Deep–Sea Research I 158 (2020) 103222

Contents lists available at ScienceDirect

Deep-Sea Research Part I

journal homepage: http://www.elsevier.com/locate/dsri

Population genomics of three deep-sea cephalopod species reveals connectivity between the Gulf of Mexico and northwestern Atlantic Ocean

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ARTICLE INFO

Keywords:
ddRADseq
Cephalopoda
Sanger sequencing
Diversity
Connectivity
Gulf of Mexico
Bear seamount
Population genetics

ABSTRACT

Despite the ecological importance of deep-sea cephalopods, little is known about their genetic diversity or population dynamics. The cephalopod species *Cranchia scabra*, *Pyroteuthis margaritifera*, and *Vampyroteuthis infernalis* are commonly collected in midwater samples from both the Gulf of Mexico and northwestern Atlantic Ocean but, despite their common appearance in trawls and important roles in marine food webs, no genetic studies of population connectivity exist for these species. Here, Sanger sequencing of three conserved genetic loci and ddRADseq techniques were used to examine population genetic dynamics in these deep-sea species. Genetic diversity is lowest in *C. scabra*, which appears to be in a population growth stage, and highest in *V. infernalis*. Population structure was unique to *V. infernalis* but does not appear to be the result of ocean-basin vicariance, thus possible alternative explanations are explored, specifically environmental variation in dissolved oxygen. The genetic connectivity between these geographically disparate sites suggests these three cephalopod species could be resilient to localized environmental disturbances in the Gulf of Mexico.

1. Introduction

Molecular approaches are powerful tools to investigate life history and population dynamics in enigmatic species, such as cephalopods (e.g. Dai et al., 2012; Vecchione et al., 2015; Judkins et al., 2016). This is especially true for midwater offshore species, which are difficult to collect and cannot be kept alive for study onshore. These difficulties have resulted in a paucity of information about their natural history (Hoving et al., 2014). Until recently, studies involving large samples of oceanic cephalopods in the Gulf of Mexico have been limited (Judkins et al., 2013, 2016). With so few specimens available, population genetics/genomics studies can provide unprecedented insight into the state and flux of diversity in these elusive species and allow us to make inferences regarding their life histories (Domínguez-Contreras et al., 2018).

Ecologically, cephalopods are crucial links in the world oceans: as predators of nekton and zooplankton, and as major food sources for nektonic fishes and mammals (Judkins, 2009). Cephalopod life-history strategies (high growth rates and short lifespans) allow them to contend with rapidly changing environmental conditions such as extreme climate change and anthropogenic influences like overfishing, pollution, etc. (Doubleday et al., 2016). Less is known about the impact of other aspects of life history (e.g., reproduction rates, development, migration patterns, diets, and diel vertical migratory (DVM) behavior) on cephalopod survival and adaptation (Hoving et al., 2014).

This study targets three cosmopolitan deep-sea cephalopod species with differing DVM behaviors: *Cranchia scabra* Leach, 1817 (Cranchiidae) (Fig. 1A), *Pyroteuthis margaritifera* (Ruppell, 1844) (Enoploteuthidae) (Fig. 1B), and *Vampyroteuthis infernalis* Chun, 1903 (Vampyroteuthidae) (Fig. 1C). These three species are abundant in the Gulf of Mexico and the western North Atlantic Ocean. Geographically, *C. scabra* is found in tropical and sub-tropical oceans worldwide (Jereb and Roper, 2010; Young and Mangold, 2016). In the Gulf, *C. scabra* occupies a large section of the water column, from the surface down to 1500 m depth (Fig. 1D), with little evidence of ontogenetic shift or vertical migration (Judkins and Vecchione, 2020, in press). *Pyroteuthis margaritifera,* the small (<5 cm mantle length) jewel Enope squid, is found throughout the water column in the North Atlantic and the Gulf,

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https://doi.org/10.1016/j.dsr.2020.103222
Received 24 March 2019; Received in revised form 19 December 2019; Accepted 7 January 2020
Available online 10 January 2020

vertically migrating from the upper mesopelagic zone (~600 m depth) during the day to the surface and epipelagic zone at night (Young and Mangold, 2009) (Fig. 1E). *Vampyroteuthis infernalis* (the vampire squid) is found worldwide in tropical and temperate oceans (Jereb and Roper, 2010; Young, 2016), between 600 m–1500 m deep (Judkins and Vecchione, 2020, in press) (Fig. 1F).Vampire squid are unique in terms of reproductive cycle (evincing multiple reproductive cycles in their lifespan, instead of the single cycle characteristic of other coleoid cephalopods (Hoving et al., 2015)), and behavior (often passively feeding on marine snow in oxygen minimum zones (OMZs), unlike the active carnivory characteristic of other cephalopods (Hoving and Robison, 2012)). To date, only a few studies have examined large numbers of oceanic cephalopods in the Gulf of Mexico (Judkins et al., 2013, 2016) and, to date, no genetic studies have been undertaken to explore genetic connectivity or population dynamics in these three species.

Population genetic studies are sometimes the only realistic means of inferring life history and broader ecology of enigmatic species that are both difficult to observe and complicated to collect (Dai et al., 2012; Vecchione et al., 2015; Judkins et al., 2016; Domínguez-Contreras et al., 2018). Two metrics targeted in population genetics studies, genetic diversity and population structure, provide especially valuable information about the species at large, namely health and resilience, respectively (Cowen and Sponaugle, 2009; Danovaro et al., 2008; Hellberg et al., 2002; Hughes and Stachowicz, 2004). Genetic diversity is the number of alleles present within a population or species (Wright, 1931). Higher genetic diversity is characteristic of a healthy population, increasing a population's or species' ability to adapt to new environments or changing environmental conditions (Cowen and Sponaugle, 2009; Danovaro et al., 2008; Hughes and Stachowicz, 2004). Population structure can provide insight into historical gene flow, migration, and demography of a population, such as recent geographic separation or re-introduction and population size changes (Cowen et al., 2007). Ecologically, population structure can inform our understanding of species resilience: in the event of a localized perturbation, lack of structure among geographically separated groups can indicate gene flow between the affected group and a functional genetic reservoir outside the affected area (Cowen and Sponaugle, 2009; Hellberg et al., 2002).

This study focuses on the population genetics of three cephalopod species in the Gulf of Mexico and northwestern Atlantic. The biological importance of the Gulf of Mexico (Backus et al., 1977; Gartner, 1988; Sutton et al., 2017), in light of the frequent natural and anthropogenic perturbations it experiences (Kaiser, 2015; Soto et al., 2014; Murawski et al., 2018), is strong motivation for comparative study of population genetics in common Gulf species. Moreover, the information generated here fills a large and important data gap, namely by establishing reference states for the targeted species. Here, we define a "reference state" as a description of standing genetic variation and inferred connectivity in a species. Because so little is known about the biological impacts of the aforementioned disturbances, much less the rates and means of recovery, it is not possible to define a normal state or biological baseline with any degree of confidence.

