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Rapid Communication

Caribbean Creeping Crabs: northward expansion of the green porcelain crab in North Carolina, USA

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Abstract

Marine species a.re expanding polewards with rising temperatures. In the N011hwest Atlantic, tropical/subtropical species native to the Caribbean have been docwnented as spreading into temperate regions. One such species, the green porcelain crab *(Petrolisthes annatus),* has been migrating up the southeastern United States coastline from Florida since the 1990s. Until 2018, the species had not been detected north of Wilmington, No11h Carolina.(NC). Here, we document the establishment of *P. annatus* populations a.long tl1e NC central coast. Owing biodiversity monit01ing, we detected the first record of *P. armatus* in 2018 in Bea.ufo11, NC, and subsequent records of the crab were detected at multiple locations in the region over a four-year time period (2018-2022), often at relatively high abundances. Morphological and genetic evidence confinned the identity of *P. annatus* in this region and distinguished it from its less abundant native congener, *P. galathinus* (banded porcelain crab). While cold winter temperatures have likely litnited ilie species' ability to spread n011hwa.rds in the past, 1ising winter temperatures in recent years may facilitate its continued expansion. It is vital that we continue monit01ing the species' population demographics in its expanding range, as well as detemune com1mmity-level impacts in the region.

Key words: climate change, non-native, N011hwest Atlantic, *Petrolisthes armatus,* range expansion

Intrnduction

Species distributions are shifting as a result of warming temperatures (Portner et al. 2022). Ocean warming has facilitated the poleward movements of numerous organisms in coastal communities around the world over the past several decades (Atkins and Travis 2010). For example, in a global meta-analysis of over 200 marine studies, Poloczanska et al. (2013) found that >80% of species distributions have shown poleward shifts consistent with climate change scenarios. In a review of marine organisms located in the eastern Atlantic, Canning-Clode and Carlton (2017) noted numerous species that have migrated from Macronesia and northern/northwestern

