Examining the Influence of Genetics on Migration and Habitat Preference in *Callinectes sapidus*

By

Megan Moran

Dr. Daniel Rittschof, Adviser

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Abstract

The Atlantic blue crab (*Callinectes sapidus*) is an ecologically and commercially fundamental species. At various life stages, crab migrations are influenced by environmental cues including light, salinity, chemistry, depth change, turbulence, and water flow. Though adult and juvenile blue crabs live in estuaries, the larval stages of all genotypes are mixed and develop in the coastal ocean. The objective of this study is to determine whether blue crab habitat use, and migration patterns are reflected in the mitochondrial cytochrome c oxidase 1 (CO1) gene region. This will be determined by examining resident blue crabs from Carrot Island, NC (29-35 PSU) and Lake Mattamuskeet, NC (0 PSU), and spawning female crabs from Beaufort Inlet, NC (29-34.5 PSU). Carrot Island had a relatively lower haplotype diversity (0.7260 ± .03900) compared to Beaufort Inlet (0.9841 ± .00021) and Lake Mattamuskeet (0.94154 ± .00118). Significant pairwise differences were found between Carrot Island and Beaufort Inlet ($Nm = 0.26018$, $p < 0.001$), as well as between Carrot Island and Lake Mattamuskeet ($Nm = 0.19482$, $p < 0.001$), indicating a lack of gene flow. Overall, blue crabs from Carrot Island had high, significant genetic differentiation when compared to crabs from both Beaufort Inlet ($Fst = 0.11830$, $p < 0.001$) and Lake Mattamuskeet ($Fst = 0.09689$, $p < 0.001$). These results support the hypothesis and provide initial evidence that genetics influence habitat preference and migration patterns in blue crabs.

Introduction

The Atlantic blue crab (*Callinectes sapidus*) is both an ecologically and commercially fundamental species. Blue crabs start their life cycle when spawning females release zoea larvae from brooded eggs near the mouth of an estuary around the time of the nocturnal high tide (Provenzano et al. 1983; Forward Jr. 2004). Zoea molt multiple times in the coastal ocean and metamorphose to the final larval stage, the megalopa. After larval mixing within the coastal ocean, megalopae migrate from the ocean back into sounds and estuaries. Once the megalopae reach the mouth of an estuary, they change behavior as a result of environmental cues, and begin a rhythmic pattern of migration in which they use selective tidal stream transport to move up estuary. Megalopae move upward during nocturnal flood tides and rest near the bottom of the water column during other diel/tidal phases (Rowe and Epifanio 1994a). The selective tidal-stream transport is derived from the vertical shear in the estuarine tidal currents (Rowe and Epifanio 1994b). The transfer from coastal ocean water to low salinity water results in a rapid onset of metamorphosis in the megalopae (Wolcott and DeVries 1994). This rapid onset of metamorphosis is also expected to be supported by the presence of various forms of sea grass and macroalgae that exist within estuarine waters (Forward et al. 1994; Brumbaugh and McConaugha 1994; Forward Jr. et al. 1996). Seagrass and oyster beds serve as critical, structural habitats for juvenile crabs. After reaching the estuary, the juvenile crabs may migrate further inland due to inadequate habitat structures, or high predation rates (Posey et al. 2005). Therefore, blue crabs have an expansive range of nursery habitats to select from during their migration inland.

The physical, chemical, and biological characteristics typical of estuarine environments vary greatly. Due to the natural environmental variation associated with estuarine habitats, blue

crabs must select from a variety of settlement sites displaying a wide range of environmental conditions. In North Carolina, when megalopae and juvenile crabs migrate up estuary, their final habitat selection can vary from 0 to 35 PSU. Since genetic diversity within blue crab populations is known to be very high, habitat selection may help explain some of this genetic variation (Feng et al. 2017; Cushman and Darden 2017). Genetic alterations have the potential to modify an organism's physiology, thereby affecting how it may respond to its natural environment. Blue crabs are capable of responding to numerous environmental cues such as salinity, depth change, turbulence, and water flow. These cues influence their final habitat selection (Welch et al. 1997; Tankersley et al. 1998). Studies have shown that blue crabs need to expend more energy in lower verses higher salinity environments after determining that the consumption of oxygen increases in salinities below 15 to 20 PSU (King 1965; Findley et al. 1978). Therefore, an increase in standard respiration rate at low salinities may negatively affect growth rate through decreased molt increment and/or an increase in intermolt period (Jobling 1994). The adaptations required to thrive at different salinities may have resulted in genetic alterations. When blue crabs exhibit habitat selection that is geographically distinct from other crabs, their preference may be represented within their genome.

Haplotype mapping allows for the categorization of genetic variants based upon single nucleotide polymorphisms. Recently, haplotype mapping has been shown to be a useful tool in the analyzation of blue crab population structure (Rodrigues et al. 2017). Haplotype mapping is often used to focus on a specific region of an organism's genome. Mitochondrial DNA (mtDNA) markers are often used to examine population origins and interrelatedness due to their maternal inheritance, rapid rate of evolution, and low rate of recombination (Wilson et al. 1985). In 2004, Darden found that blue crab gene flow was reduced along the western Gulf of Mexico

region through the analyzation of the mitochondrially encoded cytochrome c oxidase 1 (CO1). This study emphasized the significance gene flow has in population connectivity. Furthermore, mtDNA has also been used to evaluate the variation and genetic structuring of blue crab populations. One particular study was conducted on a large scale spanning multiple locations throughout the east coast; however, no geographic patterns were found. This may be due to the fact that all of the blue crab samples were taken from high salinity waters (McMillen-Jackson and Bert 2004). No comparison of genetic differentiation has been conducted in high salinity habitats versus low salinity habitats. The purpose of this study was to examine whether there is significant genetic differentiation between blue crabs that migrate to lower salinity waters versus those that stay within higher salinity waters.

Materials and Methods

Data Collection

Sampling was conducted at low tide on Carrot Island (29-35 PSU), part of the Rachel Carson Reserve in Beaufort, North Carolina. Only late stage juvenile crabs that were preterminal molt, and mating females and males were selected for, because they were known to have grown up at high salinity. Pressure was applied near the merus-basis joint of the fourth leg on the right side of each crab to trigger limb autotomy. After sampling, crabs were returned to the environment. If a crab was already missing its fourth leg on the right side, it was assumed that it had already been sampled. Each leg was stored individually with 95% ethanol. Tissue was removed from each crab sample for DNA extraction.

DNA sequences from Lake Mattamuskeet (0 PSU) and Beaufort Inlet (29-34.5 PSU) were used for a comparative analysis. A total of 26 female crabs were analyzed from Lake

Mattamuskeet. These crabs were known to have grown up in freshwater. A total of 28 spawning females were analyzed from Beaufort Inlet. These crabs were known to be spawning since they carried a visible egg mass. Crabs collected from Beaufort Inlet are representative of the entire watershed since they were migrating through the inlet to a higher salinity to release their eggs.

