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ORIGINAL PAPER

Population genetics of estuary and reservoir populations of Harris mud crabs, *Rhithropanopeus harrisii*, in Texas and Oklahoma

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Abstract *Rhithropanopeus harrisii* are small, estuarine crabs native to the Atlantic and Gulf coasts of North America. They have become an invasive species, establishing populations on the west coast of the United States, Europe, Panama, and Japan. Reproducing populations are also established in freshwater reservoirs in Texas and on the Texas/Oklahoma border. In order to compare levels of genetic diversity within introduced reservoir populations with those of native estuary populations and to determine possible source populations and routes of colonization among Texas reservoir populations, we obtained mitochondrial DNA sequences from reservoirs and several estuaries along the Texas and Louisiana coast. Overall, genetic diversity within reservoirs was lower than within estuaries; however, some reservoirs exhibited relatively high levels of genetic diversity indicating that they were founded by numerous individuals or individuals from divergent source populations. In contrast, two genetically divergent reservoir populations had greatly reduced genetic diversity suggestive of extreme founder effects. All estuary and reservoir haplotypes formed a monophyletic group separate from Atlantic coast haplotypes, thus colonization of Texas reservoirs occurred from the Gulf Coast as expected based on geographic proximity. There was minimal DNA sequence divergence among Gulf Coast and reservoir haplotypes and a lack of phylogeographic structure among estuary populations. However, there was significant population divergence among some estuaries based on haplotype frequencies. Genetic differences among estuaries were subtle in most cases, preventing identification of source populations using mitochondrial DNA sequences.

Keywords *Rhithropanopeus harrisii* · Population genetics · Estuaries · Reservoirs · Introduced

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Introduction

The introduction of non-native species often occurs by the release of small numbers of individuals, thus new populations that become established are predicted to show evidence of the founder effect and have lower

genetic diversity than that of their source populations (Holland 2000). Alternatively, if the number of founding individuals was large, the reduction in genetic diversity would be negligible. Furthermore, if non-native populations receive multiple introductions from genetically divergent source populations, the genetic diversity of introduced populations can actually be higher than that of any one native population (Roman and Darling 2007). Distinguishing between these scenarios requires thorough sampling of native populations so that patterns of genetic diversity of potential sources populations are accurately documented (Hardouin et al. 2018).

The successful establishment of introduced species requires that species quickly adapt to the newly colonized ecosystem (Lee 2002), but the reduced genetic diversity of many introduced populations is expected to limit adaptive potential. The large numbers of successful introductions despite their reduced genetic diversity has been termed the 'genetic paradox' (Roman and Darling 2007). Successful introductions often involve the transfer of organism into habitats to which they were already broadly adapted. For example, marine and estuarine species introduced into similar marine and estuarine habitats. However, there are numerous examples of saline-adapted organisms invading freshwater environments (Paiva et al. 2018). Some of these species may have been predisposed to successful invasion because of their evolutionary histories (Casties et al. 2016), thus facilitating the adaptive process. The estuarine and saltmarsh copepod *Eurytemora affinis* species complex is a remarkable and well-studied example which has successfully invaded freshwater habitats multiple times from genetically divergent clades throughout the Northern Hemisphere (Lee 1999; Winkler et al. 2008). The specific evolutionary adaptations which allowed the copepod *E. affinis* to transition from marine to freshwater environments are being investigated (Stern and Lee 2020), but the extent to which these mechanisms can be generalized could be better understood by studying additional species which have made similar adaptive leaps from marine to freshwater environments.

Another example of an estuarine species successfully invading freshwater environments is the Harris mud crab, *Rhithropanopeus harrisii* (Gould). This species has become established in freshwater reservoirs as well as other estuaries. *R. harrisii* is native to estuarine environments of the eastern coast of North America from Canada to Veracruz, Mexico (Williams 1984). These crabs are a global invader of estuarine habitats, having first been observed in the Netherlands in 1874 (Maitland 1874) but initially misidentified (Buitendijk and Holthuis 1949). Since then, they have spread to locations in the North and Baltic seas (Fowler et al. 2013), the Pacific coast of the United States (Jones 1940), Venezuela (Rodriguez 1963), the Panama Canal (Roche and Torchin 2007), and Japan (Iseda et al. 2007). These non-native populations are thought to have been established through introductions from ballast water, hull fouling, and live oyster shipments.

In addition to having invaded estuarine habitats, *R. harrisii* have become established in freshwater reservoirs in Texas and on the Texas-Oklahoma border. The first occurrence was documented in 1998, when crabs were found in Possum Kingdom Reservoir (Howells 1998). Subsequently, they have been documented in the following Texas and Oklahoma reservoirs: Lake Granbury, Lake Whitney, Lake Colorado City, E.V. Spence Reservoir, Tradinghouse Creek Reservoir, Lake Balmorhea, Squaw Creek Reservoir, and Lake Texoma (Fig. 1; Boyle et al. 2010; Patton et al. 2010). Most recently (2011), *R. harrisii* was documented in Hubbard Creek Reservoir in Texas (personal observation by TMB). The presence of gravid females, zoeae, and/or juvenile crabs in these reservoirs demonstrate that these are reproducing populations (Boyle et al. 2010). These represent the only known instances of marine or estuarine crabs establishing reproducing populations in freshwater reservoirs. How dispersal occurred is unknown, though it seems most likely to be human-mediated given the large distance between reservoirs and potential source populations.

