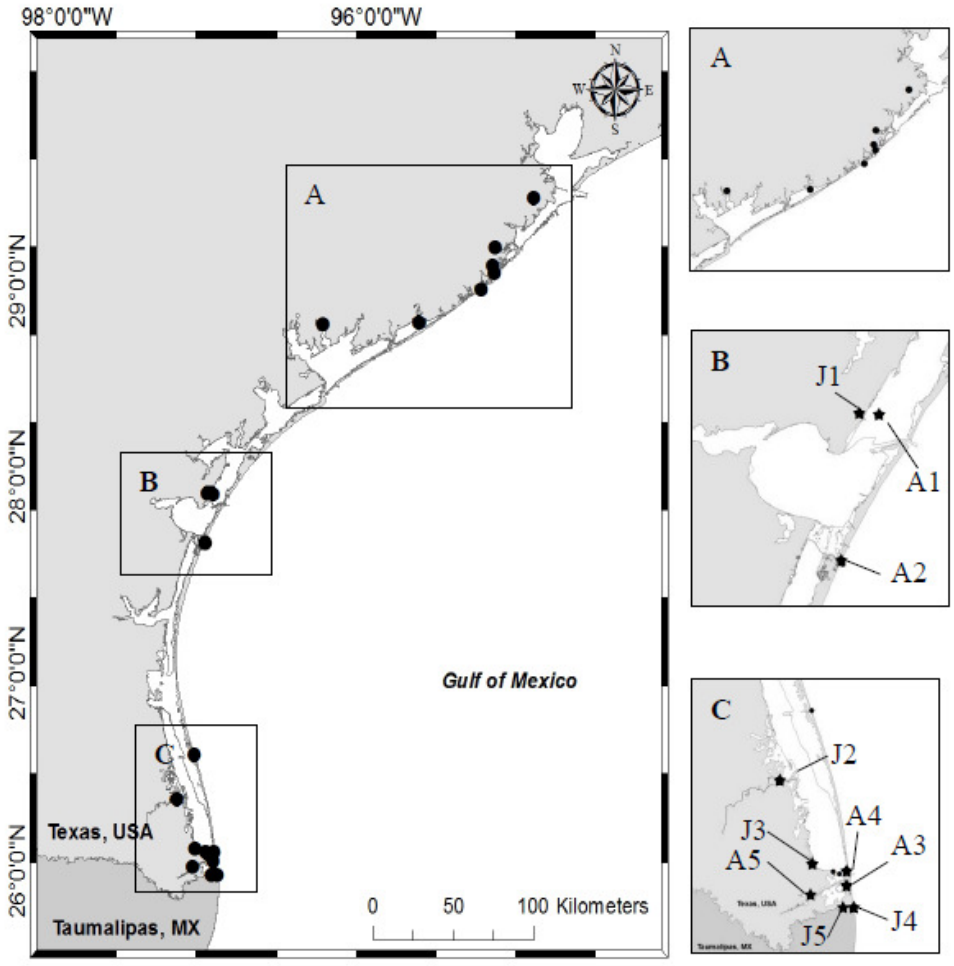

Figure 3

Map of sampling locations along the Texas coast of the Gulf of Mexico. Panels A, B, and C are magnified. 16S mitochondrial DNA was sequenced from snook collected in study sites. Sites where samples were genotyped with microsatellites in panels B and C only are marked with a star. Sample sizes were too small for population-genetic analysis at all other sites.