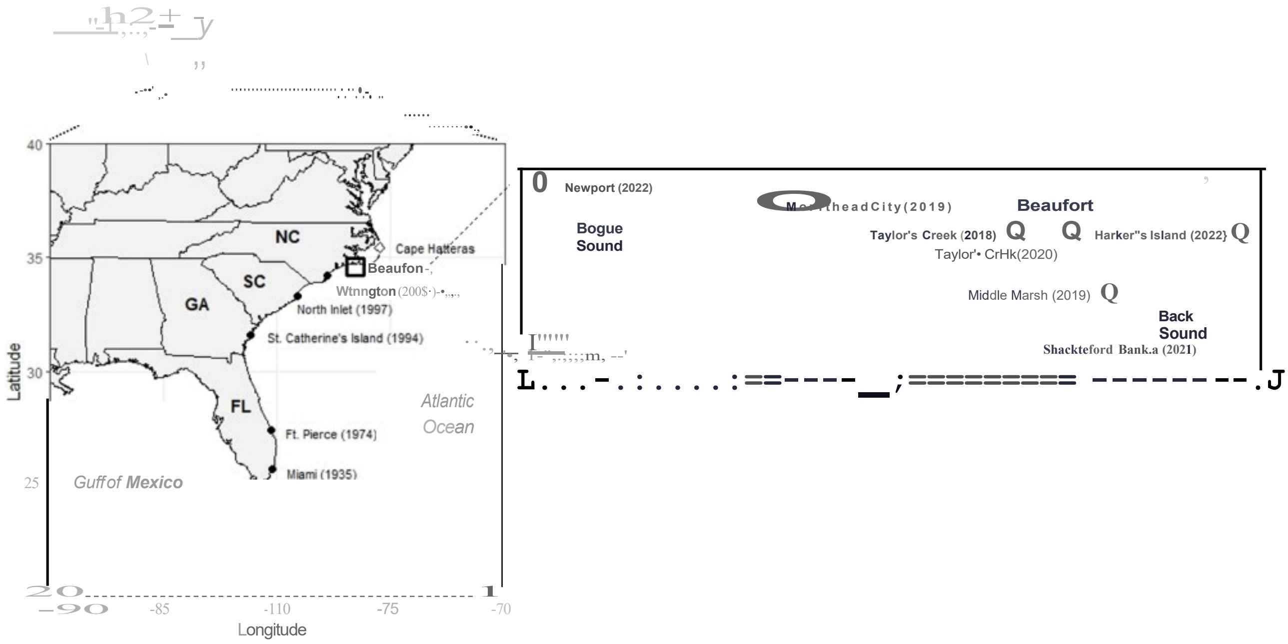

Figure 1. Spread of *Petrolisthes armatus* along the Atlantic coast of the USA through time. (A) Records of the species' presence in the southeastern USA: Miami, Florida, USA in the 1930s; Ft. Pierce, Florida in the 1970s; St. Catherine's Island, Georgia in the 1990s; No1th Inlet, South Carolina in the late 1990s; Cape Fear Sound, Wilmington, NC in the 2000s (Haig 1960; Fofonoff et al. 2018; Knott et al. 2000; Prezant et al. 2002; Rodiiguez et al. 2005); and as we report here, the newest and northernmost record of the species is in the Beaufort, NC vicinity, where it was first noted in 2018, (B) Tilis was followed by additional detections at other locations in the region from 2019-2022,

Africa to the Mediterranean Sea and Atlantic Europe, calling these northward range expansions the "African creep".Similarly, in the western Atlantic, a nwnber of tropical and subtropical species have expanded from the Caribbean into temperate regions along the Atlantic coast of the United States of America (USA). This northward march of species from the Caribbean was likewise coined the "Caribbean creep" by JT Carlton (Canning-Clode et al. 2011; Canning-Clode and Carlton 2017; Castro et al. 2022). Rising coastal temperatures resulting from human-induced climate change are thereby enabling range expansions and exacerbating the potential for biological invasions (Sorte et al. 2010).

A representative of the "Caribbean creep" is the green porcelain crab, *Petrolisthesarmatus,* which has been "creeping" northwards along the Atlantic USA coast over the past several decades (Figure 1), preswnably with warming coastal temperatures (Canning-Clode et al. 2011; Canning-Clode and Carlton 2017). *Petrolisthes armatus* is a filter-feeding anomuran crab species with a wide native distribution in tropical and subtropical waters of the southeast and southwest Atlantic coasts (Haig 1960; Gore and Abele 1976; Hiller and Lessios 2017). This intertidal/shallow-subtidal species predominantly inhabits natural and artificial structures like rocks, oyster reefs, mangrove roots, riprap, and breakwaters (Hollebone and Hay 2008; Fofonoff et al. 2018; Kinney et al. 2019). In the USA, the species was previously only known from southern Florida (Knott et al. 2000) and did not appear in any of the prominent Atlantic USA guidebooks of the 1980s or earlier, including one dedicated to crustaceans of the Atlantic coast (Williams 1984). In the 1990s, however, *P. armatus* was discovered at multiple coastal sites in Georgia and South Carolina (Hollebone and Hay 2007; Tilburg et al. 2010; Wassick et al. 2017), sometimes reaching densities as high as 4,000-11,000 m2, which is 37 times the densities that have been reported from source populations (Hollebone and Hay 2007). In a 2015-2016 survey from Savannah, Georgia to North Inlet, South Carolina, *P. armatus* was the most abundant benthic crab species in summer months among multiple native crab species (Mack et al. 2019). Similarly in 2016, field surveys and experiments carried out at 8 southeastern sites (St. Augustine, Florida to North Inlet, South Carolina) reported high densities of *P. armatus,* with little evidence of biotic resistance by predators (Kinney et al. 2019). At the time of these studies, *P. armatus* was detected in low abundances in Wilmington, North Carolina (NC) (Kinney et al. (2019); Mack et al. 2019), and in 2016, this Wilmington record represented the northernmost Atlantic population of *P. armatus* (Wassick et al. 2017). Indeed, Kinney et al. (2019) used Morehead City, NC as an uninvaded "control" site for their 2016 investigation.