Given that *R. harrisii* are native to the brackish waters of estuaries but has established reproducing populations in freshwater reservoirs, there is a potential role for adaptation facilitating these successful invasions. Like many estuarine species, *R. harrisii* exhibits relatively broad salinity tolerances. Adults have been reported to survive in fresh water for several months, although they appeared to experience disturbances in the molting cycle (Turoboyski 1973). Larvae, however, were reported to not survive at salinities less than 1 ppt, and survival of zoeae was greatly reduced at 2.5 ppt (Costlow et al. 1966;

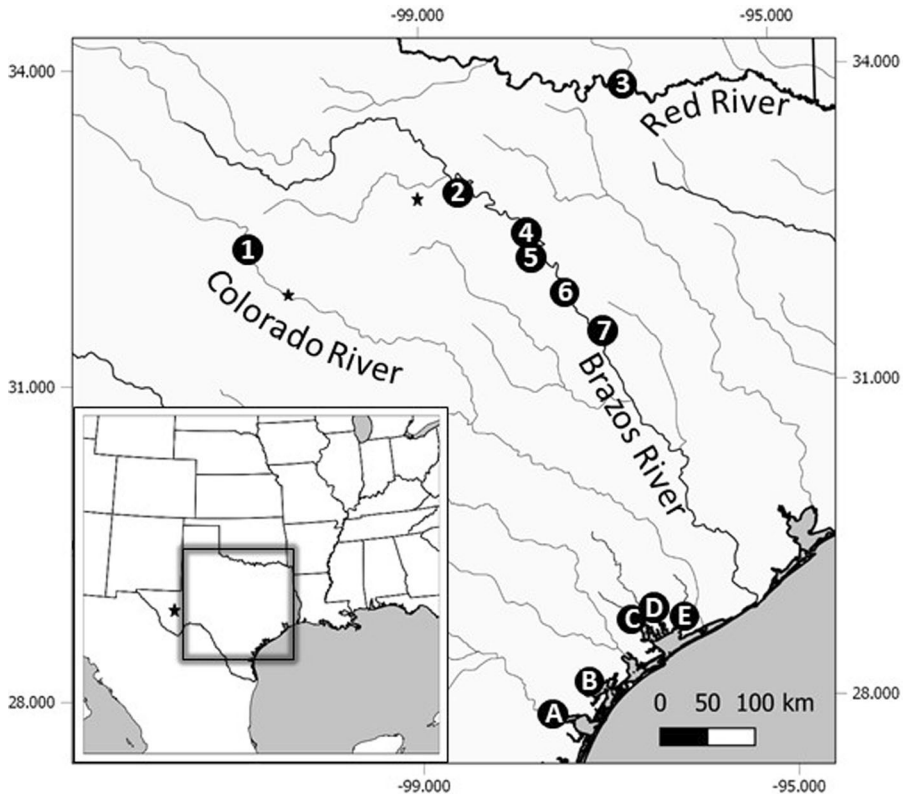

Fig. 1 Location of reservoirs and estuaries from which R. harrisii specimens were obtained for genetic analysis: (1) Colorado City Reservoir, (2) Possum Kingdom Reservoir, (3) Lake Texoma, (4) Lake Granbury, (5) Squaw Creek Reservoir, (6) Lake Whitney, (7) Tradinghouse Creek Reservoir, (A) Nueces River, (B) Mission River, (C) Lavaca River, (D) Garcitas Creek, (E) Tres Palacios River. Stars indicate additional reservoirs with documented occurrence of *R. harrisii*

Turoboyski 1973). At salinities below 15 ppt, larval survival was higher in water that was 30 °C compared to 20 °C (Costlow et al. 1966). Laughlin and French (1989) found that larvae reared in the lab at 2.0 ppt had only a 5.5% survival rate. In apparent contrast to some of these findings, gravid females and very small juvenile crabs were reported in large numbers by Boyle et al. (2010) in Possum Kingdom Reservoir at salinities of 1.4 to 2.6 and Tradinghouse Creek Reservoir which has salinities between 0.4 and 0.5. Although *R. harrisii* have been documented to die at low salinities in the laboratory, studies of salinity tolerance were apparently based on specimens native to or introduced from the Atlantic coast. Gulf coast populations from which Texas reservoir populations are thought to be derived (Boyle et al. 2010) are genetically distinct from those of the Atlantic coast populations of New Jersey and North Carolina (Projecto-Garcia et al. 2010; Tepolt et al. 2020) leaving open the possibility of geographic variation in salinity tolerance.

A better understanding of *R. harrisii* could help to elucidate the role of genetic variation and adaptive potential in the successful establishment of invasive species. Toward that goal, we collected specimens from multiple estuaries and reservoirs in Texas to determine levels of genetic diversity within introduced reservoir populations relative to their native estuaries and possible source populations and routes of colonization from estuaries to and among reservoirs. Mitochondrial DNA (mtDNA) sequences were obtained from several estuaries along the Texas coast and from additional reservoirs not examined by Boyle et al. (2010).