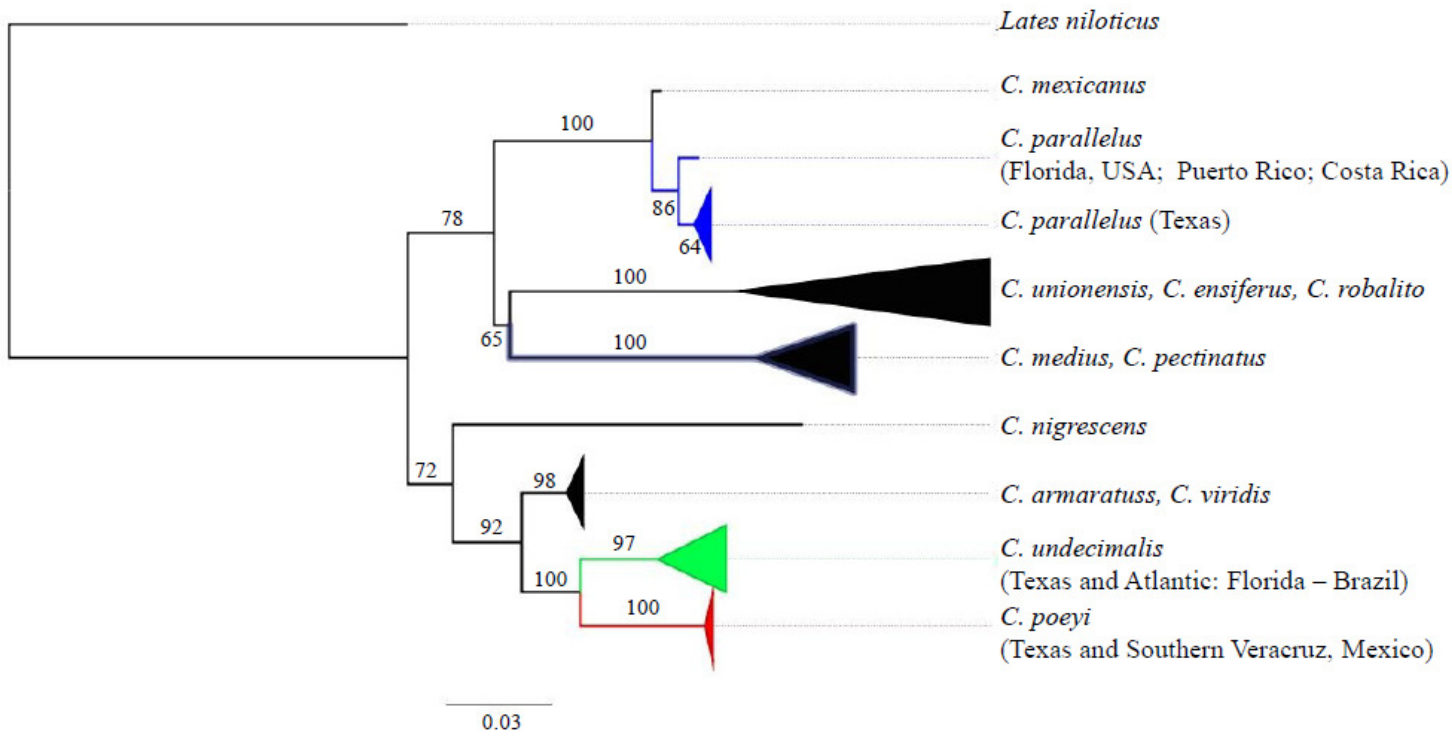

Figure 4

Maximum likelihood phylogenetic tree using l6S mtDNA sequences from *Centropomus spp*. collected in this study and all previously published 16S sequences in GenBank (see Table 2). Bootstrapped branch support values greater than 50 are shown.