DNA Extraction, PCR, and Sequencing Procedures

DNA was extracted from tissue samples using Wizard® Genomic DNA Purification Kit (Promega, USA) following the instructions set out in the technical manual. Broad spectrum primers (LC01/HCO1) were used in attempt to isolate a 710 base pair fragment of the cytochrome c oxidase 1 (CO1) gene encoded in the mitochondrial genome. Polymerase chain reactions (PCR) were performed in a thermal cycler in an 18 ul volume reaction mixture. PCR products were analyzed using agarose gel electrophoresis in Tris-acetate-EDTA buffer, stained with GelRed®, and visualized in UV light. Once it was ensured that the CO1 fragment was successfully amplified, samples were purified using ExoSAP-it (USB Corporation, Cleveland, USA). Further DNA sequencing services were performed by Eurofins Genomics LLC (Kentucky, USA). Processed sequences were edited and aligned using Codon Code Aligner 9.0.1 with Muscle algorithms.

Data Analysis

The program DnaSP 5.10.01 was used to calculate the haplotype and nucleotide diversity, variable sites, nucleotide divergence, nucleotide differences, and the net genetic distance between sample locations. Molecular variance was analyzed to examine the population and subdivision structure. Arlequin 3.5.2.2 was used to compute the Fst statistic, conduct neutrality tests, and report shared haplotypes between the sample locations. For the Fst statistic, a pairwise

Fst significance test was conducted through nonparametric permutation with 1,000 data permutations. PopArt 1.7 was used to create non-parsimonious (TCS) haplotype networks.

Results

From the Carrot Island samples, a total of 95 mtDNA CO1 sequences were generated from 113 attempted. Of which, 26 were female and 69 were male. These sequences were compared to the 26 female samples from Lake Mattamuskeet, and the 28 female samples from Beaufort Inlet. After alignment, there was a full overlap of 552 base pairs from all three locations.

Haplotype Distribution

Within all 149 samples, sequence analysis revealed 56 variable sites, 24 singleton variable sites, and 54 defined haplotypes. The total haplotype diversity was 84.4% and the total nucleotide diversity was 0.55%. Haplotype diversity ranged from 76.62% in Carrot Island females to 98.41% in Beaufort Inlet. Nucleotide diversity ranged from 0.211% in Carrot Island females to 0.933% in Beaufort Inlet. The greatest number of haplotypes was found in Beaufort Inlet, while the least number of haplotypes were found in the Carrot Island Females.

Haplotypes H3 and H8 were most characteristic of Carrot Island. Haplotype H3 had a frequency of 42.3% in the female group and 47.8% in the male group. Haplotype H8 had a frequency of 26.9% in the female group and 24.6% in the male group. Haplotypes H3 and H8 were also present within Lake Mattamuskeet samples at a frequency of 11.5% and 23.1%, respectively. There was no dominant haplotype found within Beaufort samples. Results from the statistical comparisons can be found in Tables 1 and 2. Haplotype networks were constructed to represent the genetic relationship between blue crabs from each sampling location (Figures 1-10).

Genetic Structure

An AMOVA analysis was conducted to analyze the genetic structure. Based on the results, 92.24% of the variation occurred within the individual groups, and 7.76% of the variation occurred among the three separate location groups ($F_{st} = 0.07762$, $p < 0.001$). Significant, genetic differentiation was observed among the sampled locations. The highest Fst value resulted from the comparison of Carrot Island males and Beaufort females ($F_{st} = 0.10037$, $p < 0.001$), while the lowest Fst value was found between Carrot Island males and Carrot Island females ($F_{st} = -0.01753$). The pairwise differences between each location also suggested that there was high genetic differentiation. The highest pairwise difference existed between Carrot Island males and Beaufort females ($N_m = 0.25207$, $p < 0.001$), while the lowest pairwise difference was found between Carrot Island males and Carrot Island females ($N_m = -0.01753$) (Table 3).

An overall comparison from each location shows blue crabs from Carrot Island had high, significant genetic differentiation when compared to crabs from both Beaufort Inlet ($F_{st} = 0.11830$, $p < 0.001$) as well as crabs from Lake Mattamuskeet ($F_{st} = 0.09689$, $p < 0.001$). Significant, corrected pairwise differences were found between Carrot Island and Beaufort Inlet ($N_m = 0.26018$, $p < 0.001$), as well as between Carrot Island and Lake Mattamuskeet ($N_m = 0.19482$, $p < 0.001$). Taken together, these statistics indicate that there is significant genetic differentiation in blue crab samples between Carrot Island and Lake Mattamuskeet, as well as between Carrot Island and Beaufort Inlet (Table 4).

Location	No. Individuals	No. of Haplotypes	Haplotype Diversity ± SD	Nucleotide Diversity ± SD	Average No. of Nucleotide Differences (k)
Carrot Island Females	26	10	0.76620 ± .06700	0.00211 ± .00038	1.163
Carrot Island Males	69	21	0.71700 ± .00228	0.00288 ± .00046	1.590
Carrot Island (both sexes)	95	26	0.72600 ± .03900	0.00266 ± 0.00035	1.466
Lake Mattamuskeet	26	18	0.94154 ± .00118	0.01070 ± .00277	5.905
Beaufort Inlet	28	23	0.98410 ± .00021	0.00933 ± .00198	5.148

Table 1. Haplotype statistics for each location sampled. SD = standard deviation.

Haplotype	Carrot Island Females	Carrot Island Males	Beaufort	Lake Mattamuskeet	Total
H1	0	0	2	0	2
H2	0	0	2	0	2
H3	11	33	2	3	49
H4	0	0	1	0	1
H5	0	0	1	0	1
H6	0	0	1	0	1
H7	0	1	1	0	2
H8	7	17	3	6	33
H9	0	0	1	0	1
H10	0	0	1	0	1
H11	0	0	1	0	1
H12	0	0	1	0	1
H13	0	0	1	0	1
H14	0	0	1	0	1
H15	0	0	1	0	1
H16	0	0	1	0	1
H17	1	0	1	0	2
H18	1	1	1	0	3
H19	0	1	1	1	3
H20	1	1	1	1	4
H21	0	0	1	0	1
H22	0	0	1	0	1
H23	0	1	1	0	2
H24	0	1	0	0	1

H25	0	1	0	0	1
H26	0	1	0	0	1
H27	1	0	0	0	1
H28	0	1	0	0	1
H29	1	0	0	0	1
H30	1	1	0	1	3
H31	0	1	0	0	1
H32	0	1	0	0	1
H33	0	1	0	0	1
H34	0	1	0	0	1
H35	1	0	0	0	1
H36	0	1	0	0	1
H37	0	1	0	0	1
H38	0	1	0	0	1
H39	0	1	0	0	1
H40	1	0	0	0	1
H41	0	1	0	0	1
H42	0	0	0	1	1
H43	0	0	0	1	1
H44	0	0	0	1	1
H45	0	0	0	1	1
H46	0	0	0	1	1
H47	0	0	0	2	2
H48	0	0	0	1	1
H49	0	0	0	1	1
H50	0	0	0	1	1
H51	0	0	0	1	1
H52	0	0	0	1	1
H53	0	0	0	1	1
H54	0	0	0	1	1
Total	26	69	28	26	149

Table 2. Geographic distribution of haplotypes.