Materials and methods

Sample collection

Specimens were collected from the following reservoirs by hand while examining shoreline debris: Whitney, Texoma, and Squaw Creek (Fig. 1 and Appendix). All specimens were found in water less than one meter deep in windward bays with fine sediment substrates and collected from wood and other debris. Specimens were placed on ice in the field and transferred to 95% ethanol for storage. Collection of live specimens from Squaw Creek Reservoir (the cooling impoundment for Comanche Peak Nuclear Power Plant) failed four times from March through August 2011. Collection attempts in this reservoir were challenging because wading is prohibited in the reservoir inside the only available public-access area. Claws, legs, and carapaces were found, and park personnel reported crabs on shorelines in early March, but no live specimens were found. Live trapping with dead fish as bait in wooden bundles failed also. The Aquatic Biologist for Atkins Global (Austin, Texas) supplied 80 crabs from a restricted, safe shut down impoundment adjacent to the reservoir. Lake Whitney was sampled in March 2011 (approximately 50 specimens), and Lake Texoma was sampled in June 2011 (approximately 45 specimens). An attempt was made to collect specimens at Lake Braunig in July 2011, based on an unconfirmed report from that reservoir. However, no specimens nor evidence of crabs (such as claws, legs, or carapaces) were found, and conversations with fishermen failed to reveal any knowledge of crab populations.

Specimens were collected from five coastal estuaries in July 2008: Nueces River, Mission River, Garcitas Creek, Lavaca River, and Tres Palacios River. Additional specimens were collected from the Tres Palacios River in June 2017. These estuaries exhibit varying levels of connectivity. The Nueces River flows into Nueces Bay, which in turn opens into Corpus Christi Bay. The Mission River flows into Mission Bay which opens into Copano Bay which opens into Aransas Bay. Both Garcitas Creek and the Lavaca River flow into Lavaca Bay, and the Tres Palacios River flows into Tres Palacios Bay. All three of these bays open into Matagorda Bay. Copano, Aransas, and Matagorda bays were historically connected (at least partially) by marshes behind the barrier islands and are currently fully connected by the Texas Inter-coastal Waterway.Laboratory procedures

Laboratory procedures

DNA was extracted using either phenol/chloroform and ethanol precipitation or the method of Ivanova et al. (2006) from muscle tissue removed from the cheliped of adults or from the entire cheliped and legs of smaller crabs. For very small crabs, half or even the entire crab was used. Polymerase chain reaction (PCR) was then used to amplify the mitochondrial cytochrome *c* oxidase I gene (COI) for sequencing using primers developed by Folmer et al. (1994; LC01490 5'-GGTCAACAAATCATAAAGATATTGG-3'and HC021981 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions were carried out in final volumes of 25 µL consisting of 1X buffer, 2.5 mM $MgCl_2$, 0.16 mM each dNTP, 0.1 µM each primer, and 0.05 U *Taq* DNA polymerase (Qiagen). PCR reactions were performed in thermal cyclers as follows: 95 °C for five min, twenty cycles at 95 °C for one min, 52 °C for thirty sec, 72 °C for one min thirty sec, twenty cycles of 95 °C for thirty sec, 42 °C for one min, and 72 °C for one min, followed by 72 °C for ten min. Excess primers and dideoxyribonucleotides were removed using ExoSAP-IT (Affymetrics). Separate sequencing reactions using the same forward and reverse primers as used for PCR were performed using Beckman-Coulter chemistry, cleaned by ethanol precipitation, and visualized using a Beckman-Coulter CEQ 8000 Genetic Analysis System. Sequences were aligned to a reference and visually inspected for errors and low-quality base calls using Beckman-Coulter software.

Data analysis

DNA sequences obtained as part of this study were combined with those obtained by Boyle et al. (2010; GenBank numbers EF467218-EF467227, GQ332605, and GQ332606 representing 82 specimens from Possum Kingdom Reservoir, Lake Granbury, Colorado City Reservoir, Tradinghouse Creek Reservoir, and Garcitas Creek Reservoir) and Projecto-Garcia et al. (2010; GenBank numbers FJ517513-FJ517532 representing 20 specimens collected from the vicinity of Intracoastal City, Vermilian Bay, Louisiana [J. Projecto-Garcia pers. comm.]). Sequences obtained by

Peterson (2006) from Neuse River, North Carolina (DQ094789, DQ094813, and DQ094814) and New Jersey (FJ517494, FJ517495, and FJ517511) were included as representatives of the divergent Atlantic clade (Boyle et al. 2010; Projecto-Garcia et al. 2010). A closely related species (*Necora puber;* DQ882052) was selected for use as an outgroup.

DNA sequences were aligned in the software Bioedit (Hall et al. 2011) using ClustalW. The software package Arlequin 3.5 (Excoffier and Lischer 2010) was used to calculate conventional pairwise F_{ST} from haplotype frequencies (with 16,000 permutations for significance), analysis of molecular variance (AMOVA), haplotype diversity (h), nucleotide diversity (π), and number of transitions and transversions. To protect against Type I error in the pairwise F_{ST} tests, significance levels were adjusted using sequential Bonferroni correction (Holm 1979) and the false discovery rate of Benjamini and Hochberg (1995) using the p.adjust function in the statistical program R. Tests of significance for number of haplotypes, h, and π were conducted in the statistical program R using Welch's unequal variances t-test. Hierarchical AMOVA was performed by grouping estuaries by bay (Matagorda Bay, Aransas Bay, Corpus Christi Bay) and reservoirs by river system (Red, Colorado, and Brazos). Within the hierarchical AMOVA, some groups contain only one population, but nesting with single or multiple subgroups does not influence the interpretation of the results. A median-joining haplotype network was created using PopART (Leigh and Bryant 2015). A neighbor-joining phylogenetic tree was constructed with the Kimura 2-parameter model using MEGA-X (Kumar et al. 2018).

