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GENETIC DIVERSITY OF THE BLUE LAND CRAB, *CARDISOMA GUANHUMI* LATREILLE (CRUSTACEA: DECAPODA), ON ANDROS ISLAND: IMPLICATIONS FOR SUSTAINABLE MANAGEMENT

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ABSTRACT

Androsians provide for their families by harvesting marine and terrestrial organisms on and around the island. These species, including *Cardisoma guanhumi*, the blue land crab, provide not only food, but also a substantial supplement to the annual economy of Andros Island (Rolle, 2007; ANCAT, 2010). As habitat fragmentation and harvesting pressure increase, many species have declined or been extirpated. It is becoming increasingly important to understand the genetic diversity of these cottage industry species. Andros Island is known to have the largest metapopulation of blue land crabs in The Bahamas (ANCAT, 2010), providing generations of Androsians with income derived from the bountiful harvest during the spawning migrations. Locals have reported having to travel further from their settlements to collect adequate numbers of crabs to send to market, due to increased harvesting pressure. This project analyzed genetic variation at the nuclear arginine kinase gene and microsatellite markers to identify the discrete populations of *C. guanhumi* and quantify the extent of its genetic diversity on Andros Island. Preliminary analysis of arginine kinase sequences revealed limited genetic diversity between populations in the less-settled portions of Andros. Mapping the extent of the populations and amount of genetic diversity in each will allow scientific data to be used to improve management strategies to conserve both *C. guanhumi* and its economic value to Androsians.

INTRODUCTION

Human populations have traditionally subsisted on organisms harvested within the local environment (Boone, 2002). Edible species of great supply are often the target of regular, small-scale artisanal fishery harvesting (Trosper, 2002). Eventual commercialization of artisanal fisheries has led to collapses in fishery stocks, as occurred with the anchovy (Tarazona, 1983) and is occurring with the Nassau grouper (Sadovy and Eklund 1999; ANCAT, 2011; Cornish and Eklund, 2003). Seemingly limitless resources provide a false sense of resource and economic security leading to a tragedy (*sensu* Hardin, 1968) when human use is not balanced with population sustainability of commodity species.

The islands of The Bahamas have been inhabited by humans since around 600 A.D. (Craton and Saunders, 1992). Fishery resources have been, and continue to be, an important source of food and revenue for the island nation. The spiny lobster and Nassau grouper fisheries that grew out of early artisanal small-scale harvesting now make up more than 75% of the commercial fishery resources with an estimated annual economic contribution of $105M USD (Rankine et al., 2004). Most of the commercial fisheries in The Bahamas are based out of the main population centers on Grand Bahama and New Providence. Historically, the people living on the sparsely populated islands continued to harvest in much smaller quantities, putting less pressure on fishery stocks. Increases in tourism on these less well-known islands have created a market for increased harvesting of the artisanal fishery species (Danylchuk, 2005). *Cardisoma guanhumi* Latrielle, the blue land crab,

is emerging as a profitable fishery resource as the smaller island communities struggle to remain economically viable. The large numbers of blue land crabs on Andros Island are becoming well-known and increasing numbers of land crabs are being collected for use in tourist-based heritage festivals across The Bahamas (Miller, 2005; DCL, 2005).

Andros Island is believed to have the largest population of *Cardisoma guanhumi* in The Bahamas and Androsians are known throughout the islands for their crab collecting expertise (ANCAT, 2010; Rolle, 2007). Individuals have been known to collect over 100 crabs in an evening; with groups of crabbers quickly collecting dozens in a short sweep of the migratory path (Hostetler et al., 1991; Wedes, 2004; D. Scott, pers. comm.). The blue land crab provides a good source of income, fetching up to $5 per crab at market, depending on the season and the fat content of the crab. This high price allows seasoned collectors to add an estimated $20,000 to their annual income in just 2-3 months (BIS, 2002). The artisanal crabbing industry has allowed homes to be built and provided college tuition for many Androsian students (ANCAT, 2010).

The maintenance of genetic diversity is emerging as a new paradigm for managing commodity organisms to promote economic prosperity in developing nations (e.g. Dawson et al., 2009). Conservation genetics uses genetic information to assess populations under threat of habitat loss, exploitation and/or environmental change (Frankham, 2002). The cornerstone of this type of management rests on a good understanding of the underlying genetic structure of the species in question. DeOliveria-Neto et al. (2008) found a high degree of genetic diversity throughout the blue land crab populations along the coast of Brazil.