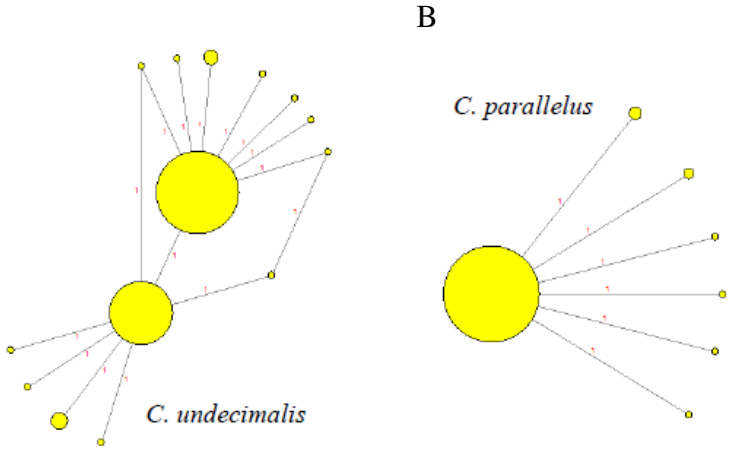

Figure 5

Haplotype network for common snook (*C. undecimalis*), and small-scale fat snook (*C. parallelus*). Size of circles indicates number of individuals with that haplotype. The number on the branches are the number of nucleotide differences between haplotypes.

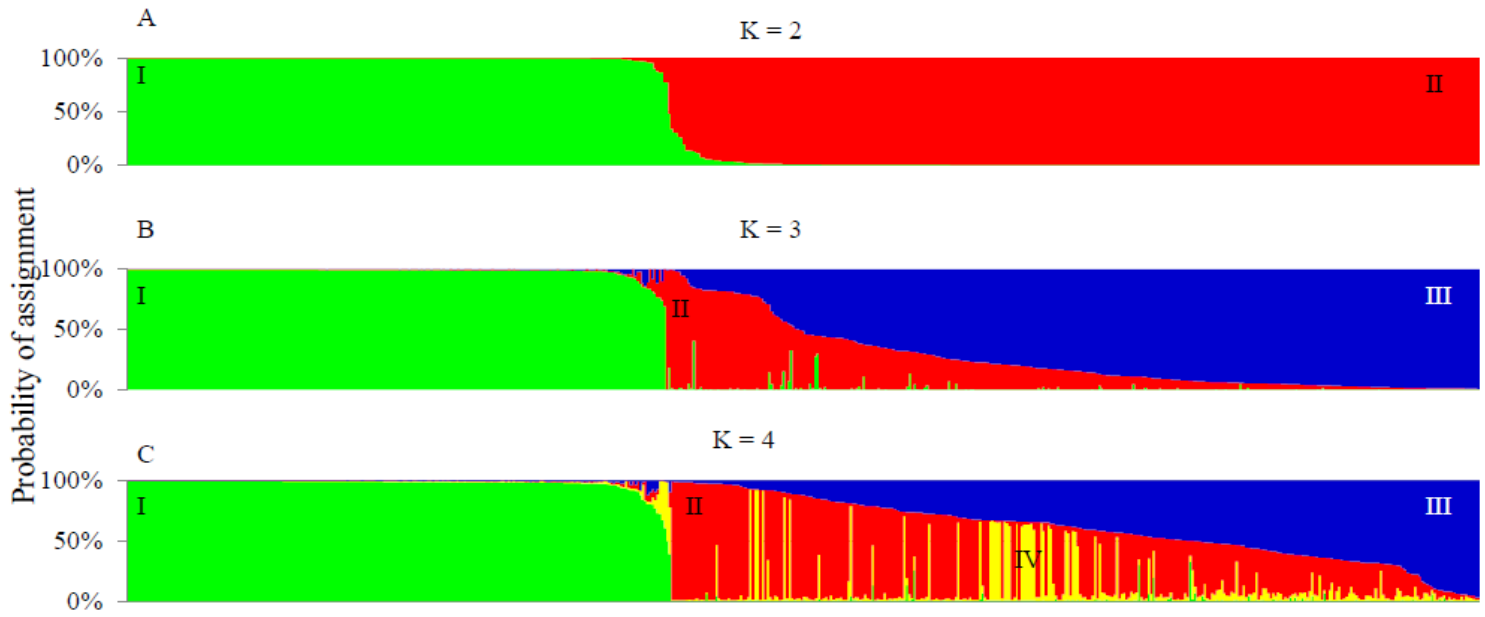

Figure 6

Results of genetic analysis with the use of STRUCTURE for K = 2 (Panel A), K = 3 (Panel B), and K = 4 (Panel C). Each column represents an individual sample and the colors represent different genetic groups: group I (green), group II (red), group III (blue), and group IV (yellow). Multiple colors within a column indicates probability of assignment to multiple genetic groups.

