INTRODUCTION

Queen conch, *Strombus gigas*, is a large tropical marine gastropod (snail) common to shallow-water seagrass habitats throughout the Caribbean. Six species of *Strombus* occur in the Caribbean region and queen conch is the largest of the recognized species (McCarthy, 2007). Their geographic range is from Bermuda to South Florida in the United States and throughout the Bahamas and the Caribbean (Stoner, 2003). Queen conch populations have supported a substantial commercial fishery through the Caribbean region; however, over the past 25 years, populations have been severely depleted due to over harvesting because of human consumption demands (Stoner, 1997). Despite the institution of strict harvesting regulations by many Caribbean nations, *Strombus gigas* was listed in Appendix II of the Convention on International Trade in Endangered Species in 1992. This classification indicates the species is not currently threatened with extinction, but the potential for extinction is high unless their trade is subject to strict regulations (Stoner, 2003). In 1985, a harvest moratorium was instituted in the United States. Despite this moratorium, queen conch populations have not recovered (Delgado, et al. 2002).

The major effort used to replenish impoverished Caribbean conch populations has been the release of hatchery-reared juvenile conch in the wild (Stoner, 2003). The goal of the hatchery is to re-establish populations and to supplement or enhance natural populations through the release and survival of hatchery-reared juveniles. In order to achieve these goals, a successful breeding program must produce large quantities of “high-quality” (genetically diverse) individuals and released juveniles must survive, grow, and reproduce.

Similar to many marine invertebrates, the queen conch has a life cycle that includes a planktonic larval stage making it ideal for hatchery breeding and release. Reproduction involves internal fertilization during the warmest months of the year (Stoner et al. 1992). Eggs are deposited in benthic masses and hatch three to five days after deposition. The larvae enter the water column where they feed on phytoplankton for the next two-five weeks (Davis et al. 1993). Larvae metamorphose and settle to the bottom. Most juvenile conch occupy shallow clear water where they consume plankton. Sexual maturity occurs at 3-4 years of age when juveniles move to deeper water and continue herbivory. Shell length can reach approximately 30-40 cm. (Stoner and Ray, 1996). The biggest obstacle to maintaining conch populations is that *S. gigas* reaches marketable size prior to the age of first reproduction.

For success in rehabilitating local conch populations, a management program is essential. Restocking programs can only be successful if seeded animals survive to maturity and reproduce sufficiently to insure continued population growth. Two methods have been suggested to restore local conch populations. One method is the release of hatchery-reared juvenile conch into the wild. The culturing of queen conch in the laboratory may provide a means to produce large numbers of conch. Because of high monetary costs, the most common strategy for conch hatchery operations is to produce the largest product in the quickest time possible. Unfortunately, this strategy has led to behavioral and morphological problems that have been associated with hatchery-reared queen conch (Stoner and Glazer, 1998). Iversen et al. (1986) observed that hatchery-reared conch had lower sand burial rates and hence higher predation rates than their wild counterparts. Additionally, Stoner and Davis (1994) reported that hatchery-reared queen conch had decreased shell weight and they correlated this difference to increased mortality.
The second method incorporates a switch from hatchery rearing to habitat-based nurseries (Stoner 2003). This strategy relies on the idea that abundance and productivity are linked directly to the amount of suitable habitat available to establish nurseries in the proper environments. However, because of our limited knowledge of habitat quality and function for the queen conch, few nurseries have been established. Obvious places warranting special protection are current spawning sites. Successful nurseries depend on selecting locations, which have historical importance for queen conch breeding. One such location is Graham’s Harbor, which is located on the north end of San Salvador, Bahamas directly across from the Gerace Research Centre. We propose, in collaboration with the Director of the Gerace Research Centre and the College of the Bahamas, to establish a nursery in Graham’s Harbor.

The ultimate aim of the nursery-breeding program is to maximize the genetic gain through accurate selection of breeding candidates. In order to accomplish this goal, selected breeding candidates and their offspring must be tracked genetically. This monitoring is important in order to maximize genetic diversity and to minimize the deleterious effects of inbreeding which can result in the reduction of fitness traits such as reproductive capacity and physiological efficiency. In order to manage nurseries with the goal of maintaining genetic diversity, it is necessary to have access to reliable parentage information for evaluating the contribution of different breeder stocks to the population. Furthermore, it is essential to assess the genetic diversity and to maintain information on the pedigree of the selected breeding founders. Once reliable pedigree information is obtained, matings can be arranged to minimize inbreeding.