Results

Haplotype abundance and distribution

Mitochondrial DNA sequences were obtained for the COI gene from 246 specimens from seven Texas reservoirs, five Texas estuaries, and one Louisiana estuary, including sequences from Boyle et al. (2010) and Projecto-Garcia et al. (2010). New haplotypes reported herein were accessioned to GenBank (MT313158-MT313172). Sequences were trimmed to 534 base pairs for analysis. Among the 34 haplotypes (Table 1), there were 30 polymorphic sites with 31 substitutions, including 26 transitions and five transversions. No insertions or deletions were observed. The most abundant haplotype (A) was found at all locations except Lake Texoma, Tradinghouse Creek Reservoir, and Louisiana (Table 1). The second most abundant haplotype (H) was found at all locations except Lake Whitney, Tradinghouse Creek Reservoir, and the Nueces River. Twenty-three haplotypes were private (unique to one site). Four of 11 haplotypes among reservoirs (36%) were private while 19 out of 29 haplotypes among estuaries were private (66%). The average number of haplotypes among reservoir populations (3.71) was significantly different (t = -2.921, P = 0.01588) than among estuary populations (7.66). Average haplotype diversity of the reservoir populations (h = 0.562 ± 0.269) was not significantly different (t = -1.521, P = 0.16) than that of the estuary populations (h = 0.745 ± 0.158). Average nucleotide diversity of the reservoir populations (h = 0.003 ± 0.002) was not significantly different (t = -0.588, P = 0.569) than that of the estuary populations (h = 0.003 ± 0.001). There was considerable variation of diversity values among reservoir and estuary populations (Table 2).

Phylogenetic structure and haplotype relationships

Based on the haplotype network (Fig. 2a), the greatest difference between any two neighboring haplotypes was two mutational steps. There were several haplotypes that were relatively abundant and scattered throughout the network. The most abundant haplotypes exhibited a star-like pattern with numerous one and two-step haplotypes connected to them. All common haplotypes were found in both estuary and reservoir populations.

All haplotypes from Texas and Louisiana formed a monophyletic clade with 81% bootstrap support (Fig. 2b). Atlantic coast haplotypes from New Jersey and North Carolina fell outside of this clade. Haplotypes from throughout the Gulf coast clade were distributed across estuary and reservoir populations without geographical partitioning in the phylogenetic tree.

Population structure

Comparisons of estuaries using AMOVA revealed that most (59.24%) of the genetic variation was found

Table 1 COI mtDNA haplotype frequencies of *R. harrisii* populations from Texas reservoirs and Texas and Louisiana estuaries

Locality	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	Y	Z	AA	CC	DD	EE	FF	HH	II	JJ	LL
Reservoirs																																		
Colorado City	6				2		3	8	2																									
Lake Granbury	10				1	1		6		1																								
Lake Texoma							16	13																										
Lake Whitney	6																																	
Possum Kingdom	9	1	1		1	1		7	1																									
Squaw Creek	7					6		4																						2				
Tradinghouse Creek				10	11																													
Estuaries																																		
Garcitas Creek	7			2	1			2			1	2								1									1		2			
Lavaca River	4			1	2			1															1									1		
Mission River	13				6			1				1											1				1	1	1				1	
Nueces River	9										2																							1
Tres Palacios River	1				3		1	8								1	1	1			1		1			1								
Louisiana								8	1				5	1	1				1			1		1	1									

Table 2 Sample size and genetic diversity of *R. harrisii* populations in Texas reservoirs and Texas and Louisiana estuaries. Sample size (n), number of haplotypes (n^{hap}), haplotype diversity (h), and nucleotide diversity (π)

Locality	River system or bay	n	n^{hap}	h (± SD)	π (± SD)
Reservoirs					
Colorado city reservoir	Colorado river	21	5	0.771 (0.057)	0.004 (0.003)
Lake Texoma	Red river	29	2	0.512 (0.031)	0.001 (0.001)
Lake Granbury	Brazos river	19	5	0.649 (0.085)	0.004 (0.003)
Lake Whitney	Brazos river	6	1	0.000 (0.000)	0.000 (0.000)
Possum Kingdom reservoir	Brazos river	21	7	0.729 (0.071)	0.005 (0.003)
Squaw Creek reservoir	Brazos river	19	4	0.749 (0.052)	0.005 (0.003)
Tradinghouse Creek reservoir	Brazos river	21	2	0.524 (0.036)	0.001 (0.001)
Estuaries					
Garcitas creek	Matagorda bay	19	9	0.854 (0.068)	0.005 (0.003)
Tres Palacios river	Matagorda bay	19	10	0.819 (0.083)	0.003 (0.002)
Lavaca river	Matagorda bay	10	6	0.844 (0.103)	0.005 (0.003)
Mission river	Aransas bay	26	9	0.714 (0.080)	0.004 (0.002)
Nueces river	Corpus Christi bay	12	3	0.439 (0.158)	0.001 (0.001)
Louisiana (Intracoastal City)	Vermilion bay	20	9	0.800 (0.073)	0.003 (0.002)

within estuaries (Table 3) with most of the remaining variation being partitioned among estuaries within bays (31.15%) rather than among bays (9.61%). *F*-statistics showed a significant difference among estuaries overall (F_{ST} = 0.140, P = 0.000) and among estuaries within bays (F_{SC} = 0.074, P = 0.011). Differences among bays (F_{CT} = 0.071, P = 0.624) were not statistically significant. Based on pairwise F_{ST} (Table 4), populations exhibiting the least divergence (F_{ST} effectively zero) were Lavaca River compared to Garcitas Creek and Mission River. The greatest divergences were Nueces River compared to Louisiana (F_{ST} = 0.359, P = 0.000) and Tres Palacios River (F_{ST} = 0.325, P = 0.000). Tres Palacios River and Garcitas Creek, despite emptying into the same bay, exhibited significant divergence (F_{ST} = 0.099, P = 0.006) while the comparison between Tres Palacios River and Louisiana was not significant (F_{ST} = 0.027, P = 0.129).