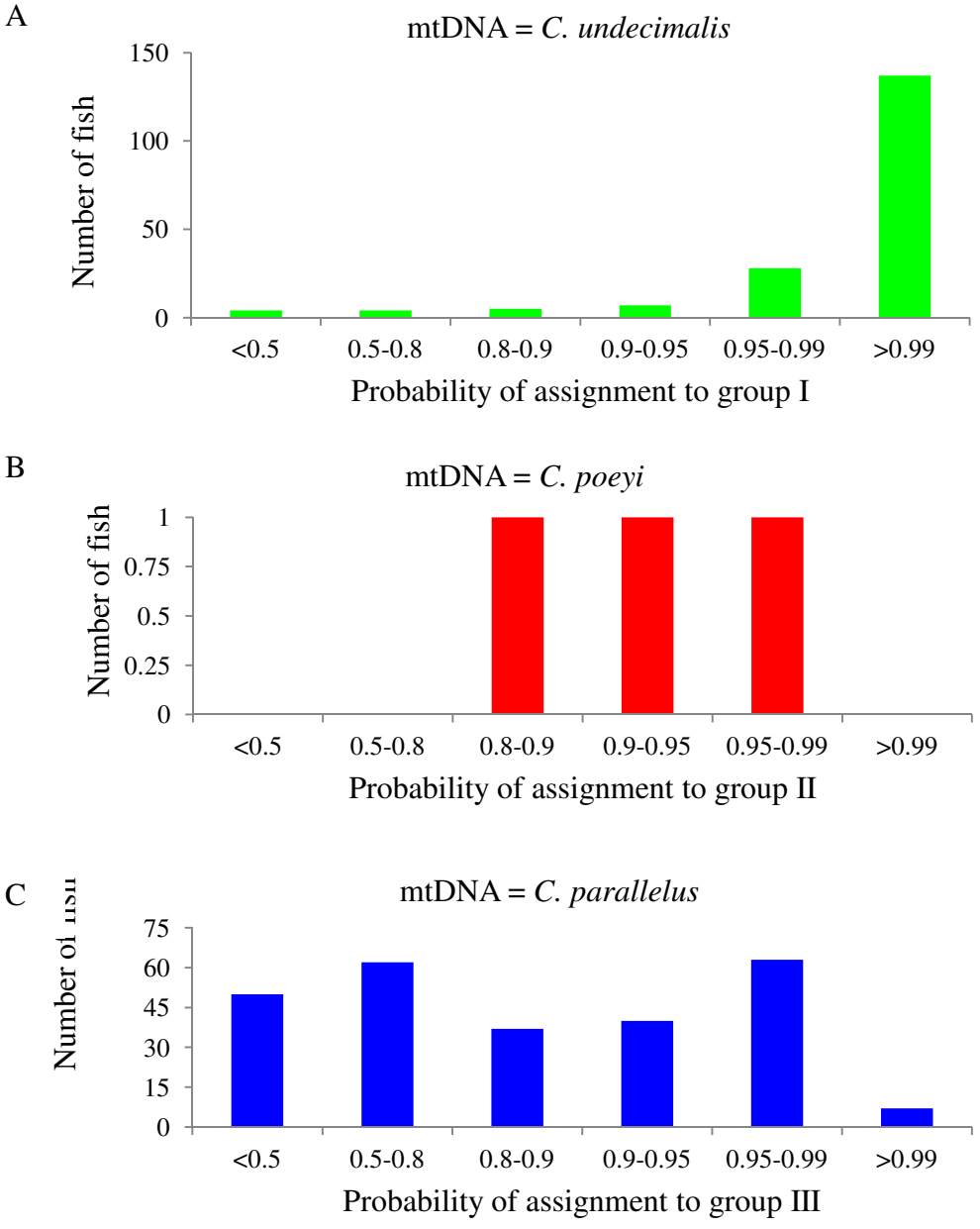

Figure 7

Histograms depicting the assignment of (A) *Centropomus unidecimalis,* (B) *C. poeyi,* and (C) *C. parallelus* identified with mtDNA to genetic groups (A) I, (B) II, and (C) (III) defined by a STRUCTURE