Since queen conch have a planktonic larval stage, it is difficult to use external tags to track offspring. Fortunately, the utilization of highly polymorphic genetic markers such as microsatellites provides an excellent tool for identifying parentage relationships among individuals and obtaining pedigree information (Dong et al., 2006). Microsatellites are simple DNA sequence repeat loci consisting of two to six nucleotides repeated in tandem and distributed throughout an organism’s genome (Chistiakov et al., 2005). Microsatellites are ideal to track parentage and estimate genetic diversity because they are polymorphic, codominant in their manner of inheritance and relatively easy to characterize by using polymerase chain reaction (Estoup et al., 1998). In the last decade, parentage determination using microsatellites has been successful in numerous aquatic species. Using multiple microsatellite loci in order to eliminate incorrect parentage assignments based on random probability, Dong et al. (2006), used five microsatellite loci and identified 97% of Chinese shrimp to their correct parents; van den Berg and Roodt-Wilding (2010) surveyed nine loci in abalone and successfully assigned parentage to 91% of the offspring; and Sourinejad et al. (2011) using nine loci, had an overall success rate of 98% of assigning progeny from brown trout to their correct parents.

As previously stated, this increased interest in using microsatellite loci as the genetic markers of choice to determine parentage is because of their high degree of polymorphism and relative ease in scoring. However, one of the major drawbacks is the need to isolate primer combinations for these markers de novo from the species of interest (Zane et al. 2002). Primers are required to amplify and characterize the microsatellite loci. Fortunately, Zomora-Bustillos et al. (2007) isolated and designed primers for eight microsatellite loci specific to pink conch. Zomora-Bustillos et al. (2007) showed all eight microsatellite loci exhibited a high level of polymorphism making these loci ideal to determine parentage and genetic diversity of S. gigas breeding founders and offspring once the parameters are developed for our particular system. We believe
that the availability of microsatellite DNA markers for genotyping makes *S. gigas* highly amenable to selective breeding research.

**PREVIOUS RESEARCH**

Prior to establishing a nursery for selective breeding in Graham’s Harbor in San Salvador, Bahamas, it is essential to develop the genetic protocols required to use microsatellites to genotype individuals. The initial step in this process is the development of a field DNA isolation protocol. We began this work in February of 2011. Samples of *S. gigas* tissue were collected from individuals residing offshore in San Salvador, Bahamas. We developed a successful DNA isolation protocol by modifying an existing protocol we use to isolate coral DNA (Squiers et al., 2011). The next step in the progression of obtaining a successful genotyping protocol was to begin to analyze the eight microsatellites developed by Zamora-Bustillos et al. (2007) to determine if the primer combinations they developed for pink conch would amplify similar microsatellites loci in queen conch. We have developed the polymerase chain parameters and successfully amplified one of the eight microsatellite loci described by Zamora-Bustillos et al. (2007). In order to confirm that we had amplified the correct locus, we cloned the amplified locus and carried out a DNA sequence analysis. A BLAST query was initiated in the National Center of Biological Information (NCBI) sequence database and we found an 88% homology match to the sequence uploaded by Zomara-Bustillos et al. (2007) for the same primer combination. The probability that our sequence matched the sequence in the database by chance was $7.0 \times 10^{-32}$. The combination of percent similarity and the low probability of a match by chance, makes us confident that we have successfully amplified the first of the eight microsatellite loci described by Zamora-Bustillos et al. (2007).

**METHODS AND MEANS TO BE UTILIZED**

In February 2012, at the Gerace Research Centre, we isolated total DNA from numerous tissue samples of *S. gigas*. DNA samples were transported back to my laboratory at Cornell College and DNA mass of each sample was obtained by spectrophotometric analysis. We propose to use the primers developed by Zamora-Bustillos et al. (2007) to amplify the remaining seven *S. gigas* microsatellite loci. The polymerase chain reaction (PCR) conditions for amplifying these remaining microsatellite loci will need to be developed by us. Once we are successful in developing these parameters, amplified PCR products can be sized by agarose gel electrophoresis and compared to the sizes published by Zamora-Bustillos et al. (2007). Once the approximate sizes are confirmed, PCR fragments will be purified and ligated into pGEM-T vectors. DNA sequence analysis will be conducted on our LI-COR 4300S DNA Sequencer. All of the cloning and sequencing protocols have been previously developed by my lab group (Squiers et al., 2011). Obtained sequences will be submitted to the NCBI database for comparison of sequences entered for these microsatellites by Zamora-Bustillos et al. (2007). We hope to complete the preliminary requirements needed to establish a genetic breeding program for *S. gigas*, in San Salvador, Bahamas.

**REFERENCES**