To estimate this reference state, we used a powerful next-generation sequencing (NGS) method, double digest Restriction site Associated DNA sequencing (ddRADseq, as described by Peterson et al., 2012), in combination with traditional single-locus Sanger sequencing of three genes. This approach provided several notable benefits: Sanger data allowed us to decisively confirm species identifications with DNA barcoding and take advantage of analyses that have not yet been optimized for NGS, such as Tajima's D (Arnold et al., 2013). By conducting ddRADseq research in parallel, we were also able to complete the most comprehensive and statistically powerful analyses of genetic diversity and connectivity to date.

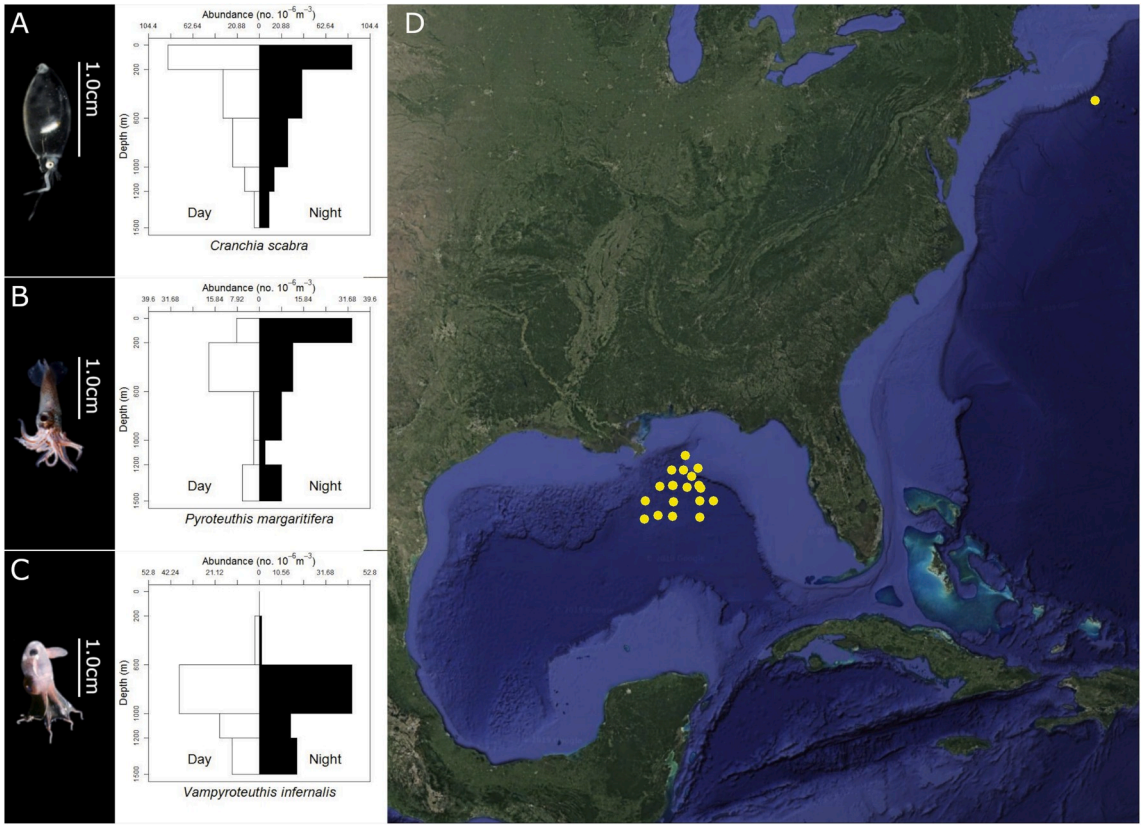


Fig. 1. Three species targeted for analysis and their associated depth distributions: (A) *Cranchia scabra*, (B) *Pyroteuthis margaritifera*, and (C) *Vampyroteuthis infernalis*. Scale bars of 1.0 cm are displayed alongside each species. Sampling locations are displayed in D. Map taken from Google Earth. *Cranchia scabra* and *Pyroteuthis margaritifera* photo credit: Danté Fenolio. *Vampyroteuthis infernalis* photo credit: David Wrobel.

Utilizing this integrative approach, our overall objective was to improve our understanding of population dynamics of midwater cephalopods in the Gulf of Mexico and the northernwestern Atlantic by establishing reference states for three species (*C. scabra*, *P. margaritifera*, and *V. infernalis*). We sought to 1) describe the organization of genetic diversity within each species between the Gulf of Mexico and the Atlantic; 2) characterize the role of population connectivity in maintaining this organization; and 3) use these insights to make inferences about life history, ecology, and species health. To the best of our knowledge, the work we present here represents the first comparative population genomics study of deep-sea cephalopods.

2. Methods

2.1. Sampling effort

Deep-sea cephalopod specimens were collected across 18 stations in the northern Gulf of Mexico over a three-year period (2015–2017) through the DEEPEND Project (Deep Pelagic Nekton Dynamics of the Gulf of Mexico), as well as near Bear Seamount in the northwestern Atlantic Ocean (39°55′N 67°30′W) in 2014 (Shea et al., 2017) (Fig. 1G). *Cranchia scabra*, *P. margaritifera*, and *V. infernalis* were common in both collections. Specimens were collected from the Gulf of Mexico with a 10 m^2 mouth area Multiple Opening/Closing Net and Environmental Sensing System (MOC-10) rigged with six 3 mm mesh nets (Sutton et al., 2015). Bear Seamount specimens were caught using a dual-warp midwater trawl. Specimens used in this study are housed at the National Museum of Natural History in Washington, D.C.

Gulf tissue samples were directly frozen at −20°C in RNALater, while tissue samples from Bear Seamount were directly frozen at −20°C in 95% ethanol. Respective voucher specimens were fixed in 10% formalin, and later transferred to 50% isopropyl alcohol. In total, 107 specimens were sampled for genetic data: 46 *C. scabra* (35 Gulf, 11 Atlantic), 29 *P. margaritifera* (17 Gulf, 12 Atlantic), and 32 *V. infernalis* (27 Gulf, 5 Atlantic). See Supplementary Table 1 for all sample metadata, GenBank Accession numbers associated with *de novo* Sanger sequences, and NCBI SRA Accession numbers associated with the NGS data set.

2.2. DNA extraction, PCR, and sanger sequencing

Tissue samples (12–24 mg) were excised for DNA extraction with the Qiagen DNeasy Blood and Tissue Kit, following the manufacturer's protocol. To maximize the information gained through Sanger sequencing, three genetic loci (cytochrome c oxidase subunit I (COI), 16S ribosomal RNA gene (16S), and 28S ribosomal RNA gene, D3 region (28S)) were targeted. Each differ in evolutionary rate (Hwang and Kim, 1999; Aguilera-Munoz et al., 2008; Lindgren, 2010; Allcock et al., 2011, 2015) and were amplified via polymerase chain reaction (PCR) prior to sequencing. Resulting sequences were used to corroborate field identifications and examine intraspecific diversity.