Comparisons of reservoirs using AMOVA showed that most (60.42%) of the genetic variation was found among individuals within reservoirs (Table 3) with the remaining variation partitioned among reservoirs within river systems (26.45%) and among river systems (13.13%). *F*-statistics showed a significant difference among reservoirs overall (F_{ST} = 0.396, P = 0.000) and among reservoirs within rivers (F_{SC} = 0.304, P = 0.000). Differences among rivers (F_{CT} = 0.131, P = 0.179) were not statistically significant. Based on pairwise F_{ST} (Table 5), Tradinghouse Creek Reservoir was significantly different from all other reservoirs. Lake Whitney was significantly different from all reservoirs except Lake Granbury (directly upstream from Lake Whitney). Lake Texoma was significantly different from all other reservoirs. Adjacent reservoirs directly connected along the Brazos River were not significantly different.

Discussion

Populations established by small founding numbers are expected to have lower genetic diversity and fewer rare haplotypes than the populations from which they were established (Roman and Darling 2007). In Texas reservoirs, we found that *R. harrisii* populations show at least some indications of lower genetic diversity than most estuary populations. All measures of average genetic diversity within reservoir populations were lower than those of estuary populations; however, not all measures were statistically significant. While lower diversity is expected for recently founded populations subjected to genetic drift there was considerable variation in genetic diversity among reservoirs, with some reservoirs (Colorado City, Possum Kingdom, and Squaw Creek) having genetic diversity exceeding that of some estuaries. Relatively high diversity within some reservoir populations could be explained by multiple introductions (perhaps from multiple source populations) or very large numbers of

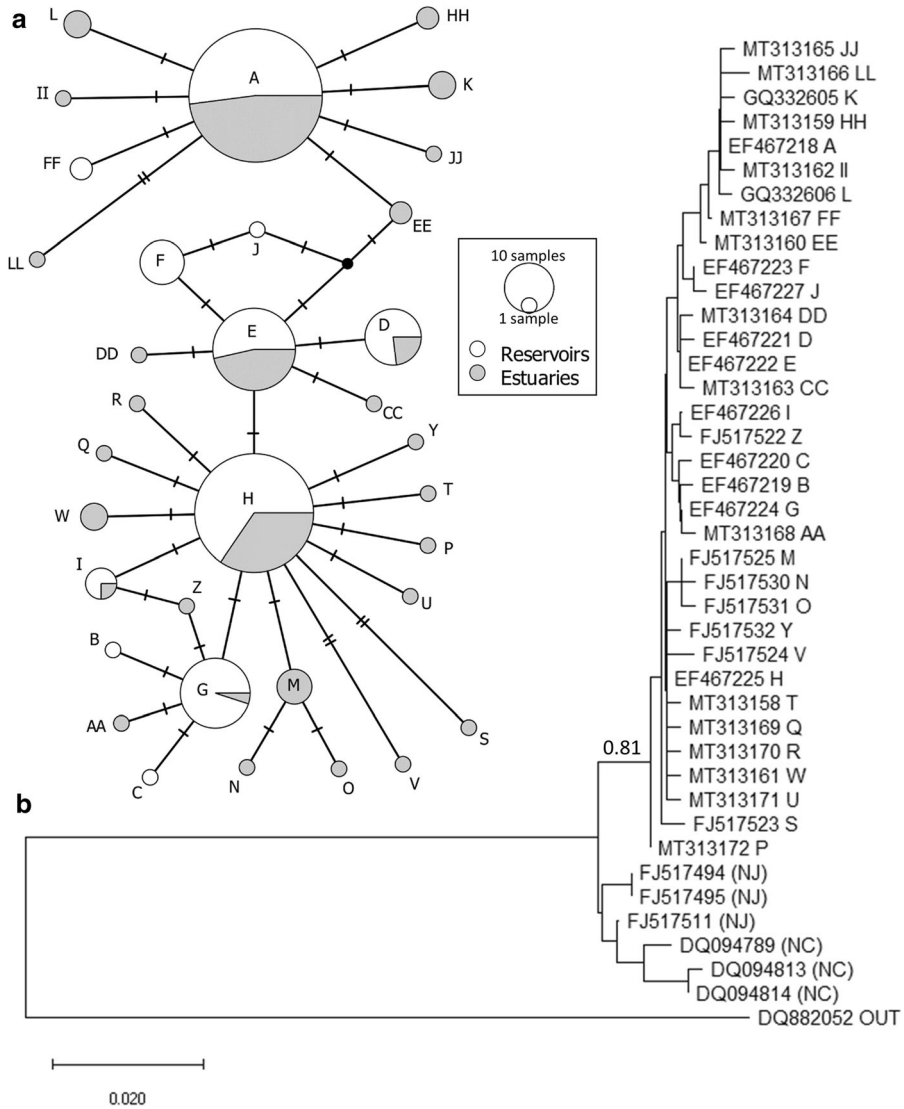

Fig. 2 Median-joining network **a** of *R. harrisii* Texas and Louisiana COI mtDNA haplotypes. Size of circles is proportional to haplotype frequency. Phylogenetic tree **b** of *R. harrisii* COI mtDNA haplotypes. The Texas/Louisiana clade received 81% bootstrap support. Sequences are identified by GenBank numbers. Letters following numbers are haplotype designations used in Table 1 (A-LL) or location designations (NJ = New Jersey, NC = North Carolina). DQ882052 (*Necora puber*) was used as an outgroup

founding individuals (propagule pressure; Allendorf and Lundquist 2003).