Location 1	Location 2	Haplotypes Shared	Fst	Nm	Dxy	Da
Carrot Island Males	Beaufort	7	0.10037***	0.25207***	0.00656	0.00046
Carrot Island Males	Lake Mattamuskeet	5	0.07969**	0.18583**	0.00713	0.00034
Carrot Island Females	Beaufort	5	0.07531**	0.26884**	0.0062	0.00049
Lake Mattamuskeet	Carrot Island Females	4	0.05503*	0.20580*	0.00677	0.00037
Lake Mattamuskeet	Beaufort	4	-0.00512	-0.02913	0.00996	-0.00005
Carrot Island Males	Carrot Island Females	5	-0.01563	-0.01753	0.00246	-0.00003

Table 3. Fst, genetic variance between the two locations; Nm, corrected pairwise difference; Dxy, nucleotide divergence; Da, net genetic distance. P-values: * < 0.05; ** < 0.01; *** < 0.001.

Location 1	Location 2	Haplotypes Shared	Fst	Nm	Dxy	Da
Beaufort Inlet	Carrot Island	8	0.11830***	0.26018***	0.00646	0.00047
Carrot Island	Lake Mattamuskeet	5	0.09689***	0.19482***	0.00703	0.00035
Beaufort Inlet	Lake Mattamuskeet	4	-0.00512	-0.02913	0.00996	-0.00005

Table 4. Fst, genetic variance between the two locations; Nm, corrected pairwise differences; Dxy, nucleotide divergence; Da, net genetic distance. P-values: * < 0.05; ** < 0.01; *** < 0.001.

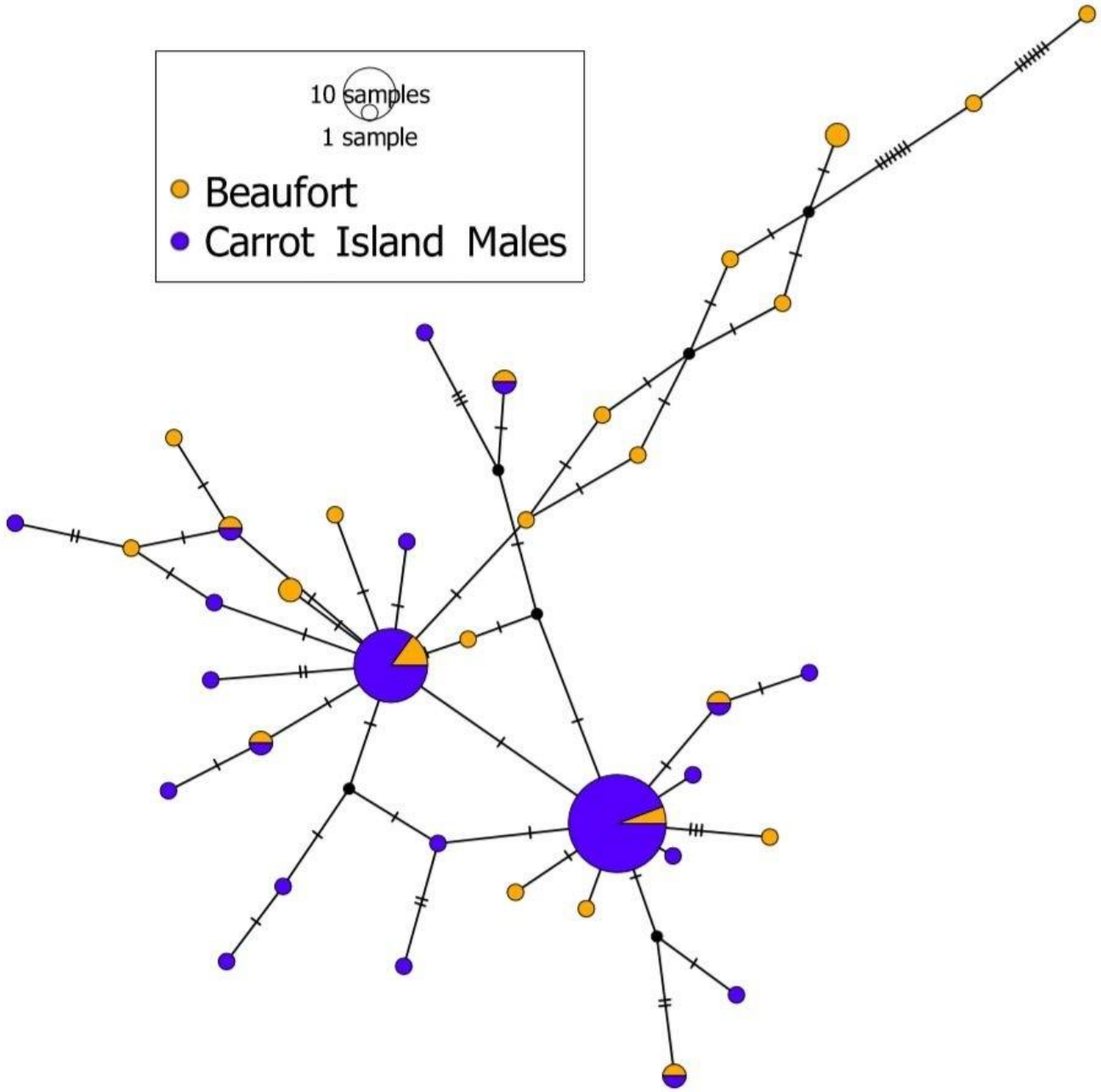

Figure 1. TCS haplotype network demonstrating the relationship between Beaufort females and Carrot Island males.