In 2018, we detected green porcelain crabs at coastal sites in the vicinity of Beaufort and Morehead City, NC while monitoring for local biodiversity (Figure 1). As there are native Porcellanidae, such as *Petrolisthes galathinus* (banded porcelain crab), in coastal North Carolina, we used morphological and genetic data to confirm the identity of *P. armatus* in the Beaufort region. In this paper, we also provide abundance data documenting further detections and spread of the species across the region over a four-year survey period (2018-2022). Importantly, our study provides early documentation of the establishment, spread, and population increase of *P. armatus* over time along the NC central coast. Our study also documents this region as the current northernmost location of the species' range expansion along the Atlantic USA seaboard.

Materials and methods

Field sampling and processing

In our study region, field surveys were conducted between 2017-2022 to assess biodiversity across several locations in the vicinity of Beaufort, NC (Figure 1) from a variety of coastal habitats, including marsh, mud.flat, seagrass, and restored/natural oyster reefs. This included a targeted survey for *P. armatus* in Middle Marsh, NC in July and October 2022 following detections of the crab during earlier sampling events (Table 1). Crabs were collected using passive sampling devices: small plastic milk crates (19 x 22 x 16 cm) with a mesh top that are filled with oyster cultch and are attached to the benthos using wooden and/or PVC stakes in the shallow subtidal zone (- 1 m depth). These crates are not traps but have been found to attract a wide diversity of

Table 1. Sample locations of biodiversity surveys conducted off the coast of Beaufo1t, NC, where *Petrolisthes armatus* (green porcelain crab) was detected. Sample locations are listed with a latitude and longitude that is representative of a central position across multiple sample sites sm-veyed per location. Locations were often visited multiple times, so the sample month and year are also listed in chronological order from 2018-2022. TI1e total (tot) number and averages (avg) across sites of *P. armatus* (PA) detected per location/sampling event are listed, along with counts of *P. galathinus* (PG) (banded porcelain crab). Tue total munber of *P. armatus* and *P. galathinus* are tallied in the last row.

Sample Location	Latitude	Longitude	SampleMonth andYear	# Sites	# *PA* (tot)	#*PA* (avg)	#*PG* (tot)
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	January 2018	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	March2018	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	May2018	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	June 2018	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	August2018	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	September 2018	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	November 2018	8	1	0.13	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	January 2019	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	April 2019	8	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	June 2019	8	0	0.00	0
Middle Marsh	34.692222°N	76.621111°W	June 2019	15	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	July 2019	8	0	0.00	0
Middle Marsh	34.692222°N	76.621111•w	July 2019	15	0	0.00	0
Middle Marsh	34.692222°N	76.621111°W	August2019	15	0	0.00	0
Middle Marsh	34.692222°N	76.621111°w	September 2019	15	0	0.00	0
Taylor Creek (Beaufort)	34.712222°N	76.648056°W	October 2019	8	0	0.00	0
Middle Marsh	34.692222°N	76.621111·w	October 2019	15	1	0.07	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	October 2020	12	7	0.58	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	November 2020	12	12	1.00	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	December 2020	12	10	0.83	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	February 2021	12	1	0.08	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	March2021	12	0	0.00	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	April 2021	12	0	0.00	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	May2021	12	0	0.00	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	June 2021	12	0	0.00	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	July 2021	12	6	0.50	0
Back Sound (Shackleford Banks)	34.655278°N	76.535833°W	August2021	18	321	17.83	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	April 2022	12	6	0.50	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	June 2022	12	17	1.42	2
Middle Marsh	34.692222°N	76.621111°W	July2022	20	165	8.25	3
Newport-I	34.719722°N	76.922778°W	August2022	4	16	4.00	0
Gloucester	34.723611°N	76.525556°W	August2022	4	2	0.50	0
Harkers Island	34.711111°N	76.564722°W	August2022	4	151	37.75	0
Newport-2	34.716389°N	76.931111°W	August2022	4	10	2.50	0
Newport-3	34.727222°N	76.8675°w	August2022	5	27	5.40	0
Newport-I	34.719722°N	76.922778°W	September 2022	4	42	10.50	0
Gloucester	34.723611°N	76.525556°W	September 2022	4	2	0.50	0
Newport-2	34.716389°N	76.931111°w	September 2022	4	19	4.75	0
Newport-3	34.727222°N	76.8675°W	September 2022	5	29	5.80	0
Taylor Creek (Carrot Island)	34.7075°N	76.630278°W	September 2022	12	272	22.67	0
Middle Marsh	34.692222°N	76.621111°w	October 2022	18	633	35.17	13
			TOTALS		1750		18