Years of overharvesting (Amaral and Jablonski, 2005) and mangrove removal (Magris and Barreto, 2010) have put additional pressure on those populations. The Brazilian Ministry of Environment includes *C. guanhumi* as an overharvested species in spite of regulations limiting harvest to only males with carapace length greater than 4.5 cm (Alves et al., 2005).

Formal studies are not readily available regarding *C. guanhumi* on Andros Island and even the number of populations on the Island is unknown. This study used nuclear and microsatellite markers to determine the population structure of *C. guanhumi* on Andros Island, The Bahamas, with the goal of long-term sustainability and science-based conservation to preserve both the crab and its economic value to Androsians.

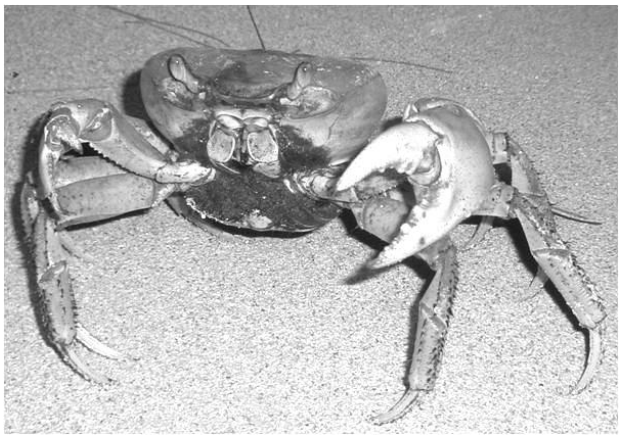

Figure 1. Cardisoma guanhumi, *ovigerous female*

MATERIALS AND METHODS

Study Organism

Cardisoma guanhumi Latreille (Crustacea: Decapoda; Figure 1) is a land crab found throughout the Atlantic coastal sub-tropic and tropic estuarine areas from Florida to Brazil (Burggren and McMahon, 1988). Juvenile crabs develop in mangrove estuaries and, with the onset of sexual maturity around four years of age, adults create burrows in the hardwood coppices (Hostetler et al., 1991). Spawning migrations typically start at the beginning of the rainy season (Witherington, 2009) which varies throughout the range based on latitude (Wedes, 2004). Females will carry a clutch of 300,000 - 700,000 fertilized eggs for about 2 weeks while development occurs (Hostetler et al., 1991) until the hatching larvae can be released into the sea. Major and minor migratory waves occur during the full and new moons (Gifford, 1962; Hill, 2001), during which the females are known to travel up to 8 Km to shed the hatching larvae into the sea (Burggren

and McMahon, 1988). Adult females will return to their burrows and await the next annual migratory spawn (Burggren and McMahon, 1988). Females can reproduce 11 times, on average, during a life-span of approximately 15 years (Witherington, 2009).

Sample collection and DNA Extraction

Geographic clines and indigenous knowledge were utilized to select thirteen potential sites on Andros Island, The Bahamas (Figure 2). Live crabs (n = 3-20) were collected by hand and a single pereopod was removed and preserved in 95% ethanol. Crabs were subsequently released as close to the point of collection as possible. Muscle was removed from each of the preserved pereopods and genomic DNA was extracted (DNeasy Blood and Tissue Kit, Qiagen, Valencia, CA). Samples were stored at -80^{o} C and vouchered with the State University of New York, College at Oneonta's Biological Field Station.

Arginine Kinase gene amplification, sequencing, and analysis

A 321-base region of arginine kinase was amplified using the AKF3 and AKR3 primers of Mahon and Neigel (2008). Amplification consisted of initial denaturation at 95^{o} C for 2 min, followed by 35 cycles of denaturation at 95^{o} C for 45 sec, annealing at 75^{o}, 70^{o}, or 67^{o} C (depending on individual) for 1 min, extension of 72^{o} C for 1 min, and a final extension at 72^{o} C for 5 min. Each 25 µL PCR was prepared according to the instructions of the manufacturer (5 Prime, Gaithersburg, MD); however, only 1 unit of HotMaster™ *Taq* DNA Polymerase was used. After purification (QIAquick PCR Purification Kit, Qiagen, Valencia, CA), successful amplicons were sequenced on a CEQ8000 (Beckman Coulter, Fullerton, CA).

Sequences were aligned using ClustalW Multiple Sequence Alignment and the open reading frame determined utilizing SIXFRAME via Bioworkbench (http://workbench.sdsc.edu/). Tests for Hardy-Weinberg Equilibrium (HWE) were performed by hand. Traditional analyses (AMOVA, F_{ST}, Pairwise differentiation) were calculated with Arlequin 3.5 (Ver 3.5, Excoffier et al., 2005).