Texas estuaries are the most likely source of founding individuals. Our additional sampling from the Gulf Coast of Texas supported the monophyly of Gulf Coast mitochondrial haplotypes separate from Atlantic Coast haplotypes (Fig. 2b) as first documented by Projecto-Garcia et al. (2010) and Boyle et al. (2010). This pattern has been confirmed with nuclear genetic data (Tepolt et al. 2020) and can be used to identify the broad geographic source (Atlantic versus Gulf coast) of globally introduced populations. While gulf coast haplotypes formed a monophyletic clade with high bootstrap support, Atlantic coast haplotypes did not. This pattern is consistent with an expansion of *R. harrissii* from the Atlantic Coast to the Gulf Coast, with modern Gulf Coast haplotypes being derived from diversification of a single, ancestral

Table 3 Hierarchical analysis of variance of R. harrisii populations from Texas and Louisiana estuaries and reservoirs

Source of variation	d.f	Sum of squares	% of total variation
Estuaries			
Among bays	3	39.95	9.61
Among estuaries within bays	2	16.49	31.15
Within estuaries	100	90.45	59.24
Reservoirs			
Among river systems	2	30.33	13.13
Among reservoirs within river systems	4	26.22	26.45
Within reservoirs	129	101.59	60.42

Estuaries were grouped by bay: Matagorda Bay (Garcitas Creek, Lavaca River, and Tres Palacios River), Corpus Christi Bay (Nueces River), Aransas Bay (Mission River), and Vermilion Bay (Intracoastal City, Louisiana). Reservoirs were grouped by river system: Brazos River (Possum Kingdom Reservoir, Lake Granbury, Squaw Creek Reservoir, Lake Whitney, and Tradinghouse Creek Reservoir), Colorado River (Lake Colorado City), and Red River (Lake Texoma)

Table 4 Pairwise FST and accompanying *P*-values (uncorrected; in parentheses) of *R. harrisii* populations from Texas and Louisiana Estuaries

	Garcitas Creek	Lavaca river	Mission river	Nueces river	Tres Palacios river
Lavaca river	− 0.034				
	(0.867)				
Mission river	0.014	− 0.040			
	(0.216)	(0.879)			
Nueces river	0.081	0.090	0.063		
	(0.041)	(0.075)	(0.082)		
Tres Palacios river	**0.099**	0.077	**0.168***	**0.325***	
	(0.006)	(0.053)	**(0.000)**	**(0.000)**	
Louisiana	**0.137***	**0.146***	**0.233***	**0.359***	0.027
	(0.000)	**(0.004)**	**(0.000)**	**(0.000)**	(0.129)

Values that were significant following sequential Bonferroni correction (Holm) are marked with an asterisk. Those that were significant following minimization of false discovery rate (Benjamini and Hochberg) are in bold

Atlantic Coast haplotype. Alternatively, the Gulf Coast population may have recovered from a bottleneck that was not experienced by the Atlantic Coast population. Because all Texas reservoir haplotypes grouped with Gulf coast haplotypes in the phylogenetic tree (Fig. 2b), Atlantic Coast populations apparently did not contribute to colonization of Texas reservoirs.

Determination of a more precise source of reservoir populations requires that Gulf Coast estuary populations show further genetic subdivision. Limited gene flow among estuaries is expected because *R. harrisii* exhibits a larval retention mechanism (Cronin 1982; Cronin and Forward 1986; Forward 2009), thus genetic subdivision among at least some estuaries would be expected. Larvae of *R. harrisii* develop through four zoeal stages and one megalopal stage which undergo vertical migration in the water column within their native environments (Cronin 1982; Cronin and Forward 1986). Larvae rise when tides are coming in and sink to the bottom when tides are going out. These vertical migrations reduce dispersal from estuaries into open water. Since *R. harrisii* are known to exhibit larval retention mechanisms, genetic structure may occur among estuaries if these mechanisms minimize gene flow (Bilton et al. 2002).