fauna, predominantly juvenile and adult crustaceans and fish, due to the structured habitat they provide (Moore et al. 2020, 2023; Blakeslee et al. 2021). Samplers were deployed within close proximity (typically< 1 m) to oyster reef, marsh, or seagrass habitats, depending on the site. Samplers were left to passively recruit fauna for 1-2 months, after which, they were retrieved and contents were sorted using a floating sieve. Recruited species were identified to the lowest taxonomic level in the field and released. However, all captured porcelain crabs were collected and brought back to

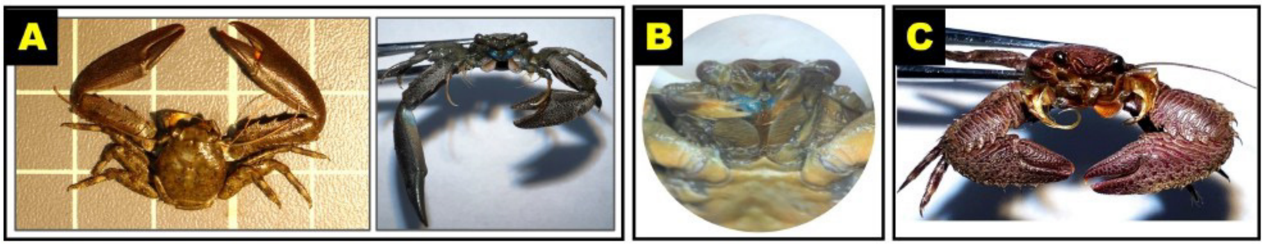

Figure 2. Identification of *Petrolisthes annatus* (green porcelain crab) from our NC sites using a combination of morphology and DNA barcoding. (A) Representative photographs of whole individuals of *P. armatus* collected from Middle Marsh and Back Sound, NC. (B) Close-up photograph of the mouthpatts of *P. armatus* showing characteristic blue coloration; (C) Photographs of a whole individual of *P. galathinus* (banded porcelain crab) detected at some of our field sites. Photographs by A. Blakeslee, C. Moore, and C. Stat1cil.

East Carolina University to be counted and identified to species level using morphological characteristics. In addition, a subset of individuals from Back Sound, NC in 2021 were saved for DNA barcoding analysis (see below). The timing of our collections occurred primarily during summer and fall months (May to November); however, at Taylor Creek, NC, surveys were also performed in winter and spring (Table 1). Altogether, our analyses included 41 sampling events (Table 1), which comprised multiple sample sites within each specific location (Taylor Creek, Middle Marsh, Back Sound, Newport, Gloucester, Harkers Island) in the Beaufort, NC vicinity at different time points over the years 2018-2022. Porcelain crabs that were detected and counted from our passive samplers at all sites were analyzed as 'Crab Abundance' because our samplers represented a standardized volume across all sampling events.

Morphological analyses and DNA barcoding

In 2021, we photographed 42 *P. armatus* individuals from Back Sound, NC (Figure 2). We also examined all crabs (n = 1768) in our surveys for two morphological traits noted as diagnostic of *P. armatus* porcelain crabs. This includes an orange spot on the claw and blue mouthparts (Mantelatto et al. 2011; Fofonoff et al. 2018) (Figure 2A, B). During our surveys, we came across 18 individuals that did not display these morphological characters, instead having orange mouthparts and banding/striations on the carapace (Figure 2C). These characters are attributed to another porcelain crab species, the banded porcelain crab *(P. galathinus)* (Hiller et al. 2006), which is considered a tropical/subtropical species native to the western Atlantic (North Carolina to Brazil) (Williams 1984).