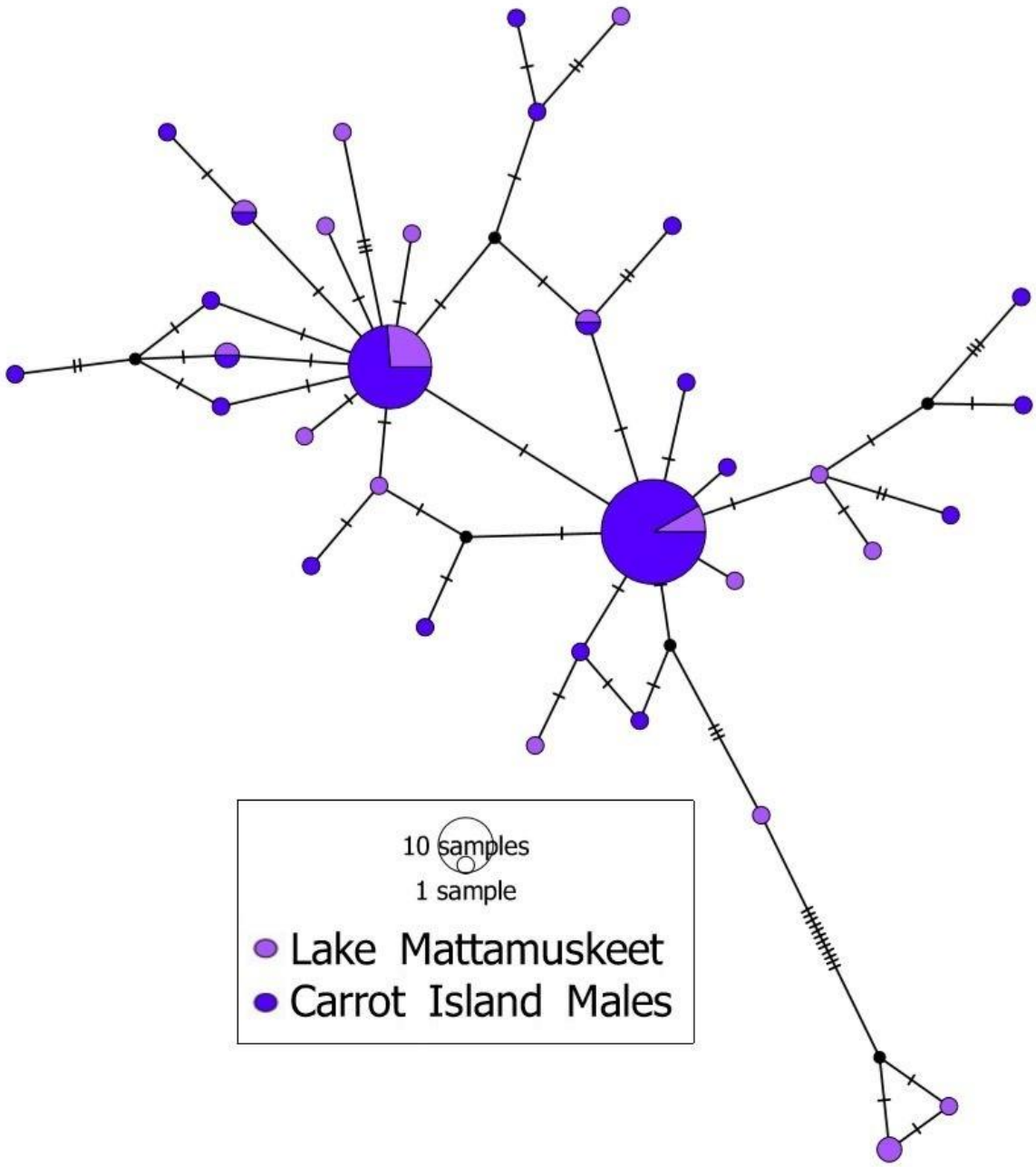

Figure 2. TCS haplotype network demonstrating the relationship between Lake Mattamuskeet females and Carrot Island males.

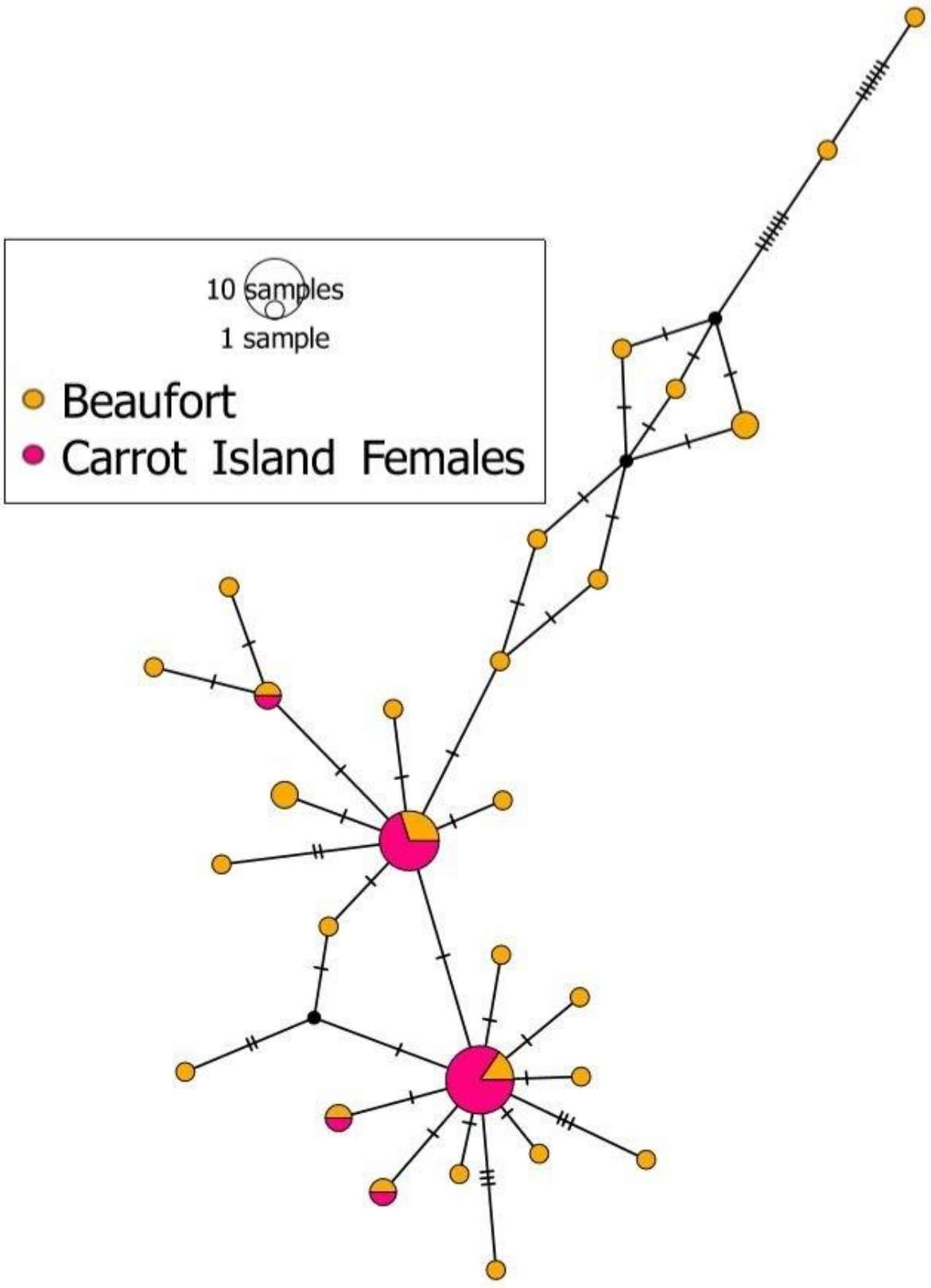

Figure 3. TCS haplotype network demonstrating the relationship between Beaufort females and Carrot Island females.