PCRs contained the following in a 25-μl final reaction volume: 12.5 μl GoTaq DNA Polymerase (Promega), 1 μl of each primer, 8.5 μl of sterile distilled water, and 2 μl of diluted template DNA. Template DNA was diluted to a 1:10 ratio for all reactions; there were some cases where template DNA had to be diluted to 1:100 and 1:1000 to reduce inhibitors and enable successful amplification. PCR conditions were as follows: 5 min at 94 °C, 35 cycles of [1 min at 94 °C, 1 min at annealing temperature, 1 min at 72 °C] followed by a 10 min extension at 72 °C. All PCR products were visualized with gel electrophoresis, run at 120 V for 60 min in a 1.5% agarose gel stained with ethidium bromide. Information on primers, annealing temperatures, and amplicon sizes are recorded in Supplementary Table 2.

PCR products were sent for commercial clean-up and bidirectional Sanger sequencing (Sanger et al., 1977) at the Genewiz sequencing facility. In cases where direct sequencing did not yield high-quality sequences, PCR products were cloned using the CloneJET PCR Cloning Kit (Thermo Scientific) and commercially sequenced using vector primers, following the same protocol used in Judkins et al. (2016). The resulting raw forward and reverse sequences were trimmed for quality and assembled into contigs using Geneious v.9.3 (Kearse et al., 2012). Assembled sequences with minimum average quality scores under 85 were excluded from downstream analyses. Sequences were imported into MEGA7 (Kumar et al., 2016) to trim primers before aligning with the 6mer-pair algorithm in MAFFT v6.2 (Katoh and Standley, 2013). All final Sanger sequences are available in GenBank (accession numbers are listed in Supplementary Material 1).

The alignment was visually inspected for errors and the reading frame and codon position of mitochondrial COI was also determined in MEGA7 to prevent the inclusion of nuclear pseudogenes (Song et al., 2008). Mitochondrial single-locus data were concatenated across genes (16S+COI) by unique individual identifier to generate mitochondrial and nuclear Sanger data sets for population genetics analysis.

Finally, the NCBI Basic Local Alignment Search Tool (BLASTN) (Altschul et al., 1990) was used to compare sequences to the GenBank database (Lindgren, 2010; Dai et al., 2012). Several sequences from individuals that were taxonomically identified in the field as *Pyroteuthis margaritifera* more closely matched *Pterygioteuthis* species in Genbank and were excluded from downstream analyses.

2.3. Next-generation sequencing with double digest RADseq

Library Preparation Of the individuals included in the Sanger dataset, 96 were found to have high molecular weight DNA in suitable quantities to be included in the ddRADseq study (Table 1), including 41 *C. scabra* (30 Gulf, 11 Atlantic), 30 *P. margaritifera* (18 Gulf, 12 Atlantic), and 25 *V. infernalis* (21 Gulf, 4 Atlantic). Reduced representation libraries were prepared according to the double digest RADseq (ddRADseq) method (Peterson et al., 2012) using EcoRI and SphI (New England Biolabs). Based on the barcoded adapters utilized by Peterson et al. (2012), custom adapters were synthesized and annealed to the fragments resulting from double digest. This allowed the pooling of individuals into sublibraries, which were size selected for 275 bp on a PippinPrep (Sage Science). Following size selection, sublibraries were amplified via PCR with Phusion Hi-Fidelity Polymerase (Thermo Scientific; 10 cycles). This step also incorporated indices (i7) and Illumina adapters into the fragments and allowed for pooling of sublibraries into the final library. Quality of the final library was ascertained on an Agilent BioAnalyzer 2100 (Agilent Technologies) before the library was sent for sequencing on an Illumina HiSeq, PE150, at the Genewiz Facility in South Plainfield, New Jersey.

Quality Filtering and Data Assembly Generally, Mastretta-Yanes et al. (2014), Paris et al. (2017), and Rochette and Catchen (2017) can

Table 1
Descriptions of the ddRADseq data sets for each species, including: the number of individuals included in the data set (N) by basin (overall/Atlantic/Gulf of Mexico), the number of single-nucleotide polymorphisms retained post-missing data filter (SNPs), the percent missing data deemed allowable for each data set (Percent Missing Data), and the number of raw reads from each species (No. Raw Reads). Tajima's D values for each species were calculated using the concatenated Sanger data. F statistics were calculated with Analysis of Molecular Variance (AMOVA) on ddRADseq data under the Infinite Allele Model. Significance was tested using 999 permutations. * is used to indicated statistically significant results.

	C. scabra	P. margaritifera	V. infernalis
N	32/23/9	23/12/11	15/11/4
SNPs	1205	1430	1542
Percent Missing Data	<10%	<15%	<10%
No. of Raw Reads	151,855,707	106,020,443	23,472,632
Tajima's D	−2.118*	−2.048*	−1.396
F_{IT}	82.5%	65.5%	64.2%
F_{IS}	17.8%*	34.1%*	37.6%*
F_{ST}	−0.3%	0.3%	−2.1%

be consulted in selecting parameter settings. Raw sequence files were quality-filtered, aligned, and assembled with the STACKS v1.45 (Catchen et al., 2013) on the Florida International University High Performance Computing Cluster (HPCC). Reads were demultiplexed, cleaned (-c), and quality-filtered (-q) with the process_radtags program. Identical reads were aligned within each individual in ustacks, and putative paralogs were excluded by setting the maximum Hamming distance between reads in a stack to 2 (-M = 2). The inclusion of paralogs can result in a stack of highly repetitive reads: the ustacks program automatically removes these stacks. Consensus reads were catalogued in cstacks. All putative loci were matched against the catalog with sstacks before individual genotype calls were corrected according to accumulated population data in rxstacks. Finally, the populations tool was used to generate a file of aligned single nucleotide polymorphisms (SNPs). For a SNP to be called, it had to meet a minimum read depth (-m = 5). SNPs also had to be present in 25% of the individuals of a population (-r = 0.25) to be called for that population. To be retained, a SNP had to be present in both the Atlantic group and the Gulf; this facilitated comparison between basins as sites only present in samples from one basin or the other would not contribute to inter-basin analyses. Only one random SNP was called per locus (–write_random_snp) to generate a final alignment of unlinked SNPs. We iteratively applied a missing data filter to remove loci and individuals with missing data above a given threshold, ultimately allowing 10%–15% missing data at each site and within each individual. We used BayeScan v2.1 (Foll and Gaggiotti, 2008) to identify F_{ST} outliers within each data set: the Reversible Jump MCMC algorithm executed 20 pilot runs of 5000 generations with a 50% burn-in. Uniform priors were placed on F_{IS} to bound it between 0 and 1. F_{ST} outliers were identified by plotting the results in R, with a False Discovery Rate of 5%, and identified sites were removed.