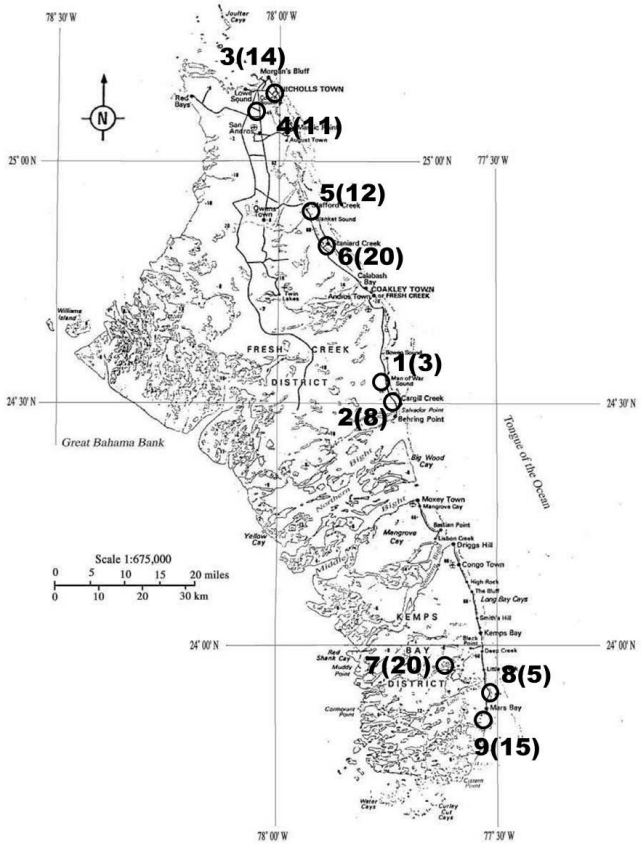

Figure 2. Andros Island Collection Site Map (modified from Nickrent et. al., 1988). Number of individuals sampled for each site indicated in parentheses after site identification number.

Microsatellite PCR and Fragment Analysis

Microsatellite loci TJH-06 of Zhang et al. (2009) and pm-109 of Fratini et al. (2006) were genotyped to assess genetic differentiation. Each 25 µL PCR contained the following concentrations: 1 unit of HotMaster™ *Taq* DNA Polymerase, 1X HotMaster™ *Taq* Buffer with 25 mM Mg^{2+}, 0.5 µM dNTPs, 0.5µM unlabeled reverse primer, 0.5 µM dye-labeled forward primer, and 30 ng genomic DNA. The thermal cycler profile consisted of an initial denaturation of 95^{o} C for 3

	1	2	3	4	5	6	7	8	9
1	*	-0.187	-0.070	-0.070	0.158	0.011	-0.042	-0.030	-0.050
2	0.991	*	-0.174	-0.174	0.186	-0.113	-0.162	-0.162	-0.163
3	0.991	0.991	*	-0.067	0.179	0.037	-0.025	-0.004	-0.039
4	0.991	0.991	0.991	*	0.179	0.037	-0.025	-0.004	-0.039
5	0.189	0.387	0.090	0.144	*	0.015	0.079	0.000	0.086
6	0.405	0.991	0.216	0.189	0.559	*	-0.021	-0.093	-0.002
7	0.658	0.991	0.748	0.703	0.297	0.667	*	-0.059	-0.030
8	0.622	0.991	0.640	0.685	0.991	0.991	0.667	*	-0.041
9	0.991	0.991	0.784	0.793	0.144	0.505	0.991	0.694	*

Table 1. Pairwise F_{ST} with corresponding p-value. F_{ST} values above the diagonal, associated p-values below the diagonal. Site numbers as per Figure 2.

min; followed by 35 cycles of denaturation of 95° C for 45 sec, annealing at 57° C for 1 min, and extension of 72° C for 1 min; and a final extension at 75° C for 15 min. Fragments were analyzed on a CEQ8000 (Beckman Coulter, Fullerton, CA) utilizing 0.5 µL of 600 size-standard, as per the instructions of the manufacturer.

RESULTS

Initial sequencing allowed analysis of a 321-base fragment of arginine kinase for 72 individuals across Andros. Allelic richness for the island was three, with two variable sites. Two rare genotypes were found in the southern portions of North and South Andros Island (Sites 1 and 9, respectively in Figure 2). Fragment analysis of 70 individuals for TJH-06 and 8 individuals for pm-109 were monomorphic for a single allele at each locus across the entire island. All populations were in HWE. The Analysis of Molecular Variance (AMOVA) indicated that almost all variation occurred within populations. Pairwise F_{ST} values ranged from -0.187 to 0.186 but were not significantly different from zero (P-value = 0.09 - 0.99, Table 1).