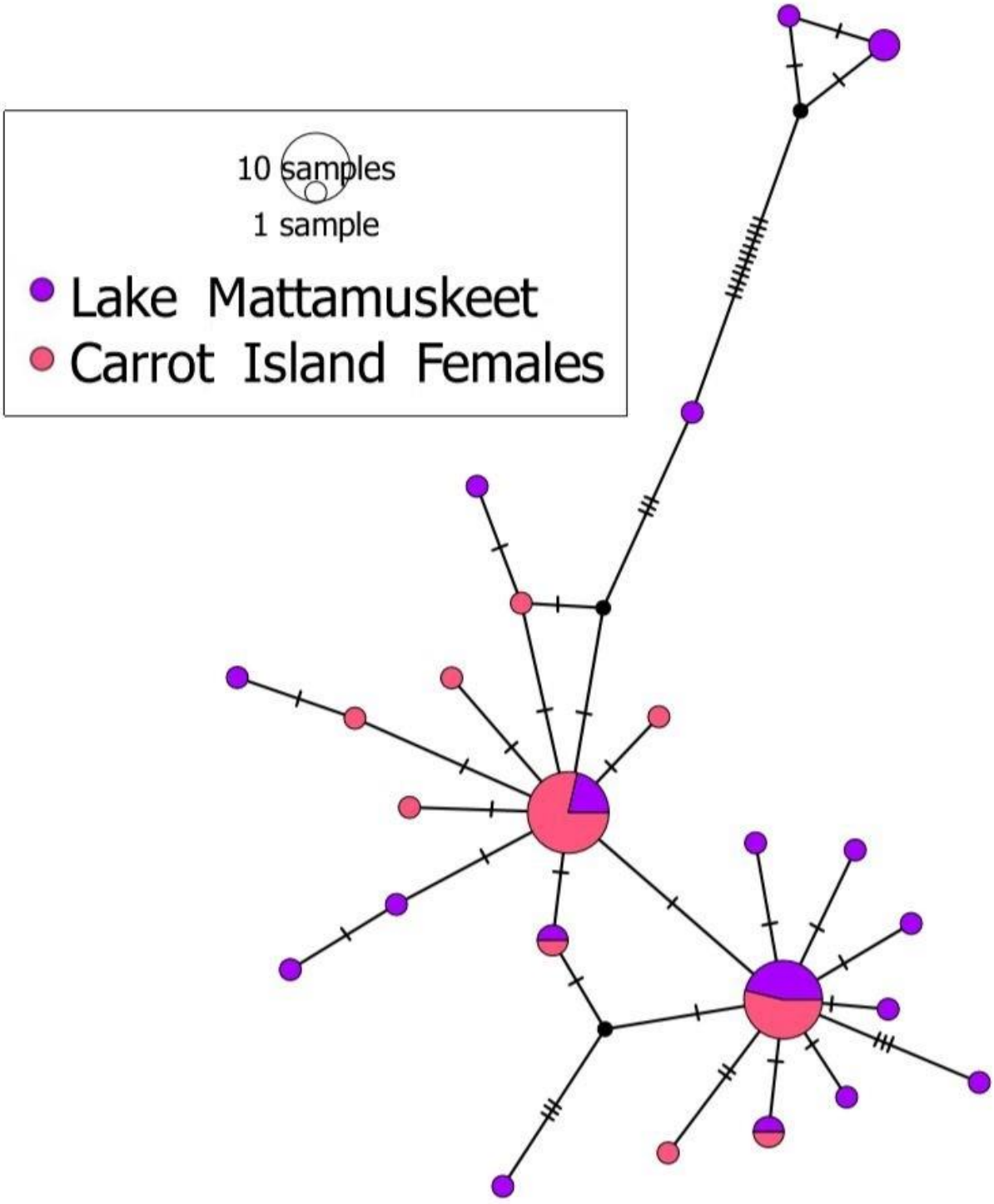

Figure 4. TCS haplotype network demonstrating the relationship between Lake Mattamuskeet females and Carrot Island females.

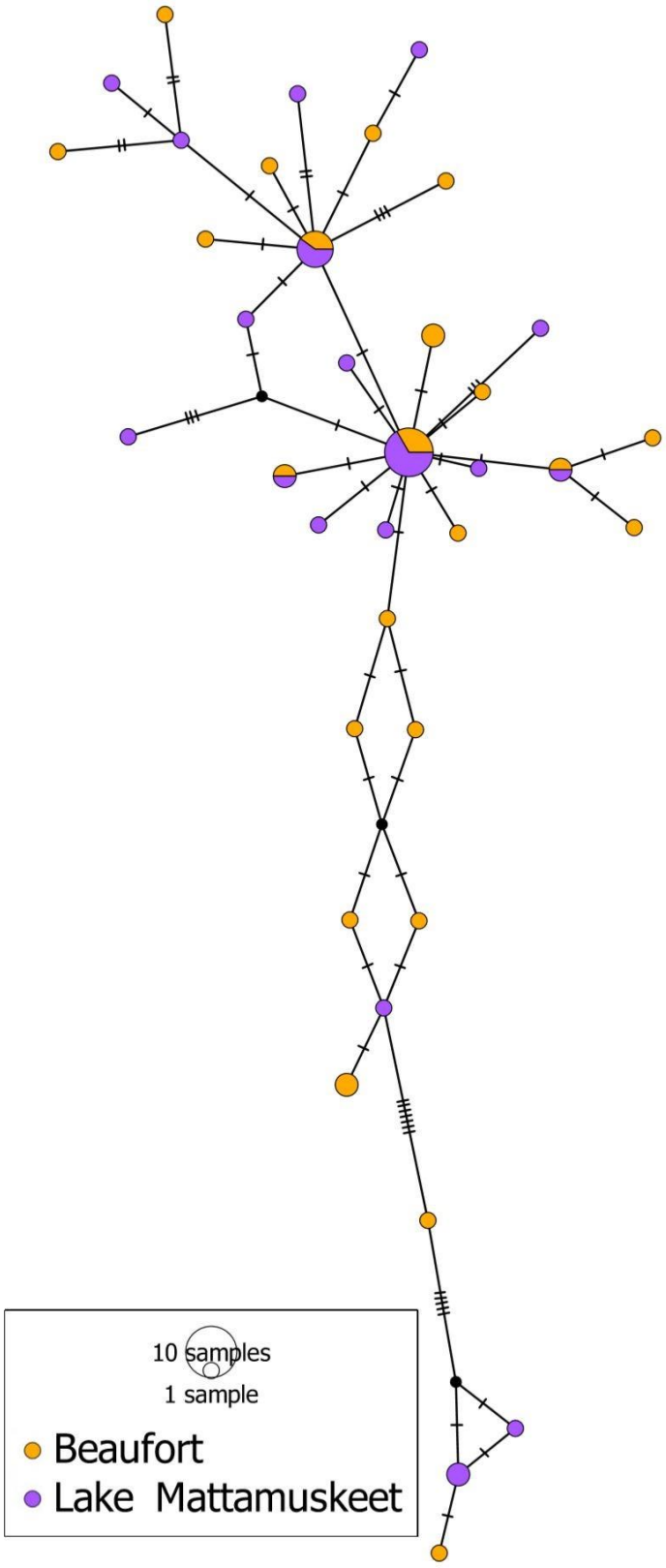

Figure 5. TCS haplotype network demonstrating the relationship between Beaufort females and Lake Mattamuskeet females.