To genetically confirm the species identifications based on morphological characters for both *P. armatus* and *P. galathinus,* we carried out DNA barcoding on: (A) 6 *P. armatus* photographed from Back Sound, NC (collected by A. Blakeslee and C. Moore); (B) 2 *P. armatus* from Morehead City, NC (collected by S. Smith and R. Aguilar); and (C) 2 *P. galathinus* detected in Middle Marsh, NC (collected by C. Stancil and A. Blakeslee). DNA was extracted using a standard cetyl trimethyl ammonium bromide (CTAB)-chloroform-ethanol precipitation protocol (France et al. 1996). PCR was performed with the following primers that amplified a 394 bp fragment of the mitochondrial COi gene: Parmatus_COIF: GCCCCTGATATAGCTTIC CCA, Parmatus_COIR:GTIGATAAAGAACTGGGTCCCC, Pgalathinus_COIF: GCGGAGTTAGGTCAACCTGG, and Pgalathinus_COIR: GAACAGGAT CTCCCCCTCCT. We used the following PCR profile: 95 °C for 2 min; 30 cycles of 95°C for 30 s, 55 °C for 30 s, and 72 °C for 60 s;and 72 °C for 5 min (Steinberg et al. 2008). PCR products were purified with EXOSAP-IT (Applied Biosystems) and submitted for sequencing at Psomagen, USA (Rockville, MD). The two *P. armatus* collected from Morehead City were processed according to Aguilar et al. (2022) and sequenced following Aguilar et al. (2017) with the exception of using Geller et al. (2013) primers: jgLCO1490 and jgHCO2198. Sequences were curated and analyzed using Geneious 2023.0.4. We utilized the NCBI BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify genetically similar individuals to our eight NC *P. armatus* (accession #s: OR822331-OR822332, OR840509-OR840514) and two *P. galathinus* (accession #s: OR840507-OR840508) sequences. Using the consensus sequence from our *P. armatus* sequences, we performed an nr/nt megablast search in BLAST, and the resulting top sequence originated from South Carolina, USA (accession#: KY857293; Hiller and Lessios 2017), matching 100% to the consensus sequence. Similarly, we used the consensus sequence from our *P. galathinus* sequences and once again performed an nr/nt megablast search in BLAST; the resulting top sequence was from Bocas Del Toro, Panama (accession #: MN183895; Venera-Pont6n et al. 2020) and matched 95% to our *P. galathinus* consensus sequence. We retrieved the FASTA files for the KY857293 and MN183895 sequences and included them with the NC *P. armatus* and *P. galathinus* sequences in a ClustalW 2.1 algorithm alignment in Geneious 2023.0.4. Using Mr. Bayes, we generated a phylogenetic tree (Huelsenbeck and Ronquist 2001) with the substitution model set to GTR with four gamma categories; the MCMC settings were set to default. To obtain a sequence to root the tree, we identified the closest sequence match to *P. armatus* in Genbank by performing an nr/nt megablast search in BLAST using our consensus *P. armatus* sequence and excluding *P. armatus* sequences. This resulted in a top match of 87% to *P. robsonae* from Panama (accession#: KY857550; Hiller and Lessios 2017).

Results and discussion

We surveyed multiple locations along the central North Carolina coast and first detected *P. armatus* beginning in 2018 (Figure 1). During these surveys, *P. armatus* was first found on the Beaufort side of Taylor Creek, NC, in November 2018 (C. Moore *pers. obs.).* The crab was then detected at the Center for Marine Sciences and Technology (CMAST) shoreline in Morehead City, NC in October 2019 (S. Smith and R. Aguilar *pers. obs.)* during sampling for the Chesapeake Bay Barcode Initiative (Aguilar et al. 2017, 2022). In that same month, the crab wasdetected in Middle Marsh, NC (Moore et al. 2023). Next, it was found on the Carrot Island side of Taylor Creek, NC, in October 2020 (N. Woodard *pers. obs.),* then Back Sound near Shackleford Banks, NC in August 2021 (R. Gittman, A. Blakeslee, C. Moore *pers. obs.),* and finally at shoreline locations in Newport, Gloucester, and Harkers Island, NC, in August 2022 (M. Geesin, R. Gittman, A. Blakeslee *pers. obs.)* (Figure 1; Table 1). These biodiversity surveys have been ongoing since 2017, particularly at Taylor Creek and Carrot Island, but until October 2018, there was no evidence of *P. armatus* at any of our field sites, even though the same sampling methodologies were employed in these prior surveys (Moore et al. 2020). Moreover, an earlier *P. armatus* study from 2016 used Morehead City as an uninvaded control (Kinney et al. 2019). Thus, it seems unlikely that the crab was present-at least in appreciable numbers-in the region prior to 2018.

