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Can Headstart Programs Truly be Effective? Genetic Assessment of Cyclura Collei, The Headstarted Jamaican Iguana

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Can Headstart programs truly be effective? Genetic assessment of *Cyclura collei*, the

Headstarted Jamaican iguana

By

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Can Headstart programs truly be effective? Genetic assessment of *Cyclura collei*, the

Headstarted Jamaican iguana

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Headstarting is one of the more recent practices being used to maintain endangered populations in the wild. A headstart program was developed for the Jamaican iguana, *Cyclura collei*, in 1991 after its rediscovery in 1990. This current study tests the hypothesis that this population is experiencing a reduction in effective population size in spite of an increasing census population size due to the small number of nests available in the early years of the headstart program. A total of 875 individuals collected from 1991 to 2011 were genotyped at twelve variable microsatellite loci. Results from this study indicate a slight but significant decline in genetic variation (3% loss), and a modest proportional reduction in effective population size (0.075), since the initiation of the program. However, it is important to note these data also suggests that effective population size of this population is stabilizing.

Key words: headstart, *Cyclura collei*, effective population

DEDICATION

I dedicate this text and my cumulative determination towards its final production to some of the closest friends that I have made during my years as a graduate student, Hanna Dorman, Giuliano Colosimo, and Rosanna Carreras. Without their continued friendship, support, and advice, I would have had little chance at succeeding in my attempt at completing my thesis.

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CHAPTER I

INTRODUCTION

The extinction rates associated with the current global biodiversity crisis are accelerating due to the cumulative effects of competition for resources, habitat destruction and fragmentation, spreading of invasive species, and climate change (Butchart et al., 2010; Hanski, 2011). Some of these losses might be mitigated if appropriate active management practices can be established (Dawson et al., 2011). For many species, the forces driving extinction have disproportionate effects on a single life history stage, and management practices that enhance survivorship and recruitment at that stage may facilitate species conservation until the initial factors driving extinction can be rectified (Muths et al., 2011). Headstarting is one of the more recent practices being used to maintain animal populations in the wild.

Headstarting involves the reintroduction of hatchlings or juveniles of an endangered species into the wild once they are large enough to experience increased survivorship in their native habitat (Alberts, 2007; Pérez-Buitrago et al., 2008). This technique is currently being used on several species including the black-footed ferret (*Mustela nigripes*), the golden lion tamarin (*Leontopithecus rosalia*), and the California condor (*Gymnogyps californianus*). Positive results, including significant increases in population sizes, and the down listing of some species from critically endangered to endangered, has occurred due to the implementation of some of these programs (Biggins

et al., 1999; Meretsky et al., 2001; Williams, 2008). While some current headstart programs have generated positive results, others have not been as successful. For example, some sea turtle headstart programs have received criticism. When these programs were originally developed they were designed to help reverse the dwindling numbers of sea turtles found in the wild (Pritchard, 1979; Bowen et al., 1994; Fontaine et al., 2005). These programs were initially deemed appropriate because early assessments indicated that the reduction of sea turtle populations was due to increased egg and hatchling mortality (Fontaine & Shaver, 2005). This limited recruitment of individuals to their respected breeding populations (Fontain & Shaver, 2005; Frazer, 1992).

While headstart programs may help to reverse dwindling census sizes, there are risks that come with their use. Some headstart programs designed for sea turtles were criticized for not addressing key human and environmental impacts (Fontaine & Shaver, 2005; Frazer, 1992). It was found that some programs were increasing the survival rates of hatchlings and juveniles but the same factors that caused a decline in the population of adults were not being addressed (Dodd & Seigel, 1991). Other studies have revealed that headstarting might be associated with a loss of genetic variation in some managed populations (Jansson et al., 2012; Pérez-Buitrago et al., 2008). In some of these cases a captive breeding population is used to help increase the number of individuals released back into the wild. If certain precautions are not taken a reduction of genetic variation can occur in the wild population through excessive inbreeding, mating of genetically similar individuals (Jansson et al., 2012; Pérez-Buitrago et al., 2008). In spite of these criticisms, headstart programs may be appropriate for other endangered species, and positive results

have been observed for endangered Caribbean iguanas in the genus *Cyclura* (Alberts, 2004; Alberts, 2007; Pérez-Buitrago et al., 2008; Welch et al., 2012).