analysis of the nuclear microsatellite loci. .

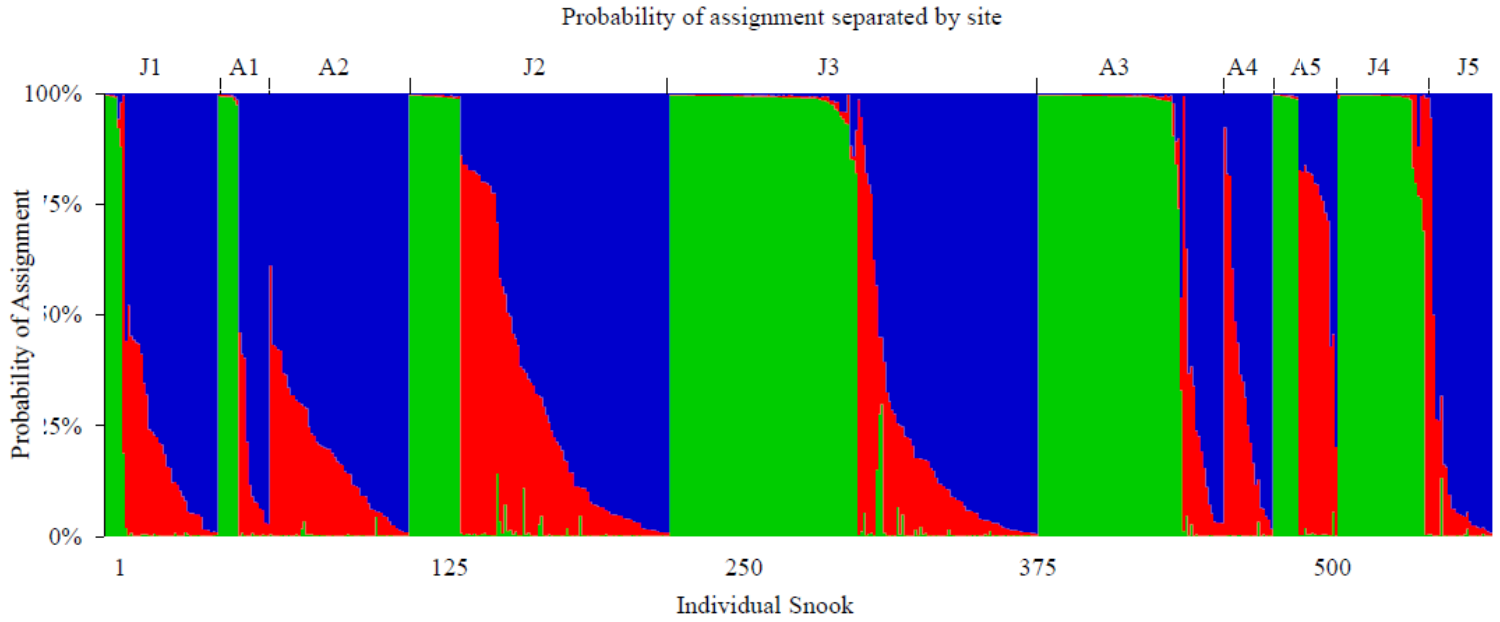

Figure 8

STRUCTURE results for K = 3 were separated by site to check for geographic bias in genetic group assignment. The pattern remained generally the same.