Based on characteristic morphological features (orange spot on claw and blue mouthparts: Figure 2) (Mantelatto et al. 2011) and confirmation using DNA barcoding techniques (discussed below), we determined that the majority (99%) of porcelain crabs detected at our sample sites were *P. armatus.* In contrast, out of the 1768 porcelain crabs we surveyed across all sites and time points (Table 1), the native porcelain crab, *P. galathinus,* was only detected 18 times (1%). Interestingly, the sites where we detected *P. galathinus* predominantly came from our interior sound sites in Middle Marsh (Figure 1). *Petrolisthes galathinus* is found associated with multiple structured habitats, including rocks, sponges, corals, anemones, mangrove roots, and mussel beds, and inhabits shallow depths down to 54-m (Gore and Abele 1976; Williams 1984). It may be that the diversity of habitats found at our interior sound sites, as well as greater water flow, resulted in detection of *P. galathinus* at these sites, as opposed to our nearshore sites. *Petrolisthes armatus* also inhabits a wide variety of habitat types, and studies in South Carolina and Georgia have found the species strongly associated with oyster reefs and marsh habitat (Mack et al. 2019), as we also found in our investigation.

Throughout our studyregion, we observed increasing abundance of *P. armatus* over time using our standardized passive sampler design (Figure 3). In 2018 and 2019, we only detected two instances of *P. armatus* at our sample sites; however, in Fall 2020, several crabs were found on the Carrot Island site of Taylor Creek (Table 1; Figures 1, 3). Crabs were absent from Taylor Creek in Winter/Spring 2021and reappeared again in Summer 2021.Thenin August 2021 in Back Sound, relatively large abundances of the crab were detected (upwards of 60 crabs per sampler), and in 2022, surveys of Taylor Creek and Middle Marsh in Summer and Fall detected upwards of 90 crabs per sampler (19 x 22 x 16 cm). Though we predominantly sampled in the summer and fall, these data are suggestive of a seasonal effect on *P. armatus* abundance, with more limited abundances in the winter and spring. Prior studies have also observed peaks in *P. armatus* abundance in summer months and lowest abundances

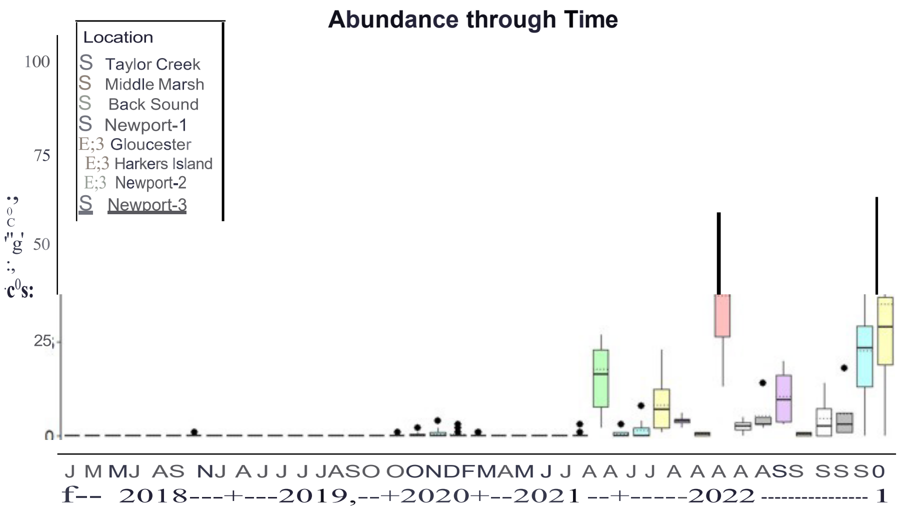

Figure 3. Crab abundances from standardized passive samplers (plastic milk crates filled with oyster cultch; 19 x 22 x 16 cm) over a four-year time period (2018-2022). Letters below the X-axis represent months. See Table 1 for month and year combinations for each of these 41 sampling events in chronological order across our different sampling locations. A Kruskal-Wallace test demonstrated a significant effect of location and time on crab abundance (Chi-squared = 379.98, df = 40, $p < 0.001$).