As with sea turtles, several iguana species have experienced significant reductions in recruitment from the hatchling life history stage to the adult breeding populations (Alberts, 2007). The main factor driving down recruitment rates has been predation by a variety of invasive mammals including dogs, mongooses, pigs, rats and feral cats (Iverson 1978, Alberts 2007). One species of Caribbean iguana has gone extinct (Henderson and Powell 2009), and several are currently listed by the International Union for Conservation of Nature (IUCN) as Endangered or Critically Endangered (Alberts, 2004). With near zero recruitment and estimated population sizes for several of these species dropping below critical levels in the wild, four headstart programs were initiated to bolster population numbers until the factors that limit recruitment can be rectified (Alberts, 2007). These ongoing programs on these species, *C. collei, C. cornuta stejnegeri, C. pinguis*, and *C. lewisi*, have built on lessons from earlier attempts at headstarting and have focused on ecological factors that might limit success. Specifically, habitat quality has been a major focus, and the effects of capture and release of juveniles were found to be tolerable in all four of these species (Alberts, 2004; Alberts, 2007; Wilson et al., 2004). Although most of these iguanas are still listed as critically endangered, headstarting has been extremely effective at increasing breeding population sizes (Alberts, 2007).

While population growth is encouraging, headstarting began after most of these populations had fallen to crippling levels. With this clear evidence of a bottleneck occurring and significant losses in population sizes in a few generations, there is an

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increasing probability of inbreeding (Thornhill, 1993; Wright et al., 2008). Extinctions can occur as a result of both biotic and abiotic processes following population bottlenecks. Environmental stochasticity, inbreeding, demographic sochasticity, and the loss of beneficial behaviors through adaptation have all been observed to drive extinctions or increase the chance of extinction (Fagan & Holmes, 2006; Gilpin & Soulé, 1986). Extinctions are, in theory, more likely when multiple factors contribute to a negative feedback loop that reduces population size and mean individual vigor (Fagan & Holmes, 2006). These occurrences were termed extinction vortices by Gilpin and Soulé (1986). Some of the factors thought to contribute to extinction vortices include reductions in population size and genetic variation, which can result in a loss in fitness (Thornhill, 1993). Inbreeding is thought to play a central role in extinction vortices and can be measured as a function of heterozygosity found in populations (Wright et al., 2008). With the potential of these negative impacts occurring in headstarted species, more work is needed in terms of genetic analysis of these programs. This study attempts to address this issue for one species by examining the genetic makeup of one of the rarest species of iguana for which a headstart program has been established, the Jamaican iguana, *Cyclura collei*.

After being thought extinct for over 40 years, a population of *C. collei* was rediscovered in 1990 in a remote region of Jamaica known as the Hellshire Hills (Vogel, 1994; Wilson et al., 2004). The headstart program initiated in 1991 began the work of raising 31 hatchlings captured in the Hellshire Hills at the Hope Zoo in Kingston, Jamaica (Vogel, 1994). Only two nest sites were located in 1990, and all hatchlings collected for the headstart program, in its earliest years, were collected from this limited

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source (Vogel, 1994; Wilson, 2004). Due to the small population size and this being the only population still existing in the wild, it is believed that all individuals were captured since the start of the program. During the first few years (1991-1996) of the program, all hatchlings captured were taken to the zoo for captive rearing. In 1996, the space available in the zoo became limited, and few additional individuals were collected for headstarting from 1997 to 2001 (Table 1).

Conservationists have improved the survival rate of the wild population by keeping people, particularly charcoal burners, out of the core area and trapping invasive mongooses and cats that prey on young animals (Vogel, 1994; Wilson et al., 2004). Additional enclosures have been constructed at the zoo. The number of hatchlings emerging each year has increased dramatically due to the apparent success of the headstarted individuals joining the breeding population in the wild. The expanded facilities at the Hope Zoo can now no longer house all hatchlings that are captured each year. Hence, a representative group of hatchlings captured annually is taken to the zoo for headstarting, and the rest are released.

While headstarting has been overwhelmingly successful at growing the census size of the Hellshire Hills population, this population has passed through a serious bottleneck. The severity of that bottleneck has potentially been exacerbated by the limited sample of hatchlings initially available for establishing the headstart population. It is conceivable that the headstart program has contributed to a loss of genetic variation in this population in spite of the growing census population size. Since individuals used in the program have a survival advantage over those found in the wild, if headstarted individuals are more inbred than the wild population the program could be having a

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negative impact on the genetic makeup of the population. The goal of this study is to assess the genetic impact of headstarting in this population in general and to specifically test the hypothesis that this species is experiencing a reduction in effective population size. Effective population is debated as being the most significant population parameter in evolutionary biology as well as conservation practices. While census population size is the total number of individuals in a population, effective population size defines the rate at which genetic variation is projected to be lost at each generation, and ultimately limits the rate at which it can respond to changes in the environment (Turner et al., 2006). If this hypothesis is correct, a significant loss in genetic variation in this population over time should be observed. This assessment was achieved by contrasting neutral molecular genetic variation observed in animals captured before and after headstarters had the opportunity to reproduce in the population.