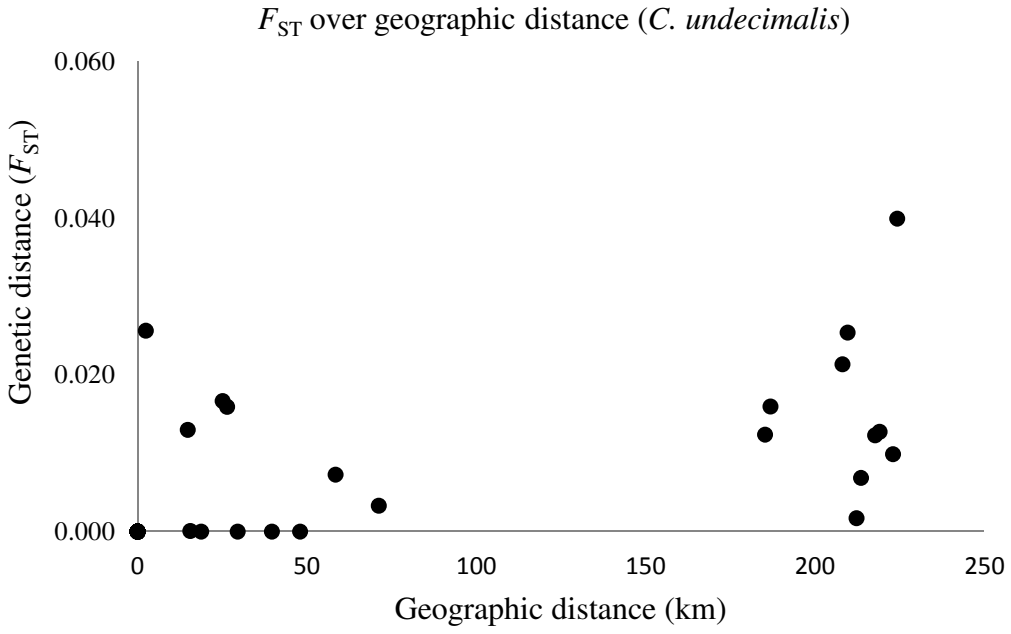

Figure 9

Pairwise F_{ST} values plotted over absolute minimum travel distance by water reveal no evident pattern of isolation by distance ($r^2 = 0.15$, $P = 0.0704$) for common snook (*C. undecimalis*).