in winter (Hartman 2003; Hollebone and Hay 2007; Wassick et al. 2017; Mack et al. 2019). For example, surveys in Georgia have revealed the highest densities of the crab during warmer months which dropped by 64-99% in cooler months (Hollebone and Hay 2007). It is unclear whether the lower abundances we detected in the winter and spring are because the crab buries itself and/or moves into deeper waters with greater temperature stability; whether it utilizes other habitats during cooler months not in close proximity to our samplers; or whether there is greater mortality during the coldest parts of the year (Mack et al. 2019). Lab studies have indicated upper and lower thermal tolerances of 40.5 °C and 16 °C for the crab, respectively (Oliveira and Masunari 1995; Stillman and Somero 2000). As winter temperatures can drop below this threshold in our study region (Moore et al. 2020, 2023), it may be that crabs face strong environmental stressors during the coldest part of the year, leading to greater mortality. Field experiments in the region examining temperature stress on crab mortality could help to resolve this question, while tagging experiments could determine whether crabs move to different habitats or deeper waters during winter and spring months.

Finally, in the phylogenetic tree comprised of our NC porcelain crab sequences, two major clades were apparent that also contained the *P. armatus* and *P. galathinus* sequences from Genbank that were top matches to our NC sequences (Figure 4). Our two *P. galathinus* sequences had the same sequence as one another, and they were 95% similar to a *P. galathinus* sequence from Genbank which originated from Panama (Venera-Pontón et al. 2020). The *P. armatus* clade showed some genetic diversity among the sequences, which were 98% to 100% similar to each other and also to the closest matching *P. armatus* sequence in Genbank, which originated from

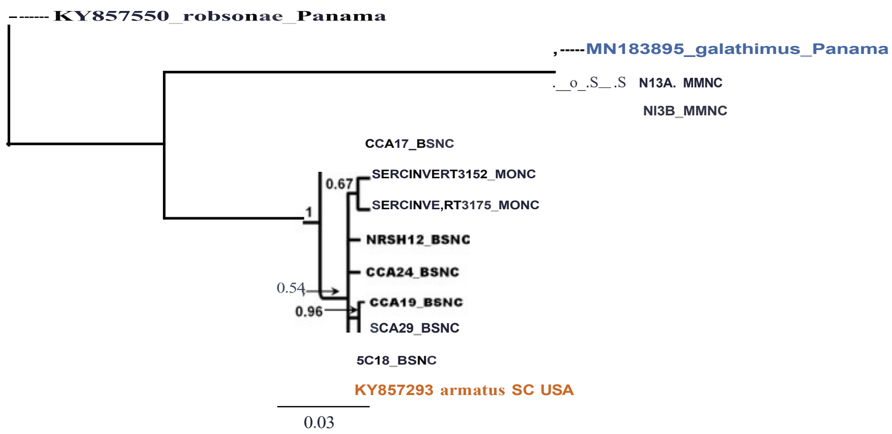

Figure 4. Bayesian phylogenetic tree produced using Mr. Bayes in Geneious 2023.0.4. TI1e tree includes 8 *P. annatus* and 2 *P. galathinus* sequences from our NC sample sites, along with *P. annatus* and *P. galathinus* sequences extracted from GenBank. TI1eorange sequence (accession# KY857293; Hiller and Lessios 2017) represents the closest match on Genbank to our consensus sequence of NC *P. armatus,* and the blue sequence (accession #: MN183895; Ve11era-Pont611et al. 2020) represents the closest match on Geubank to our consensus sequence of NC *P. galathinus.* TI1e values at nodes are poste1ior probabilities. The tree is rooted with au overlapping *P. robsonae* sequence (accession #: KY857550; Hiller and Lessios 2017). All sequences ending in "MMNC" are from Middle Marsh, NC; those ending in "BSNC" are from Back Sound, NC; and those ending in "MONC" are from Morehead City, NC.