DISCUSSION

Preliminary analysis suggested *C. guanhumi* on Andros Island comprise one population with limited genetic diversity. Both AMOVA and tests of pairwise differentiation support the lack of genetic differentiation. That the whole island is in HWE for both expressed and unexpressed markers further suggests a single undifferentiated population. Preliminary analyses of two microsatellite loci also supported lack of diversity, as both loci were monomorphic across Andros Island.

Anecdotal evidence suggests that the blue land crab population has been steadily declining over the last several decades. Daytime sightings along the spawning path have become rare in the last decade (G and A. Colebrooke, pers. comm.).

The cyclical nature of blue land crab migrations has historically been a time of intense collecting of *C. guanhumi*. The blue land crabs are collected on their way to the sea and upon return to their burrows, with no regard to sex or reproductive status. Mangrove estuary habitat has been eliminated to make way for exclusive resorts and luxury home sites (pers. obs.). In 1998, the "All-Andros Crab Fest" was established to celebrate the crabbing legacy of Andros Island and the "Opening of the Andros Bank" (i.e. withdrawing the crabs from Andros as one would money from a savings account) with 4 days of festivities high-lighted by a plethora of crab dishes and an influx of tourism from throughout The Bahamas (BIS, 2002). People travel from other islands to collect crabs (pers. comm. G. Douglas) and anecdotal evidence indicates there are no crabs to cook by the last day of the festival, suggesting that the addi-

tive effect of changes in both habitat and collection has been disastrous.

Land Crab National Park was established in 2002 on Andros as a means to preserve *C. guanhumi* habitat and migratory pathways (BNT 2009), but its borders are not enforced, since it was never the intention of the Bahamas National Trust (BNT) to limit land crab harvest in Land Crab National Park (S. Buckner, pers. comm.).

There is currently no season, limit, or size and/or sex restrictions on harvesting *C. guanhumi* on Andros despite similar measures in other countries. Florida Fish and Wildlife currently regulates not only the allowable harvest but also an established season for allowable collecting (Hostetler et al., 1991). Puerto Rico prohibits collection during the local spawning season (Hostetler et al., 1991), though enforcement is ineffective (Govender, 2008) and harvesting more intense and destructive outside of the management areas (Rodríguez-Fourquet and Sabat, 2009). The Brazilian Ministry of the Environment restricted collection of *C. guanhumi* with carapace widths less than 8 cm and established an off season during the annual spawn (IBAMA, 2011, Ministério do Meio Ambiente, 2011), although artisnal fishers continue to harvest without regard to the regulations put into place by the Ministry of the Environment (Alves et al., 2005).

Management strategies on Andros include educational resources distributed throughout the local communities (Witherington, 2009) and through the BNT. Formal regulations have not been established through the BNT, though encouragement exists for collection of females only after eggs have been released into the ocean (Witherington, 2009). Androsians indicate that females inadvertently caught are immediately released due to lack of quality for consumption (A. Colebrooke, P. Riley, E. Wallace, pers. comm.). Protection of habitat through the Land Crab National Park continues; although with no prohibition of harvest in the management areas, only habitat is being protected (BNT, 2009).

Local stakeholders will need to be consulted in future management plans for the land crab throughout the range and more importantly, Andros. The "All-Andros Crabfest" provides a prime opportunity to serve as a checkpoint during the harvest season to survey those involved in the industry to collect data on harvest numbers and the dispersion of collection sites to manage the seasonality of the harvests. Sustainable harvest education can also become a highlight of the festival with the optimal placement of the land crab education/conservation booths. During the "All-Andros Crabfest 2011", all educational displays were located in the far reaches of Queen's Park (pers. obs.) beyond where most visitors would go. While it is important to highlight the habitat being protected, education of the local and tourist population is necessary for successful management strategies.

Our study indicated a lack of genetic diversity in the *C. guanhumi* population on Andros. Further work with polymorphic markers and on different islands in The Bahamas will assist in the establishment of Evolutionary Significant Units (Crandall et al., 2000) to aid future conservation of the species through the BNT. Any management strategy developed for *C. guanhumi* must include sustainable harvesting with input from the local collectors. The combination of habitat preservation, resource monitoring and conservation genetics utilized to develop regulations may aid long-term sustainability of the blue land crab on Andros.

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