Table 5 Pairwise FST and accompanying *P*-values (uncorrected; in parentheses) for *R. harrisii* populations from Texas reservoirs

	Whitney	Squaw Creek	Texoma	Possum Kingdom	Granbury	Colorado city
Squaw Creek	**0.266**					
	(0.020)					
Texoma	**0.621***	**0.320***				
	(0.000)	**(0.000)**				
Possum Kingdom	**0.218**	0.024	**0.285***			
	(0.031)	(0.197)	**(0.000)**			
Granbury	0.179	0.033	**0.337***	-0.037		
	(0.063)	(0.188)	**(0.000)**	(0.951)		
Colorado city	**0.313**	0.067	**0.159**	-0.011	0.019	
	(0.005)	(0.048)	**(0.005)**	(0.631)	(0.223)	
Tradinghouse Creek	**0.627***	**0.366***	**0.485***	**0.358***	**0.398***	**0.318***
	(0.000)	**(0.000)**	**(0.000)**	**(0.000)**	**(0.000)**	**(0.000)**

Values that were significant following sequential Bonferroni correction are marked with an asterisk. Those that were significant following minimization of false discovery rate are in bold

Our results showed evidence of genetic divergence among some estuaries, but not all. The two locations furthest apart (Nueces River and Louisiana) exhibited the highest F_{ST}. The most unexpected pattern of divergence was the comparison of Tres Palacios with Garcitas Creek (adjacent estuaries within Matagorda Bay) and Louisiana. F_{ST} values were relatively high (and significant) between Tres Palacios and Garcitas Creek but low (and not significant) between Tres Palacios and Louisiana, which is surprising based on the large geographic distance separating them (612 km) and the lack of continuity caused by the termination of interconnected bays and the Texas Intercostal Waterway. All Louisiana haplotypes were either the abundant and widely distributed haplotype H or within two mutational steps of haplotype H; however, Louisiana lacked the most abundant and widely distributed haplotype A which was present in all other estuaries (albeit rare in the Tres Palacios River). These haplotype distributions and F_{ST} values cannot be explained by geographic distance between estuaries alone—genetic drift, human-mediated transport, and unexpected patterns of connectivity may contribute to the observed patterns.

Studies of estuarine species often show genetic divergence among at least some estuaries as predicted by the presence of barriers to dispersal (Bilton et al. 2002). The naked goby (*Gobiosoma bosc*) is an estuarine-dependent, euryhaline fish with a distribution similar to that of *R. harrisii* and which also exhibits a genetic break at Florida (Milá et al. 2017; Moore et al. 2018), albeit of much greater divergence than that of *R. harrisii*. At a regional scale (within major clades), significant geographic structure was documented (Milá et al. 2017). On a smaller scale, however, *G. bosc* exhibited extensive connectivity between two adjacent estuaries (Moore et al. 2018). Burridge et al. (2004) and Burridge and Versace (2007), examining patterns of divergence within another estuarine fish (the black bream, *Acanthopagrus butcheri*) in Australia, found a significant pattern of isolation-by-distance consistent with the stepping-stone model of infrequent gene flow among estuaries.

Species in the Gulf of Mexico less restricted to estuaries have shown contrasting results. McMillen-Jackson and Bert (2003, 2004) studied genetic structure in three species of shrimp inhabiting the coasts of the Gulf of Mexico and Atlantic Ocean. All three species have a planktonic larval stage that, unlike *R. harrisii*, recruit to estuaries as post-larvae then return to live and spawn offshore. Brown and pink shrimp (*Farfantepenaeus aztecus* and *F. duorarum*) live and spawn further offshore than white shrimp (*Litopenaeus setiferus*), which prefers lower salinity waters and inhabits shallower nearshore waters as adults. McMillen-Jackson and Bert (2003, 2004) found genetic structuring in white shrimp, but a lack of genetic structuring in brown and pink shrimp

populations. White shrimp were divided into two distinct lineages, with frequencies of haplotypes separating western Gulf samples from Atlantic and eastern Gulf samples and exhibited a significant relationship between genetic and geographical distance.

Patterns of genetic structure among estuarine-dependent species is largely dependent on dispersal ability. While *R. harrisii* exhibits a larval retention mechanism (Cronin 1982; Cronin and Forward 1986), it is not certain how frequently larvae exit estuaries, or to what extent they are able to return. Peterson (2006), based on studies of *R. harrisii* at Atlantic and European sites, proposed either a small fraction of larvae may exit home estuaries each year or large fractions may be washed out during rare flooding events. Although off-shore currents in the Gulf of Mexico flow from south to north and east (Lin et al. 2010), in-shore currents are more complex (Smith 2008). Given that larvae can survive oceanic salinities, Costlow (1966) assumed it reasonable that *R. harrisii* could survive being carried between estuaries by offshore currents. Furthermore, metamorphosis of megalopae is known to be delayed by exposure to offshore (high salinity) water and adult odor cues (Fitzgerald et al. 1998). If crab larvae leave estuaries (either regularly or periodically due to floods), in-shore currents could result in dispersal in either direction depending on the direction of currents at that particular time and location.

Following the initial founding of reservoir populations, dispersal from one reservoir to another is possible either naturally, by way of interconnecting rivers, or by human-mediated transport. Because reservoirs within river systems were not more similar to one another than reservoirs in separate river systems (Table 3), natural dispersal among reservoirs did not appear to play a major role in the partitioning of genetic diversity. Although many reservoirs had similar haplotype frequencies (Table 1) there were three notable exceptions: Lake Whitney, Tradinghouse Creek Reservoir, and Lake Texoma. The Lake Whitney sample was fixed for a single haplotype (A) common in most other reservoirs and the south-western-most estuaries. Tradinghouse Creek Reservoir had only two haplotypes (at almost equal frequency), neither of which was common among other reservoirs. One haplotype (D) was rare even among estuaries and absent from any other reservoir. Lake Texoma is in a separate river system and had only two haplotypes (at almost equal frequency), one was common among other reservoirs and the north-eastern estuaries and the other was rare among reservoirs and estuaries. Over half of the comparisons among reservoirs revealed significant genetic divergence (F_{ST}). Based on the AMOVA, 26% of variation was explained by differences among reservoirs within river systems. Examining patterns of pairwise F_{ST}, much of this variation appeared to be due to Tradinghouse Creek which is not connected to the other reservoirs by natural inflow from the Brazos River. Possum Kingdom Reservoir flows into Lake Granbury, which flows into Lake Whitney. Tradinghouse Creek Reservoir is an off-channel reservoir receiving water via a pipeline from the Brazos River below Lake Whitney rather than a natural connection. The significant divergence of this reservoir from Lake Whitney (no haplotypes are shared) indicates that dispersal between these reservoirs has not occurred. Squaw Creek, a cooling reservoir for the Comanche Peak Nuclear Power Plant, which receives water via pipeline from Lake Granbury, does not differ significantly from Lake Granbury. Furthermore, Possum Kingdom and Lake Granbury do not differ significantly. It is possible that dispersal occurs between some reservoirs but not others due to differences in dam structure or river ecology, or that insufficient time has elapsed to allow gene flow to homogenize haplotype frequencies.