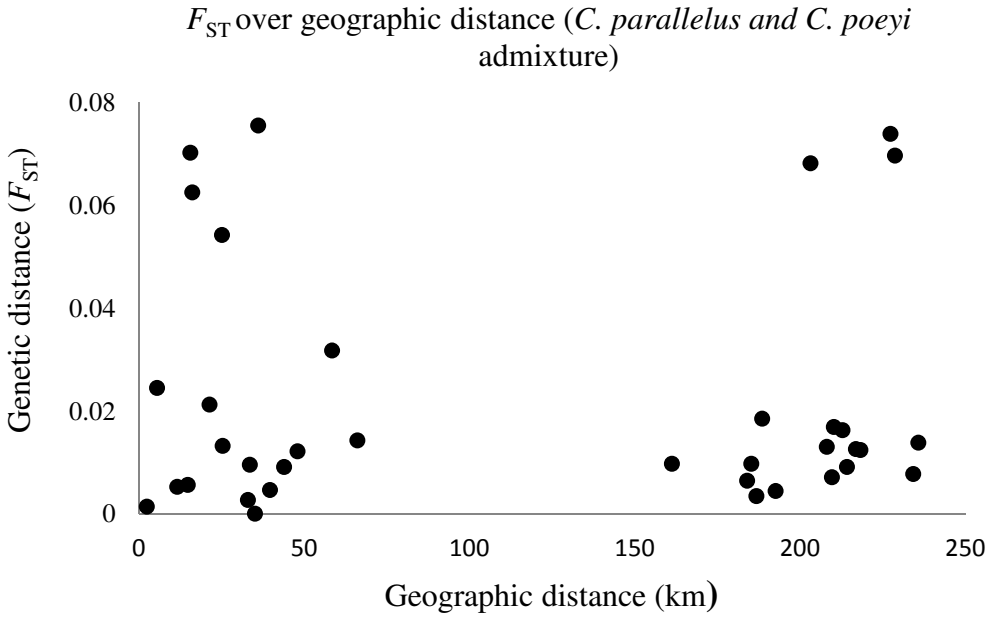

Figure 10

Pairwise F_{ST} values plotted over absolute minimum travel distance by water reveal no evident pattern of isolation by distance ($r^2 = 0.001$, $P = 0.41$) for small-scale fat snook (*C. parallelus*) and Mexican snook (*C. poeyi*) mix.

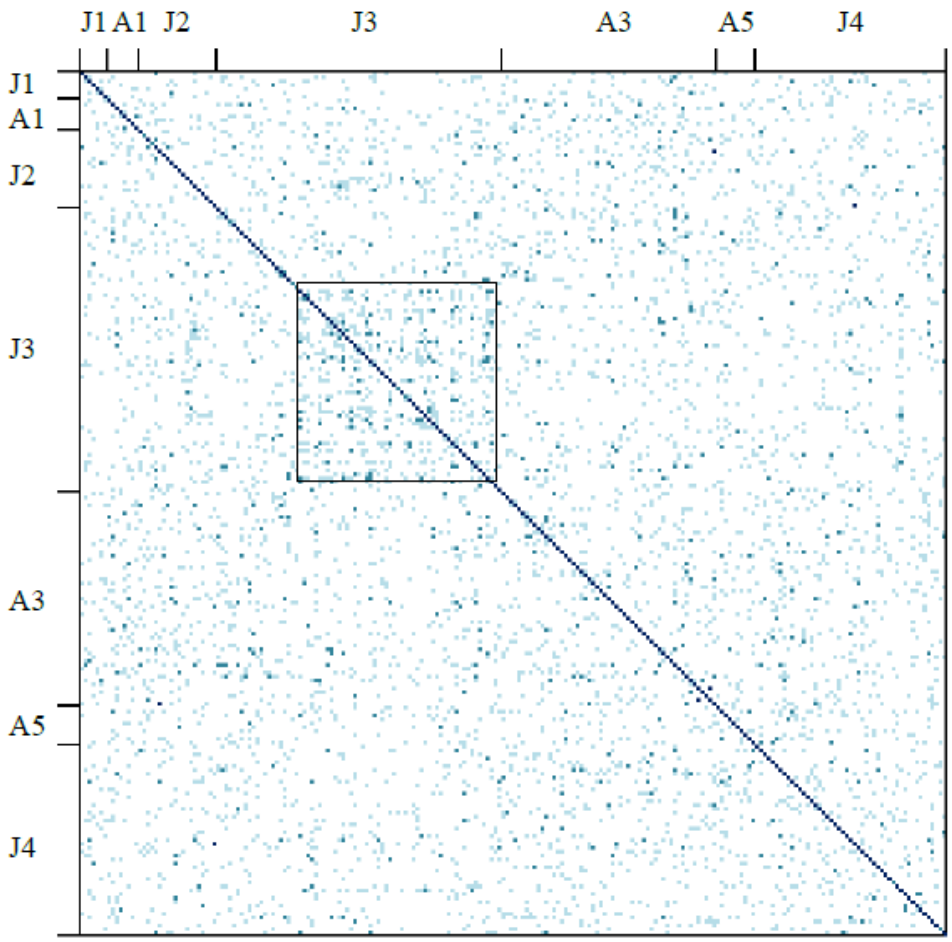

Figure 11

Pairwise kinship heatmap for *Centropomus undecimalis*. Coancestry coefficients were binned as follows: 0.57 > k > 0.375; 0.375 > k > 0.1875; 0.1875 > k > 0.09375; 0.09375 > k > 0.047; and k < 0.047. Darker colors represent higher coancestry coefficients while lighter colors represent lower coancestry coefficients. Sampling sites are separated by tick marks on the left and top of the heatmap. There is a concentration of elevated kinship above the random expectation within sample site J3 (juvenile site#3, Laguna Vista Ditch).