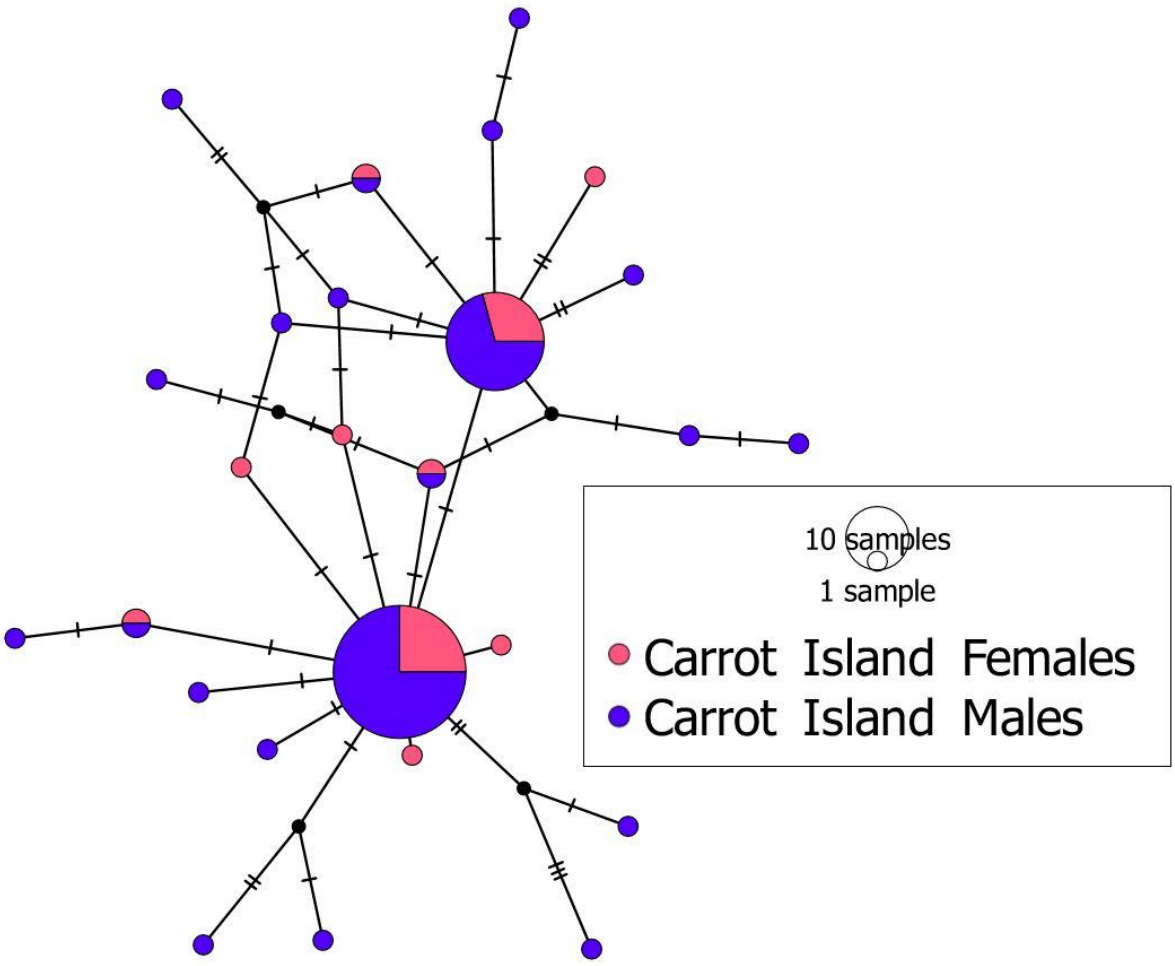

Figure 6. TCS haplotype network demonstrating the relationship between Carrot Island females and Carrot Island males.

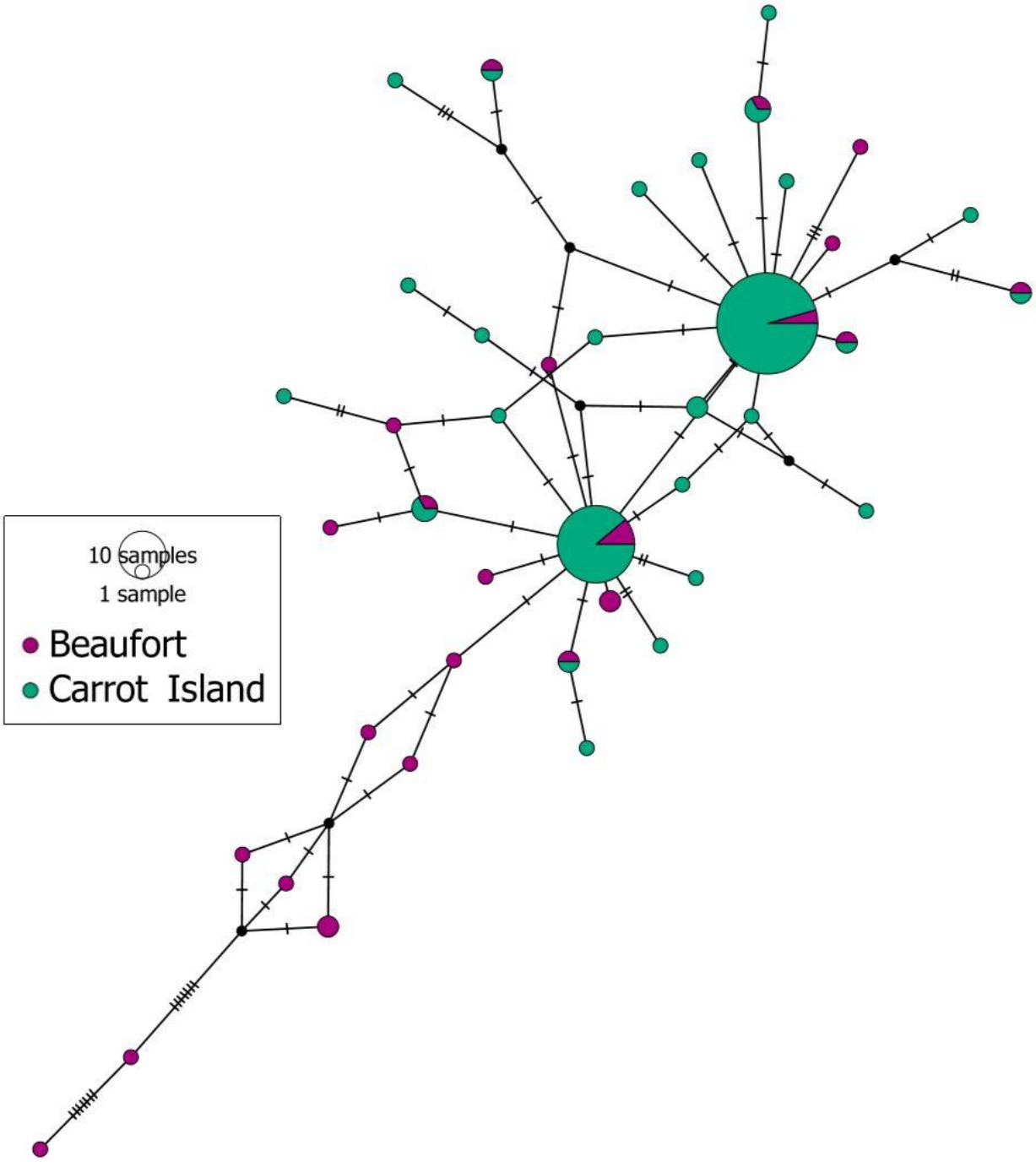

Figure 7. TCS haplotype network demonstrating the relationship between Carrot Island and Beaufort females.