CHAPTER II

METHODS AND MATERIALS

Figure 1   An adolescent female *Cyclura collei* in Hellshire Hills

Study System

The Jamaican iguana (*Cyclura collei*; See Figure 1) is a large species of lizard endemic to Jamaica and currently listed as critically endangered (Lemm & Alberts, 2011). This species was once found throughout Jamaica, but the numbers started to decline drastically around the second half of the nineteenth century after the introduction of the Indian mongoose (*Herpestes javanicus*) for rat and snake control (Lemm & Alberts, 2011; Woodley, 1980). *Cyclura collei* was assumed to be extinct following the

loss of the population on the Goat Islands of Jamaica in the 1940's (Woodley, 1980).

Following the 1990 discovery of iguanas in the Hellshire Hills, a subsequent examination

of the area revealed two active nesting sites and a small remnant population of fewer than

50 to 100 iguanas (Vogel, 1994; Lemm & Alberts, 2011). This species remains in the

area of the Hellshire Hills despite the continued threat of habitat destruction by charcoal

burning and predation by feral mammals, including mongooses, cats, rats, dogs, and pigs

(Wilson et al., 2004; Vogel, 1994). The Hellshire Hills is located along the south coast of

Jamaica and is roughly 110 km^2 in total area (Figure 2) (Vogel, 1994). These hills are an

example of a Caribbean dry forest still standing in the West Indies (Vogel, 1994). Not

only is Hellshire Hills essential to the future of *C. collei*, it is has also been identified as a

crucial site for the maintenance of several other species endemic to Jamaica (Wilson et

al., 2004; Wilson & Vogel, 2000).
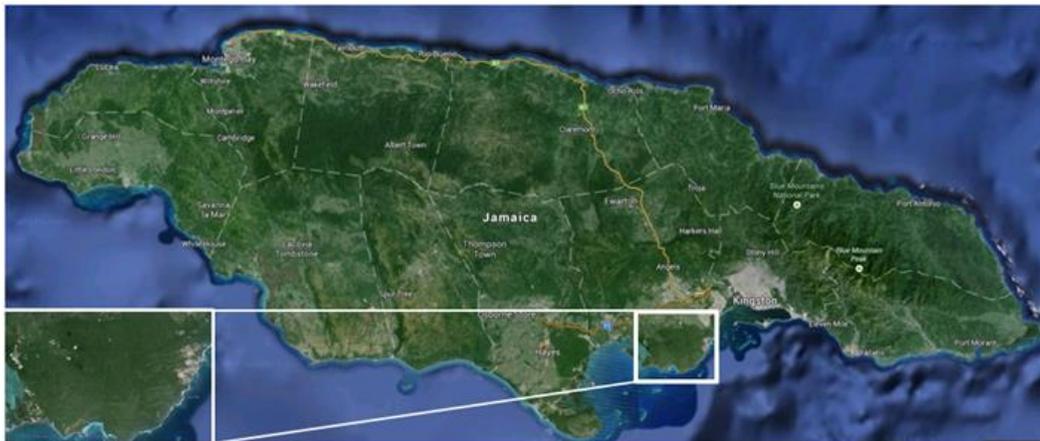
Figure 2   The location of Hellshire Hills on the island of Jamaica

The Jamaican iguana is a medium-sized species with individuals weighing up to 9 kg (Vogel, 1994). They are usually green but can also be salty blue in color with a darker olive green coloration on their shoulders. Wild nesting females can often appear reddish-brown due to digging in the soils of the Hellshire Hills. Adult males can reach 42.8 cm (16.9 in) SVL (snout-vent-length), while females usually reach a size of about 37.8 cm SVL (14.9 in) (Vogel, 1994). *Cyclura collei* usually feeds on a wide variety of leaves, flowers, and fruits. Males are territorial throughout the year and, in May and June, compete with neighboring males for mates. Females start reproducing at around 7 years of age (Vogel, 1994). They prefer to burrow communally in areas filled with loose soil. Egg deposition usually occurs in mid-June, and hatching occurs 85 to 87 days later. Clutch sizes can range from 6 to 20 eggs depending on the size and age of the female (Vogel, 1994).

Figure 3   Enclosure on nests used for catching hatchlings

Sample Collection

Hatchlings were captured during September as they emerged from their nests by constructing enclosures of aluminum flashing (Figure 3). Approximately 0.5 ml of blood was collected from each hatchling and placed in a 2% SDS lysis buffer (Longmire et al, 1992). Blood samples are collected from all captured hatchlings, including those that were never reared in captivity. Blood is collected from the caudal vein, accessed either dorsally or laterally. For smaller iguanas, particularly hatchlings, a 1cc (cubic centimeter) syringe and 23 gauge needle is used, while a 1 or 3cc syringe is used with a 23 or 25 gauge needle for larger animals. Buffer is added at equal to twice the volume of blood. DNA isolation was completed for all samples collected in the wild using either an ABI 6100 Nucleic Acid Prepstation with the NucPrep™ gDNA Isolation Kit (Applied Biosystems, Foster City, CA), or a Maxwell® 16 Research Instrument and Tissue DNA Purification Kit (Promega Corporation, Madison, WI).