2.4. Data analysis

Genetic Diversity and Historical Population Dynamics The selection coefficient (Tajima's D) was calculated for the concatenated Sanger data sets in DNAsp v5 (Librado and Rozas, 2009). Several genetic diversity indices were also calculated from the full NGS data sets in GENODIVE v2.0b23 (Meirmans and Van Tienderen, 2004), including observed, expected, and total heterozygosity (H_O, H_E, and H_T, respectively). Standard deviation was calculated through jackknifing. Genetic diversity was visualized with haplotype networks of the mitochondrial (16S and COI) and nuclear (28S) alignments in PopART (Leigh and Bryant, 2015).

To estimate historical population size for each species, we executed Extended Bayesian Skyline Plot (EBSP) analyses in BEAST2 (Bouckaert et al., 2014). Single-locus alignments were loaded individually (16S, COI, and 28S) into BEAUTi2 to set parameters. The COI alignment was divided by codon position (1+2+3) and site model parameters were set according to the results of PartitionFinder (Lanfear et al., 2012). For all data sets, the clock rate was set by the 16S alignment with a clock rate of 0.5 and the clock rates for the COI and 28S alignments were estimated in relation to the 16S alignment, as this was likely to be the fastest evolving locus. All additional parameters were set according to the manual, with the exception of the MCMC parameters: 200,000,000 generations were run, logged every 5000th. To ensure convergence was reached, trace files were examined in Tracer v1.6 (Rambaut et al., 2014).

Population Structure Analysis Genetic distances due to population differentiation (F_{ST}) were calculated in GenoDive v2.0b23 (Meirmans and Van Tienderen, 2004) with 999 permutations to assess significance. A hierarchical Analysis of Molecular Variance (AMOVA) was calculated using the Infinite Allele Model with 999 permutations to assess significance. Missing data were replaced with randomly drawn alleles determined by overall allele frequencies.

To test for population structure, *K*-means clustering was conducted in the Bayesian program STRUCTURE v2.3.4 (Pritchard et al., 2000). K = 1–7 were each tested 10 times under the admixture model with 200,000 Markov Chain Monte Carlo generations following a burn-in of 20,000 generations. STRUCTURE results were collated in STRUCTURE HARVESTER v0.6.94 (Earl and VonHoldt, 2012) wherein *ad hoc* posterior probability models (Pritchard et al., 2000) and the Evanno method (Evanno et al., 2005) were used to infer the optimal K value. The final DISTRUCT plots were generated and edited using STRUCTURE PLOT v2.0 (Ramasamy et al., 2014).

We also applied two additional, non-model based methods for inferring and visualizing population structure: multi-dimensional scaling (MDS) plots were rendered for each data set using the R package MASS (Venables and Ripley, 2002). MDS is very similar to Principal Component Analysis (PCA), with the exception that PCA preserves covariance within the data while MDS preserves distance, or dissimilarity, between points. Finally, we utilized affinity propagation (Frey and Dueck, 2007) to infer the number of clusters found within each data set. Affinity propagation clusters data based on similarity between loci, and unlike k-means clustering, selects all data points as exemplars as opposed to one data point. This allows us to ascertain whether $K = 7$ is a suitable cutoff for k-means clustering and provides additional support for the number of true populations within the sample.

3. Results

3.1. Data set descriptions

Sanger In *Cranchia scabra*, 127 *de novo* sequences were generated: 42 of 16S (514 bp), 46 of COI (657 bp), and 39 of 28S (620 bp). Sequence analysis of *Pyroteuthis margaritifera* resulted in 86 *de novo* sequences: 29 of 16S (507 bp), 29 of COI (657 bp), and 28 of 28S (519 bp). Finally, sequencing efforts in *Vampyroteuthis infernalis* yielded 81 *de novo* sequences: 26 of 16S (507 bp), 31 of COI (656 bp), and 24 of 28S (467 bp).

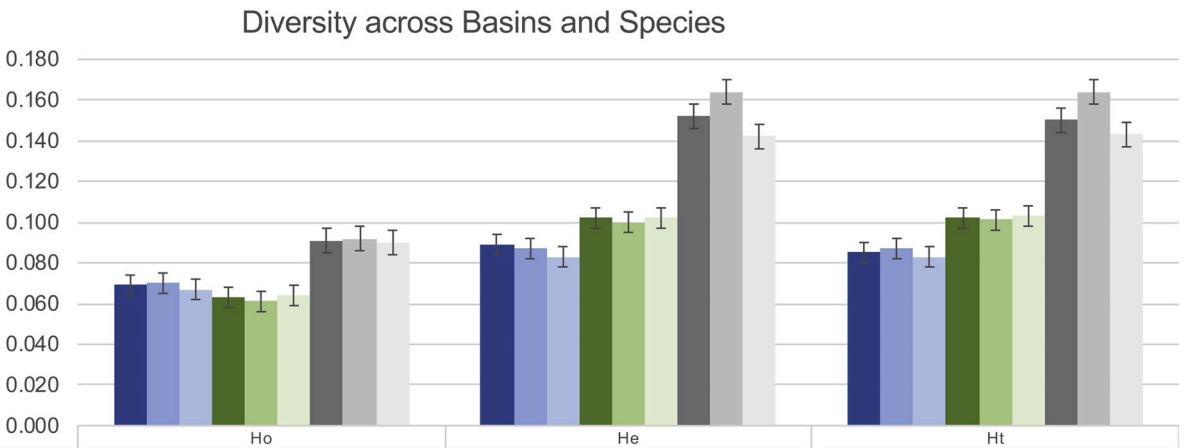
ddRADseq The initial data set for *C. scabra* included 16,229 SNPs across 41 individuals. After removal of loci and individuals with >10% missing data, 1,205 SNPs remained across 32 individuals (Table 1). The aligned and assembled *P. margaritifera* data set consisted of 12,348 SNPs across 30 individuals. After removal of loci and individuals with >15% missing data, 23 individuals were retained in an alignment of 1,430 SNPs (Table 1). After assembly and initial quality filtering in STACKS, the *V. infernalis* data set contained 5,295 SNPs across 25 individuals. Fifteen individuals passed through the missing data filter with <10% missing data and 1,524 SNPs were retained (Table 1). Using BayeScan, we were able to confirm the final data sets did not include F_{ST} outliers. Results from analysis of the NGS data sets (Timm, 2018) are presented in Figs. 2 and 5.