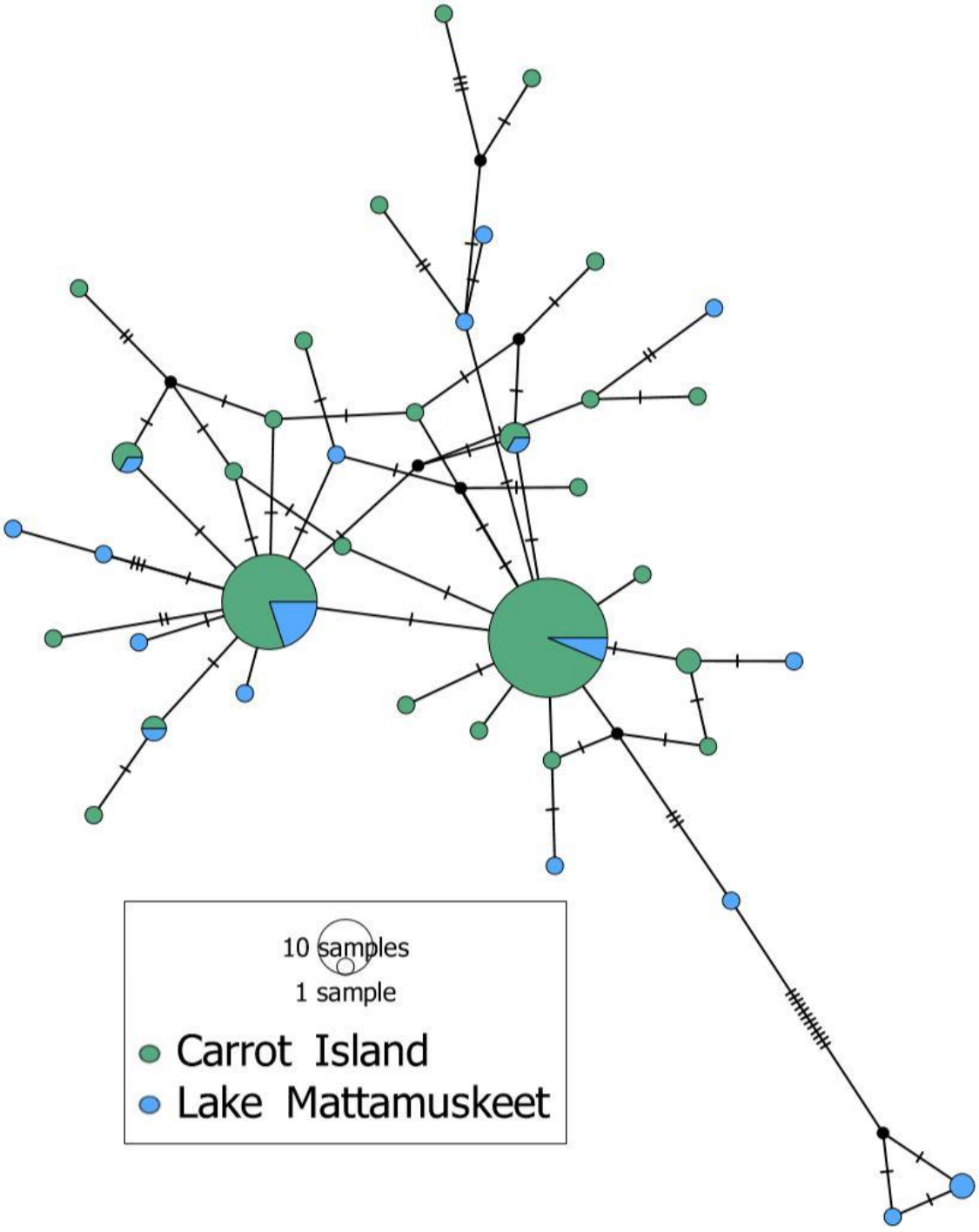

Figure 8. TCS haplotype network demonstrating the relationship between Carrot Island and Lake Mattamuskeet females.

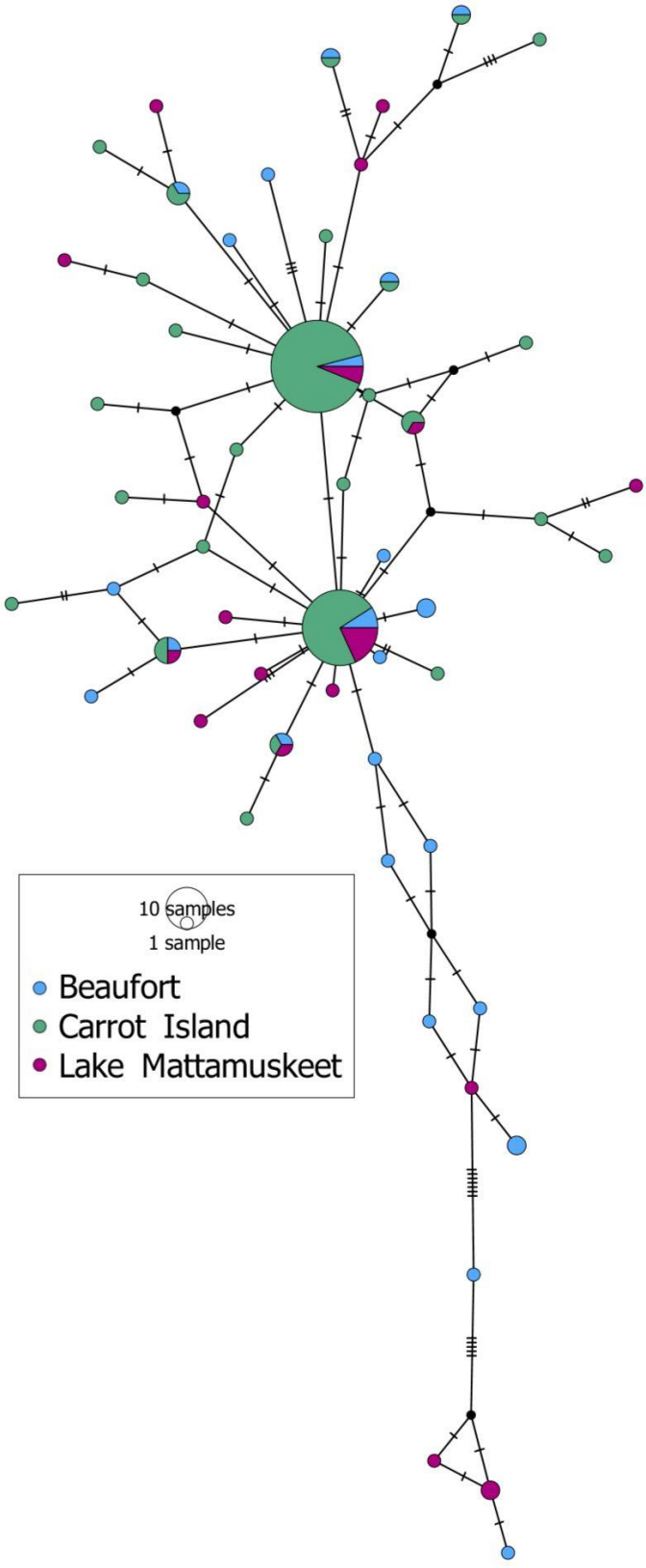

Figure 9. TCS haplotype network demonstrating the relationship between Carrot Island, Lake Mattamuskeet, and Beaufort Inlet.