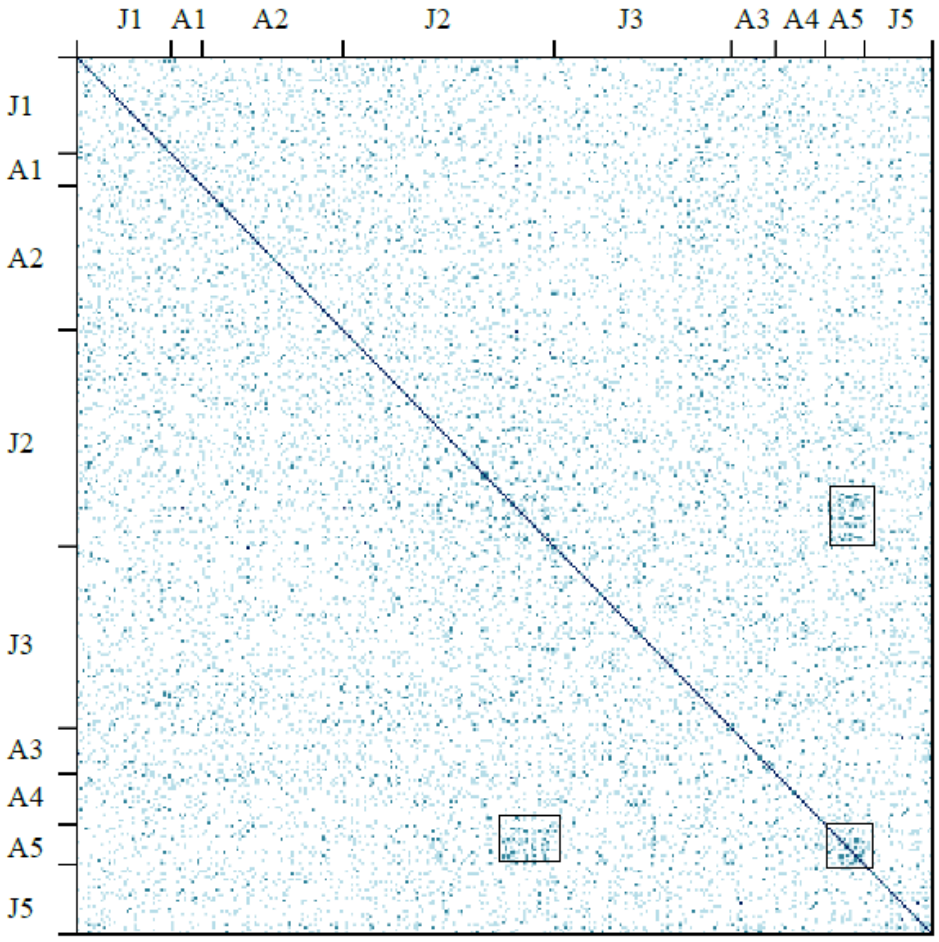

Figure 12.

C. parallelus and *C. poeyi* pairwise kinship matrix featuring coancestry coefficients binned following same parameters as in Figure 11. Darker colors represent higher coancestry coefficients while lighter colors represent lower coancestry coefficients. Sampling sites are separated by tick marks. Elevated kinship coefficients, marked by black boxes, were detected between site A5 (adult site#5, San Martin) and a subsection of site J2 (juvenile site#2, Arroyo Colorado), within site A5.