3.2. Cranchia scabra – A shallow non-vertically migrating species

Genetic Diversity and Historical Population Dynamics Species-level diversity calculated from the ddRADseq data set was $H_E = 0.089$ (Fig. 2). When genetic diversity was compared by basin, the differences were not statistically significant (H_E Gulf = 0.083; H_E Atlantic = 0.087). Both nuclear and mitochondrial haplotype networks exhibited a single, common haplotype and several closely related haplotypes (Hamming distance between the common haplotype and each "satellite" haplotype = 1; Fig. 3A and B).

Historical population growth (analyzed from the Sanger data set) was indicated in *C. scabra*: EBSP estimated ~10% population growth over the last 900–1000 years (Fig. 4A). This was supported by a statistically significant selection coefficient, Tajima's D = −2.118. For all three species, examination of the trace files generated during EBSP analysis showed that the 95% high posterior density excluded 0, suggesting that population sizes were not constant for any species included in this study.

Population Structure Analysis All genetic variance within *Cranchia scabra* was attributable to variance among individuals within populations (F_{IS} = 17.8%; statistically significant) and variance among



	Ho	He	Ht
C. scabra - Atlantic + Gulf	0.069	0.089	0.085
C. scabra - Atlantic	0.070	0.087	0.087
C. scabra - Gulf	0.067	0.083	0.083
P. margaritifera - Atlantic + Gulf	0.063	0.102	0.102
P. margaritifera - Atlantic	0.061	0.100	0.101
P. margaritifera - Gulf	0.064	0.102	0.103
V. infernalis - Atlantic + Gulf	0.091	0.152	0.150
V. infernalis - Atlantic	0.092	0.164	0.164
V. infernalis - Gulf	0.090	0.142	0.143

Fig. 2. Diversity metrics for each species, calculated using the ddRADseq data, including: observed heterozygosity (Ho), expected heterozygosity (He), and total heterozygosity (Ht). Error bars denote standard deviations, which were calculated by jackknifing over loci.

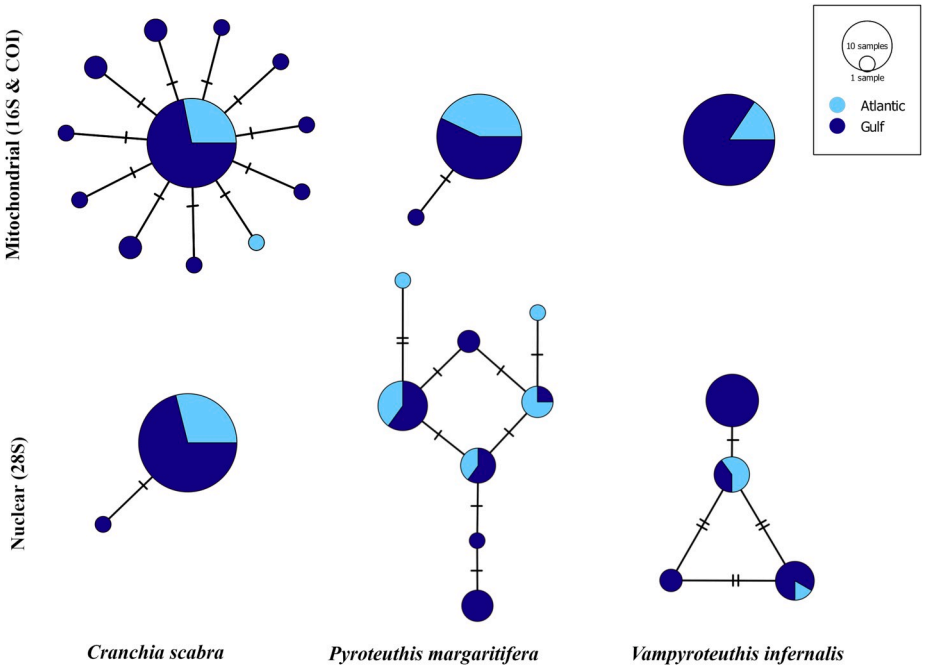


Fig. 3. Haplotype networks generated from Sanger data alignments: a concatenated 16S & COI 'mitochondrial' set and a 28S 'nuclear' set. Haplotypes are denoted with circles, with the size directly related to the abundance of the haplotype. Solid black lines indicate the most parsimonious relationships between haplotypes (routes requiring the fewest base-pair changes, illustrated as tick marks along the connecting line). From left to right, networks for *Cranchia scabra,* mitochondrial (A) and nuclear (B); *Pyroteuthis margaritifera,* mitochondrial (C) and nuclear (D); and *Vampyroteuthis infernalis*, mitochondrial (E) and nuclear (F).

individuals (F_{IT} = 82.5%). We found no evidence of population differentiation between individuals collected from the Gulf and those collected from the Atlantic (Table 1). This result was also confirmed in cluster analysis. The MDS plot (Fig. 5A) consists of a primary cluster containing the majority of individuals and five more distant individuals. These individuals do not cluster together, nor are they from a common basin. Affinity propagation identified three clusters in *C. scabra*, but two of these contained one individual each (Fig. 5). Analysis of *K*-means clustering indicate admixture from two ancestral populations, with no discernible difference in admixture proportions between the Gulf of Mexico and Atlantic (Fig. 5D). To ensure that population structure analyses were not overly influenced by structure within a subset of the data, *K*-means clustering analyses were also conducted on each basin independently (Gulf and Atlantic) for every species. In all cases, results from analyzing the Gulf and the Atlantic samples separately agreed with results seen when the full data set was analyzed.

3.3. *Pyroteuthis margaritifera* – A diel vertically migrating species

Genetic Diversity and Historical Population Dynamics Overall

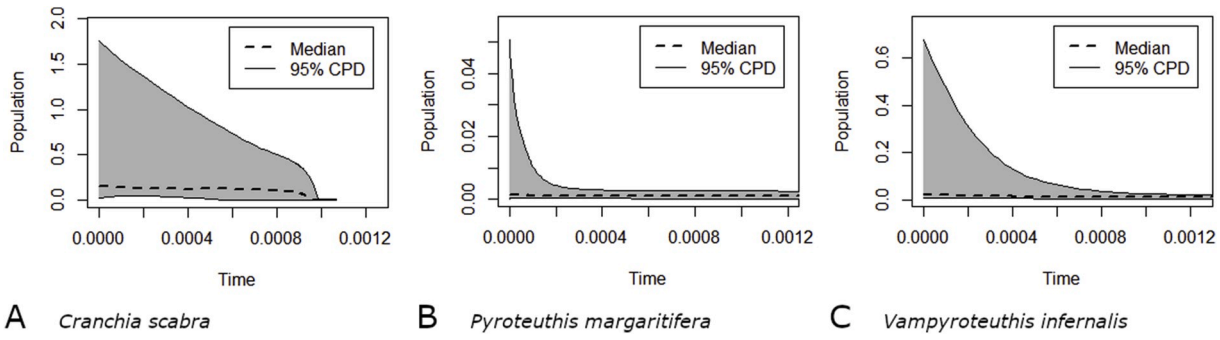


Fig. 4. Extended Bayesian Skyline Plots (EBSPs) generated using the concatenated Sanger data sets associated with *Cranchia scabra* (A), *Pyroteuthis margaritifera* (B), and *Vampyroteuthis infernalis* (C). The horizontal axis describes time (in hundreds of years) and the vertical axis measures population size. The solid lines define the 95% central posterior density (CPD) and the dotted line traces the median value over time.