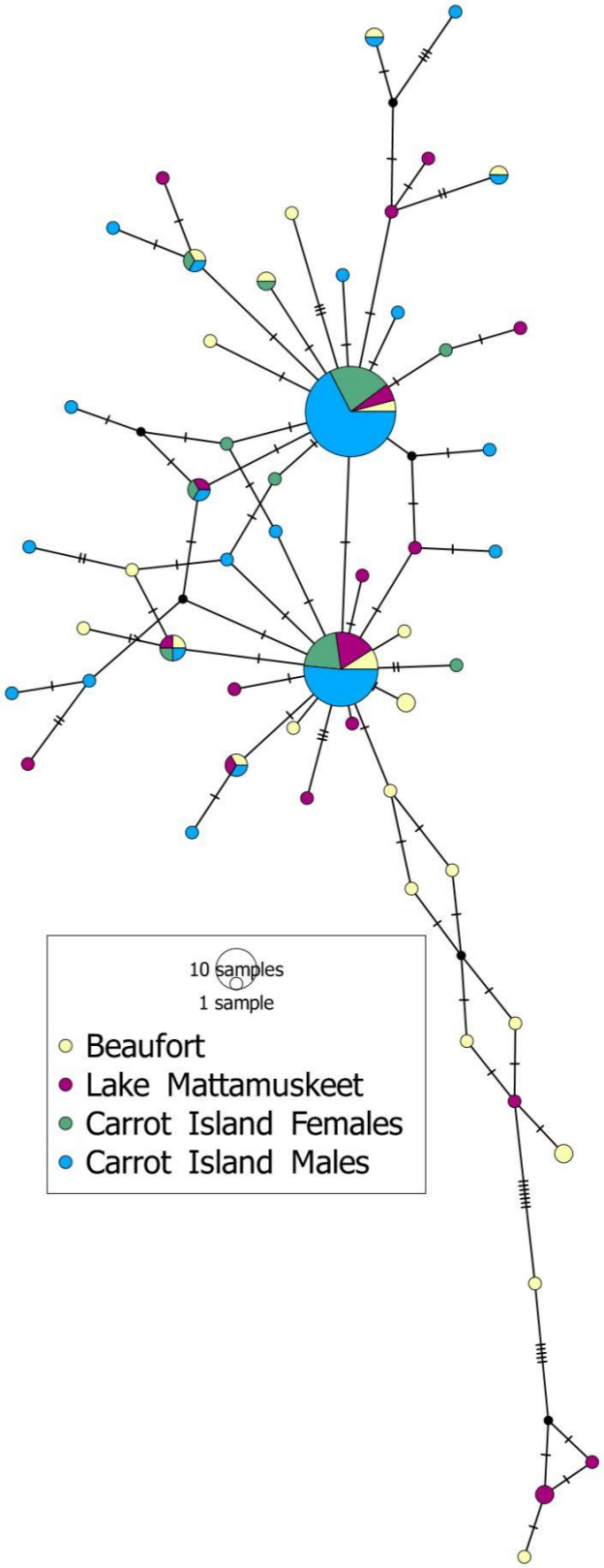

Figure 10. TCS haplotype network demonstrating the relationship between Carrot Island, Lake Mattamuskeet, and Beaufort Inlet.

Discussion and Conclusion

This study tested the hypothesis that migration and habitat preference in blue crabs are influenced by genetics. Blue crabs exhibiting different habitat preference and migration patterns were examined for genetic differentiation within the mitochondrial cytochrome c oxidase 1 (CO1) gene region. This marker has been used extensively within population and phylogeographic studies due to its maternal inheritance, rapid rate of evolution, and low rate of recombination (Wilson et al. 1985).

The spawning females from Beaufort Inlet had the highest haplotype and nucleotide diversity. Since the crabs collected from this location were spawning crabs, it was not certain which settlement habitat they had previously selected before heading to the open ocean to spawn. These crabs had migrated from various settlement locations throughout the watershed, including the Neuse and White Oak River Basins, before stopping at Beaufort Inlet on their way out to the coastal ocean to spawn. The high haplotype and nucleotide diversity found within the Beaufort Inlet samples is most likely derived from the large geographic expanse the sampling had covered. On the other hand, the haplotype and nucleotide diversity from the Carrot Island samples were much lower since these crabs were known to be residents.

Genetic diversity may be influenced by population size, immigration rates, as well as mutation rates. On the other hand, the rate of gene flow among populations and the history of ancestral populations can directly influence genetic diversity (Gompert et al. 2010; Yednock and Neigel 2014). AMOVA results suggested a pattern of genetic differentiation by geographic location, and significant geographic genetic structuring among the crabs sharing the same habitat location. In addition, significant pairwise values between Carrot Island and Beaufort Inlet, as

well as between Carrot Island and Lake Mattamuskeet samples indicated the lack of strong gene flow among the crabs from those locations over time. This indicated that crabs migrating to these separate locations are acting independently of each other.

To analyze genetic differentiation and structure between blue crabs collected from different locations, the Fst statistic was utilized (Weir and Cockerham 1984). High genetic differentiation was found between Carrot Island and Beaufort Inlet, as well as between Carrot Island and Lake Mattamuskeet. Furthermore, there was no significant genetic differentiation between Lake Mattamuskeet and Beaufort Inlet. In addition, the sex of the crabs did not provide any further source of genetic variation. The samples from Carrot Island were all centered around two major haplotypes, regardless of sex. There was a correlation between genetic differentiation and geographic distance suggesting significant geographical structuring. This suggests that specific genotypes select high versus low salinity habitats. The lack of differentiation between males and females further supports this concept.

This structuring strongly reflects the migration to contrasting environmental conditions since Carrot Island (29-35 PSU) and Beaufort Inlet (29-34.5) had much higher salinities than Lake Mattamuskeet (0 PSU) (Welch et al. 1997; Tankersley et al. 1998). A lack of gene flow and high genetic differentiation may be a result of adaption to different salinity environments. Different salinities pose different kinds of challenges for marine organisms. In fact, previous studies have indicated that blue crabs are required to expend more energy at lower salinities versus higher salinity environments. Expending more energy could negatively affect growth rate and result in a decrease in molt increment and/or an increase in intermolt period (King 1965; Findley et al. 1978). On the other hand, it may also be beneficial to stay within higher salinity waters due to decreased exposure to predation and less energy required to reach the open ocean

to spawn. Some blue crabs may be more adapted to a higher salinity as opposed to other crabs who may be better adapted to a lower salinity. The genetic differentiation found between geographically distinct blue crab samples supports the hypothesis there is a genetic component within blue crabs that influences migration and habitat selection. In the future, the entire genome of the blue crab should be analyzed to examine whether or not there are any single nucleotide polymorphisms occurring at high rates around enzymes or proteins that may be responsible for detecting or living at high versus low salinity habitats.

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