South Carolina (Hiller and Lessios 2017). Given our limited sequencing to date in NC, it is beyond the scope of this study to examine or interpret the NC data in terms of the species' population genetics. However, past studies have investigated the phylogenetics and population genetics of the two species in other parts of their ranges. For example, past work on *P. galathinus* has found considerable morphological and genetic evidence of a species complex, and thus *"P.galathinus"* is believed to be multiple species (Hiller et al. 2006). In addition, Hiller and Lessios (2017) examined sequence data from multiple populations throughout the widespread distribution of *P. armatus,* finding southeastern USA sequences (including samples from Florida, Georgia, and South Carolina) to represent their own clade that was distinct from populations further south in Mexico and Brazil. The closest match to our NC *P. armatus* samples was a Hiller and Lessios (2017) sequence from South Carolina; this may suggest that the source of the NC *P. armatus* populations are from locations further south within the species' expanded southeastern USA range. However, more extensive sequencing from NC populations is needed, which can be compared to *P. armatus* and *P. galathinus* sequences from other studies (e.g., Hiller et al. 2006; Mantelatto et al. 2011; Hiller and Lessios 2017i Venera-Pont6n et aL 2020) to resolve phylogenetics and population genetics questions pertaining to porcelain crabs found along the central NC coast.

Conclusions

Annual and seasonal field surveys of the NC central coast allowed for the detection of *P. armatus* when it first appeared in the region. Observations of relatively high abundances at multiple field sites, as well as year-to-year detections since 2020, including recent surveys (September and October 2023) at Taylor Creek, Carrot Island and a new record in Pine Knoll Shores, NC (G. Loonam, M. Schulte *pers. comm.),* have found *P. armatus* **in** relatively high abundances and suggest it is established and spreading in this region. The NC central coast now represents the northernmost location of the species' northwest Atlantic range expansion. It is important to continue to monitor the area and determine whether *P. armatus* can continue to "creep" its way further northwards, possibly to other suitable locations in North Carolina or potentially Virginia. While temperature appears to limit its ability to spread and establish further north (Canning-Clode et al. 2011), recent environmental evidence suggests that summer length in coastal North Carolina has increased significantly over the past 10 years (Bartenfelder et al. 2022). These warming trends are a likely mechanism for *P. armatus'* recent expansion into our region, and continued warming could allow access to more northern regions in the future. Moreover, *P. galathinus'* northernmost extent is also North Carolina (Williams 1984), and thus, it would be important to monitor whether this species may also spread further northwards. Though *P. armatus* is a suspension feeder, and as such,does not impart strong predatory impacts on native species like other non-native crabs, it is unclear whether high densities of *P. armatus* could impart strong competition for space or other resources with its native congener *P. galathinus* or other oyster reef inhabitants and oyster predators (e.g., stone crabs and mud crabs), thereby altering community and food web dynamics (Hollebone and Hay 2008). Future work should aim to resolve these underlying questions, as well as investigate the population genetics of *P. armatus* in established populations of its expanding range - now to the central North Carolina coast. Altogether, *P. armatus* represents a valuable case study of tropical/ subtropical species expanding into temperate regions, like North Carolina, and highlights the importance of early documentation of population- and community-level responses at the leading edge of a species' expanding range.

Authors' contribution

Research conceptualization: AMHB, CSM, RKG; sample design and methodology: AMHB, CSM, RKG; investigation and data collection: all authors; data analysis and inte1pretation: AMHB; ethics approval: AMHB, CSM, RKG; ftmding provision: AMHB, CSM, RKG; roles/wiitiug - original draft: AMHB; vni.ting - review and editing: all authors.

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Ethics and permits

Field collections were authorized by the North Carolina Division of Marine Fishe1ies (Scientific or Educational Permit #706671 and #1913987) and by the North Carolina Coastal Reserve (Permit #13-2019). Animal husbandry and dissection protocols were approved by East Carolina University's (ECU) Institutional Animal Care and Use Committee (Animal Use Protocol #D346, #D358).

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