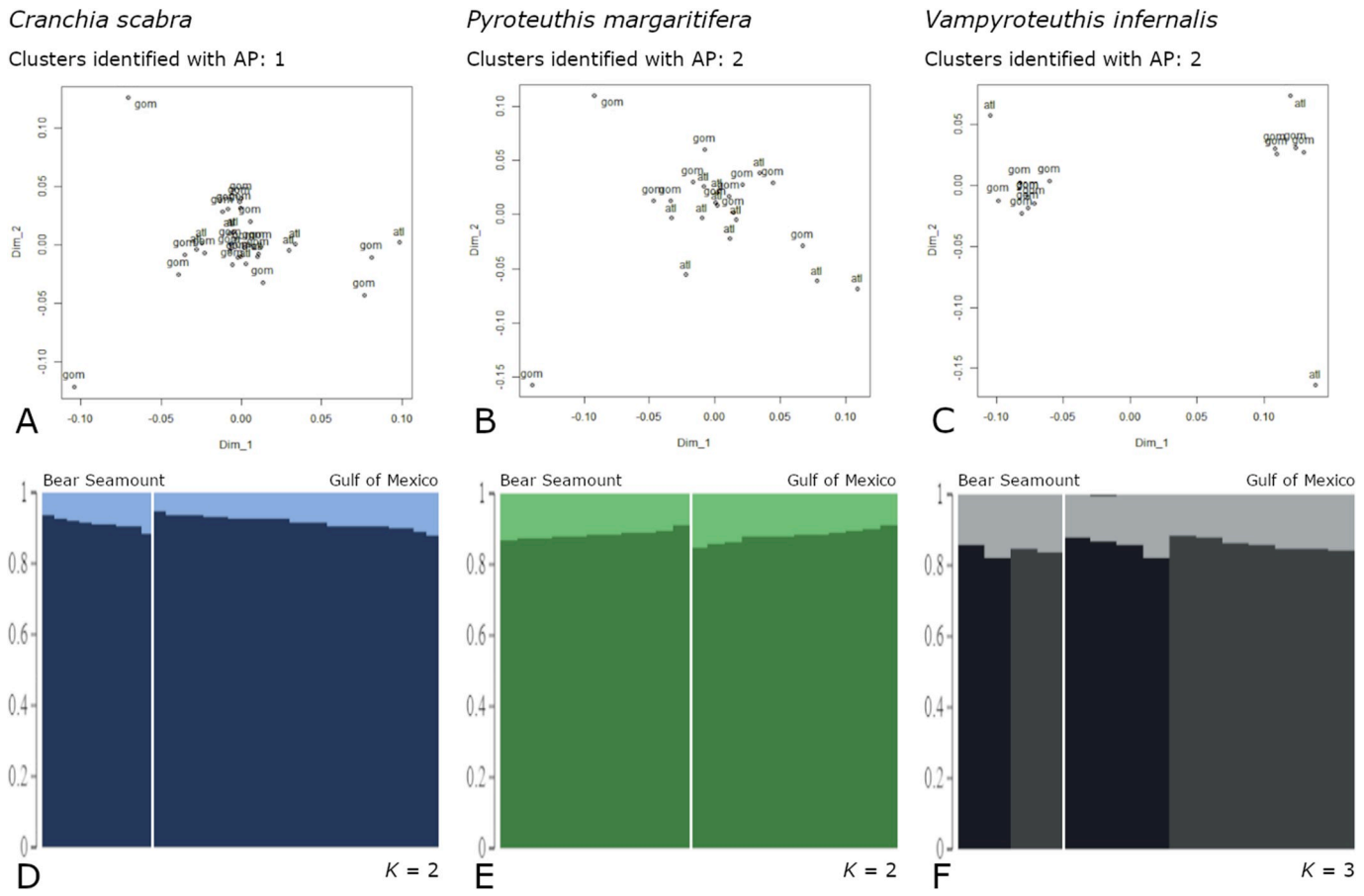


Fig. 5. Results of population structure analyses of *Cranchia scabra* (left), *Pyroteuthis margaritifera* (middle), and *Vampyroteuthis infernalis* (right). Affinity propagation (AP) identified 1–2 clusters (with membership >1) in each species. Multi-dimensional scaling plots built on genetic distance between individuals (A-C, respectively). Points are labeled with the collecton location of the individual (Atlantic = "atl", Gulf of Mexico = "gom"). Using STRUCTURE, k = 1–7 were tested ten times each, with 20,000 generations of burn-in and an additional 200,000 MCMC generations. The optimal k, determined with the Evanno Method and deltaK, is displayed (D-F, respectively).

diversity for *Pyroteuthis margaritifera*, calculated from the ddRADseq data set, was $H_E = 0.102$ and, when analyzed by basin, did not differ significantly (H_E Gulf = 0.102; H_E Atlantic = 0.100) (Fig. 2). The mitochondrial network from *P. margaritifera* reflects a common haplotype, shared by all but one individual collected from the Gulf of Mexico (Hamming distance between haplotypes = 1). The nuclear data exhibits a more complex network of eight haplotypes, four of which are only represented by 1–2 individuals. Three of the remaining, more common haplotypes are found in both basins (Fig. 3C and D).

We find evidence of historical population growth in *P. margaritifera*, though the EBSP suggests the growth is much smaller than in *C. scabra* (<0.1% over the last 1200 years; Fig. 4B). A statistically significant Tajima's D value (−2.048) confirms population growth and examination of the trace plot resulting from EBSP analysis confirms population size fluctuation.

Population Structure Analysis Molecular variance was primarily attributable to F_{IT} (65.5%) and F_{IS} (34.1%; statistically significant), though 0.3% of variance was due to differences between populations (F_{ST}; not statistically significant) (Table 1). Because the AMOVA was calculated under the Infinite Allele Model, the variance between

populations metric is equivalent to population differentiation. Thus, F_{ST} also describes the genetic distance between populations as 0.3%. This result is also supported by the MDS plot (Fig. 5B), which illustrates a primary cluster, containing the majority of individuals, and several outliers. *K*- means clustering results also agree: while two ancestral populations are inferred, there are no distinct patterns of admixture proportion between individuals, suggesting a single homogeneous population (Fig. 5E). Affinity propagation, however, identified two clusters (Fig. 5). Both clusters contained several individuals from the Gulf and several individuals from Bear Seamount.