It is not known how *R. harrisii* became established in reservoirs, though likely mechanisms include introductions through "bait bucket" releases and/or game fish stocking (Howells 2001; Boyle et al. 2010). Additionally, it remains an avenue for future research to determine if estuarine populations of *R. harrisii* are historically capable of reproducing at the lower salinities of reservoirs or if this is a more recent adaptation associated with their colonization of reservoirs. Rapid adaptation from standing genetic variation and from new mutations is thought to contribute to the success of many invasive species (Bock et al. 2015; Stern and Lee 2020). Although *R. harrisii* have been shown to not survive at low salinities, our knowledge of salinity tolerance of *R. harrisii* is based on studies of specimens native to or introduced from the Atlantic coast (Costlow et al. 1966, Turoboyski 1973). Because of the genetic divergence between Atlantic and Gulf coast populations, it is possible that

salinity tolerances differ between these two clades. Differences in salinity tolerance among genetic lineages of the amphipod *Gammarus tigrinus* was suggested by Kelly et al. (2006) as an explanation for their observed patterns of habitat colonization. If the source populations of *R. harrisii* occupying freshwater reservoirs were already tolerant to a broader range of salinities, their establishment in freshwater reservoirs would only have required human-mediated dispersal.

In conclusion, *R. harrisii* in Texas reservoirs almost certainly originated from Gulf coastal estuaries, with no evidence of introductions from other coastal populations. The subtle and complex patterns of haplotype frequencies among estuaries precluded a more specific identification of source populations. Even in ideal situations, the identification of source populations can be challenging because confounding factors (including founder effects and multiple introductions) can result in similar patterns of haplotype frequencies (Estoup and Guillemaud 2010). However, there does appear to be genetic divergence among some Gulf coast estuaries that could prove useful in identifying source populations if multi-locus, nuclear genetic markers are used (Tepolt et al. 2020). With some exceptions, genetic diversity was lower for reservoir populations relative to estuaries as expected of populations founded by a relatively small number of individuals (though not all measures of diversity were statistically different). Moderately high diversity within freshwater reservoirs may have contributed to their successful establishment. Whether *R. harrisii* along the Gulf coast were already tolerant of very low salinities, thus requiring only transport to reservoirs, or if propagule pressure and standing genetic variation (Bock et al. 2015) contributed to adaptation during the founding events remains an open question. Comparative studies of salinity tolerance between reservoir and Gulf coast estuarine populations may shed light into the mechanisms that allowed this species to become established in freshwater reservoirs.

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Availability of data and material DNA sequences are available on GenBank.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

Appendix: Locality information for specimens collected as part of this study

Reservoirs

Squaw Creek Reservoir (March through August 2011): safe shut-down impoundment adjacent to reservoir (32.2957785, − 97.7868482). *Lake Whitney* (March 2011): Lake Whitney State Park on Farm to Market Road 1244 in a bay on the northern boundary of the park (31.923169, − 97.3749368). *Lake Texoma* (June 2011): west boundary of The University of Oklahoma Biological Station on the north shore of Lake Texoma east of State Highway 377 (33.8802968, − 96.8022547). *Lake Braunig* (July 2011): Lake Braunig Park, 17,500 Donop Road, San Antonio, Texas (29.2543883, -98.3920036).

Estuaries

Nueces River (July 2008): bridge of Interstate Highway 37 over the Nueces River in Patricio County (27.8919027, − 97.6292523). The Nueces River flows into Nueces Bay. *Mission River* (July 2008): at the intersection of Farm to Market Road 2678 crossing the Mission River in Refugio County (28.1834974, − 97.2141456). The Mission River flows into Mission Bay and then into Copano Bay and then into Aransas Bay. *Garcitas Creek* (July 2008): bridge of Farm to Market Road 616 over Garcitas Creek on the border of Victoria and Jackson Counties (28.7776815, -96.6995914). Garcitas Creek flows into Lavaca Bay then into Matagorda Bay. *Lavaca River* (July 2008): bridge of Farm to Market Road 616 crossing the Lavaca River in Jackson County (28.8316365, − 96.5790748). The Lavaca River flows into Lavaca Bay and then into Matagorda Bay. *Tres Palacios River* (July 2008 and June 2017): bridge of Farm to Market Road 521 over the Tres Palacios River in Matagorda County (28.7861209, − 96.1511496).

The Tres Palacios River flows into Tres Palacios Bay and then into Matagorda Bay.

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