3.4. *Vampyroteuthis infernalis* – A deep non-vertically migrating species

Genetic Diversity and Historical Population Dynamics *Vampryoteuthis infernalis* exhibited the highest genetic diversity of the three species ($H_E = 0.152$) and was the only species to have genetic diversity differ significantly between basins (H_E Gulf = 0.142; H_E Atlantic = 0.164) (Fig. 1). The mitochondrial haplotype network represents a single haplotype, common to the Gulf and the Atlantic (Fig. 3E and F). The nuclear haplotype network, however, includes four haplotypes. Two of these are unique to the Gulf of Mexico and two are shared between the Gulf and the northwest Atlantic.

Analysis of historical population growth did not yield significant results (Tajima's D = −1.396), though evaluation of the EBSP indicated some growth over the past thousand years (Fig. 4C). We found evidence for population size fluctuation as described in *C. scabra* and *P. margaritifera*.

Population Structure Analysis As in the other species, molecular variance in *V. infernalis* is primarily due to F_{IT} (64.2%) with the remaining variance described by F_{IS} (37.6%; statistically significant) (Table 1). The MDS plot (Fig. 5C) was markedly different from those of *C. scabra* and *P. margaritifera*: two distant clusters are illustrated, each with a nearby outlier, and a third distant outlier. These two clusters were also identified with affinity propagation and *K*-means clustering (Fig. 5): though STRUCTURE analysis indicated admixture between three ancestral populations, there are two distinct groups identifiable in the DISTRUCT plot (Fig. 5F). Despite the evidence for two groups, membership in these groups does not seem to be determined by source basin. That is, both groups include individuals from the Gulf and from Bear Seamount. In light of the results, we closely re-examined the specimens. However, due to the degradation of taxonomically informative soft tissue, no morphological differences were identified.

4. Discussion

Previously, knowledge of deep-sea cephalopod species in the Gulf of Mexico was limited to two comprehensive census studies conducted by Voss (1956) and Judkins (2009). Our study investigated population dynamics in three cephalopod species common in the mesopelagic Gulf. We find significantly higher overall genetic diversity in *V. infernalis*, the only species included that occurs entirely below 600 m. *Cranchia scabra* and *P. margaritifera;* however, both occur throughout the water column (0–1500 m) and returned similar, low diversity values. In comparing genetic diversity between basins, we find *V. infernalis* exhibits a significant difference in diversity between basins (Atlantic > Gulf of Mexico), while *C. scabra* and *P. margaritifera* do not. Our results indicate both *C. scabra* and *P. margaritifera* exist in the study range as a single, homogeneous population. *Vampyroteuthis infernalis*, however, exhibits population structure. We find evidence of two populations in this species, which are not segregated by basin. Further investigation is necessary to identify the prevailing driver of population differentiation in this species; depth separation between populations, prey selection by each population, or different reproduction strategies all represent testable hypotheses for future studies.

4.1. Genetic diversity and historical population dynamics

Previous studies of population genetics of cephalopods have primarily focused on shallow water species. Cheng (2015) and Sanchez et al. (2016) examined fisheries-targeted squid: the opalescent inshore squid, *Doryteuthis opalescens,* and the epi/mesopelagic Humboldt squid, *Dosidicus gigas*, respectively. *Doryteuthis opalescens* is an inshore squid that represents the most valuable squid fishery along the California coast (Vojkovich, 1998; Zeidberg, 2013). However, this fishery has collapsed several times in the past few decades and a population genetics study of *D. opalescens* uncovered very little differentiation between populations and very similar expected heterozygosity values as those we found in our target species (Cheng, 2015). The population genetics study completed by Sanchez et al. (2016) focused on *D. gigas* along the coast of Peru and uncovered high genetic diversity and no population differentiation. Gao et al. (2016) examined 10 populations of the whip arm octopus (*Octopus minor*) and found that eight populations had high allele diversity and that heterozygosity varied. They concluded that the genetic structure of this species was related to combined effects of geographical barriers, current features, and life history characteristics. Most recently, a study of three species of octopods linking population genetics to life history found some correlation between the life history characteristics such as fecundity and developmental mode to genetic diversity and population structure (Domínguez-Contreras et al., 2018). These studies targeting shallow-water species provide valuable context for our results, however, the meso- and bathypelagic environments constitute unique habitats, with fluid structure and high connectivity to other oceanic regions. To build a more complete conceptualization of this vast habitat, additional studies are needed.

Investigating selection and changes in overall population sizes may provide further context for the trends seen in diversity. The low diversity seen in *C. scabra* is coupled with population growth, both in Tajima's D and the EBSP. Typically, population growth is diagnosed with an excess of rare alleles in an otherwise genetically homogeneous population, which may also cause low genetic diversity. *Pyroteuthis margaritifera* and *V. infernalis* both had little population size changes, indicating no significant excess or deficit in rare alleles, which would also be associated with high diversity.

While seamounts were once considered to primarily host endemic species (Wilson and Kaufmann, 1987), a 15-year study by Shea et al. (2017) demonstrated that Bear Seamount has cephalopod assemblages similar to the neighboring continental slope and supports many cosmopolitan cephalopod species. While it is an advantage for these deep-sea cephalopod populations to have geographically widespread gene pools to replenish populations after disruptions and isolated environmental disturbances, this can result in low genetic diversity (Vecchione, 2001). This reduction in genetic diversity can have crucial, detrimental impacts on a species' ability to survive and recover from environmental perturbations.

4.2. Population connectivity and structure

Across all species, we found evidence of homogeneous populations and significant inbreeding. In *C. scabra* and *P. margaritifera*, we found admixture between two ancestral populations. The proportions of admixture in each individual are nearly identical, regardless of the basin from which they were collected, Gulf of Mexico or Atlantic. *Vampyroteuthis infernalis*, however, was found to be composed of admixture from three ancestral populations. Moreover, there were two distinct patterns of admixture representing two populations, though they were not differentiated by basin. This result helps further explain the high diversity present in this species: these two populations, defined by differing admixture proportions, may represent some form of local adaptation or other genetic differentiation. However, without the addition of more Atlantic samples, we are unable to decisively address what is promoting this population structure.

The population differentiation identified in *V. infernalis* may be explained by environmental factors, specifically oxygen availability and depth. However, as dissolved oxygen concentrations vary, we also recognize a potential (indeed, likely) interaction among environmental factors. In the Monterey Submarine Canyon, off the California coast, *V. infernalis* inhabit the midwater OMZ between 600m and 900m (Hoving and Robison, 2012). While lower dissolved oxygen levels may be important to the species in the Pacific Ocean, neither the Gulf of Mexico nor the area around Bear Seamount have described OMZs (though the northern Gulf of Mexico hosts the second-largest hypoxic "Dead Zone" in the world (Joyce, 2000; Rabalais et al., 2001; Rabalais and Turner, 2001; Rabalais et al., 2002; Bianchi et al., 2010), it is highly localized to coastal waters). This clear ecological difference in a worldwide species may result in genetic differentiation. Another possible explanation for differentiation into two populations of *V. infernalis* in the Gulf/North Atlantic basins could relate to water depth. The geologic sill of the Florida Straits, which is located between the tip of Florida and Cuba, is 800 m deep (Athearn, 1963). This could serve as a barrier, limiting paralarval dispersal and resulting in two populations of *V. infernalis*: a "shallow" population (occupying 600 m–800 m depth and acclimated to higher dissolved oxygen concentrations) and a "deep" population (occupying 800 m and below). Even a single migrant per generation from the "deep" population across the geologic sill would be adequate to maintain population connectivity between basins (Spieth, 1974, but see Mills and Allendorf, 1996 for additional considerations and caveats), while differentiating from the "shallow" population.

Past literature on cephalopod population genetics has focused primarily on epipelagic and coastal cephalopods, contextualizing the results we present here, but complicating direct comparisons. However, our findings of genetic homogeneity between basins are consistent with a recent review of deep-sea population genetics studies targeting benthic invertebrate species across the world's oceans (Taylor and Roterman, 2017). Many studies discussed in this review found strong connectivity between populations separated by hundreds to thousands of kilometers. Taylor and Roterman (2017) also discuss widespread horizontal connectivity at the regional and oceanic scale, but limited vertical connectivity, presumably due to dispersal patterns, deep-water current patterns, and disturbance activity. Etter et al. (2005, 2011) examined population differentiation and genetic divergence for various deep-sea bivalves and discovered that the bathyal depths have higher genetic differentiation than abyssal zones. Possible reasons for this finding include restrictions to gene flow, bathymetric features of the region, and historical events. It is important to note that these studies frequently mention the need for additional data to clarify trends and possible comparisons among pelagic and benthic taxa.

The Gulf Stream acts as a dispersal route, flowing toward Bear Seamount from the Gulf of Mexico (Markle et al., 1980; Harold and Clark, 1990; Moore et al., 2004). Both benthic and pelagic cephalopod species found in the deep waters of the Caribbean and Gulf have been found in the vicinity of Bear Seamount (Moore et al., 2004; Shea et al., 2017). The three species examined in this study vary in vertical migration patterns (Fig. 1, Judkins and Vecchione, 2020, in press): while *P. margaritifera* is a classic example of a vertical migrator, living in the upper mesopelagic zone during the day and migrating to the epipelagic zone nightly, *C. scabra* is found throughout the water column with minimal diel vertical migration.

Occurrence above the sill depth would increase the chance of gene flow from the Gulf to the Atlantic Ocean for both of these species. Although past work suggests an ontogenetic shift for *C. scabra* in the Atlantic Ocean (Clarke and Lu, 1975), the authors concede that they had limited specimens to verify the trend. Their findings suggest that dispersal through the Florida Straits would likely occur for early developmental stages of *C. scabra*, with juveniles and paralarvae in the epipelagic to mesopelagic while adults descend to the mesopelagic to bathypelagic zones (Clarke and Lu, 1975). However, ontogenetic shift has not been described for *C. scabra* from the northern Gulf of Mexico with specimens with lengths up to 40 mm. Larger specimens of *C. scabra* may exhibit a shift in depth preference, but this has not been documented. *Pyroteuthis margaritifera,* which has a short life span but is a known diel vertical migrator (Roper and Young, 1975), would likely be able to disperse through the Florida Straits at any point in its life. In contrast, *Vampyroteuthis infernalis* lives at deeper depths (600 m–1500 m) with no evidence of vertical migration. The gene flow for this deep dweller would be strongly limited by the sill at the Florida Straits.

4.3. Health and resilience of cephalopods in the Gulf of Mexico

The lack of knowledge regarding deep pelagic environments, the largest habitat on Earth, represents an extensive and pressing data gap (Webb et al., 2010). Only recently have comprehensive programs, such as the NOAA/NRDA Offshore Nekton Sampling and Analysis Program and the DEEPEND consortium (www.deependconsortium.org), begun to rigorously survey the deep water column of the northern Gulf of Mexico. The present study analyzes population dynamics in three cephalopod species and establishes a biological reference state for these species in the Gulf. We specifically target genetic diversity and population connectivity as proxies for species' health and resilience in the Gulf of Mexico and greater Atlantic Ocean. The regions sampled in this study are separated by ~3000 km, yet we find no significant population differentiation between the Gulf and the northwestern Atlantic region, indicating high gene flow. This study also demonstrates that, on an evolutionary timescale, all three species are in periods of population growth, not decline. Our findings suggest the existence of a genetic reservoir for the Gulf: *C. scabra* and *P. margaritifera* have similar diversity values within or between basins and high connectivity between basins. Deep-water currents move south along the eastern U.S. coast and could aid in dispersal of these species. *Vampyroteuthis infernalis*, however, may be vulnerable to localized disturbances as there are two populations at each site: without a more thorough understanding of the environmental and ecological factors associated with population differentiation in this species, undue pressure may inadvertently be applied to either population, especially if the individual populations are managed as one. To determine the extent and pressure points of this vulnerability, more research is needed to identify the driver(s) differentiating populations in this species.

The present study, as well as the Shea et al. (2017) findings, support the hypothesis that Bear Seamount is an intermediary in oceanic-scale dispersal (Wilson and Kaufmann, 1987). An interesting addition to this work would be to collect and analyze Caribbean Sea specimens as the Caribbean Current feeds into the Gulf of Mexico. Another angle of study could include sampling across other ocean basins to provide a broader perspective on the state and flux of genetic diversity in these species. Inclusion of additional species at a variety of depth zones would possibly shed light on trends in genetic diversity and population dynamics. Defining the two populations of *Vampyroteuthis infernalis* uncovered in our study and performing a linear discriminant analysis could determine the extent to which ecological factors differentiate populations.

Acknowledgements

The authors thank the DEEPEND and Bear Seamount research teams and crews of the R/V *Point Sur* and NOAA Ship *Pisces* for their assistance in collecting these specimens for examination. This research was made possible by a grant from The Gulf of Mexico Research Initiative. Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org https://doi.org/10.7266/N70P0X3T, https://doi.org/10.7266/N7XP7385, and https://doi.org/10.7266/n7-05f6-th15. We would also like to extend gratitude to Dr. Timm's PhD committee: Dr. José Eirin-Lopez, Dr. Mauricio Rodriguez-Lanetty, Dr. Eric von

Wettberg, and Dr. Wensong Wu; as well as to Dr. Emily Warschefsky for assistance in library preparation, and Dr. Joseph Ahrens and Mr. Jordon Rahaman for bioinformatics support and advice. Finally, we thank the anonymous reviewers for their feedback on earlier versions of this manuscript. This is contribution#173 from the Division of Coastlines and Oceans in the Institute of Environment at Florida International University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr.2020.103222.

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