Molecular Procedures

Thirty microsatellite loci originally characterized in *C. cychlura* (Malone et al., 2003), *C. pinguis* (Lau et al., 2009), and *C. cornuta* (Rosas et al., 2008) were analyzed to determine if they could be amplified by means of PCR and were polymorphic in *C. collei*. PCR was performed in 10 μL reactions with approximately 10 ng of template DNA. As a cost saving approach M13 tags (CACGACGTTGTAAAACGAC) were used to facilitate three-primer PCR with a fluorescently labeled (FAM or HEX) M13 primer (Schuelke, 2000). Additionally, labeled forward primers were available for the loci initially characterized in *C. pinguis*. These loci underwent traditional two-primer PCR with primer concentrations of 0.3 μM. For three-primer PCR, reverse primer and M13

primer concentrations were 0.2 μM. Concentrations of forward primers for three-primer

PCR were 0.04 μM (Ccste2 and F519), 0.06 μM (Ccste1, F436, Ccste4, and Ccste76), or

0.08 μM (Ccste6) dependent on PCR amplification success in *C. collei* (Table 2). PCR

was conducted with 2 mM MgCl2, 30 mM Tricine (pH 8.4-KOH), 50 mM KCl, 100 μM

of each dNTP and 0.4 U of Taq DNA polymerase. The thermal cycling profile included 3

min at 95^0 C, 30 cycles of 95^0 C for 15 sec, annealing temperature for 15 sec, and

elongation at 72^0 C for 45 sec, and then a final 7 min period at 72° C to end each run.

PCR products were pooled without dilution, and electrophoresed at Arizona State

University's DNA lab with Mapmarker 1000 size standard (Bioventures) in each well.

Peak Scanner software v. 1.0 (Applied Biosystems) was used for scoring.

Genetic Analysis

Since release of individuals back into the wild did not begin until 1996, after the

breeding season, samples from 1991 to 1996 are known to have parents that were not

themselves headstarters. This group, pre-headstart, was used to estimate genetic variation

in the breeding population before the headstart program could influence the genetics of

the population. Larger cohorts of samples collected from later years had the potential of

being sired or damed by headstarters and were treated as the post-headstart group (2002-

2011). Close examination of the changes in heterozygosity over time was used to

estimate the change in effective population size across annual cohorts (yearly samples).

This approach is deemed appropriate because theory suggests that heterozygosity is

dependent on effective population size (Frankham, 1996; Reed & Frankham, 2003).

Further, significant correlation between heterozygosity and N_e can be assumed when

using empirical data because mutation rates should be relatively constant in diploid

eukaryotic species (Frankham, 1996; Reed & Frankham, 2003). An estimate of effective

population size was calculated assuming that heterozygosity for neutral alleles is

expected to decline over time as a function of effective population size (N_e), seen in

Equation (1) (Falconer and Mackey, 1996; Montgomery et al., 2000):

$$\frac{H_t}{H_0} = (1 - \frac{1}{2N_e})^t = 1 - F \qquad (1)$$

where H_t is the heterozygosity at generation t, F is the mean inbreeding

coefficient, and H_0 is the initial heterozygosity of the population. This calculation

assumes one generation has occurred (pre-headstart vs. post-headstart) in this population

since the initiation of the headstart program, and that the change in heterozygosity is

caused by random genetic drift. Another calculation was made to determine the

proportional loss in effective population size since the start of the program. This estimate

assumes that the initial breeding population is in drift-mutation equilibrium (Nei &

Roychoudhury, 1974). This relationship can be seen in Equation (2):

$$H = \frac{4N\mu}{4N\mu + 1} \qquad (2)$$

where H is heterozygosity, N is effective population size, and μ is the mutation rate. By

solving for N in this equation and assuming a generation has passed a calculation for

proportional loss of effective population size was made using Equation (3):

$$\frac{N_t}{N_0} = \frac{\frac{H_t}{1 - H_t}}{\frac{H_0}{1 - H_0}} \qquad (3)$$

where N_t is the effective population size at generation t and H_t is the heterozygosity at

generation t.

Another estimation of effective population size was made using NeEstimator (version 2.0; Do et al., 2014). NeEstimator estimates effective population size using multi-locus diploid genotypes of samples from a population based on the amount of linkage disequilibrium in the population and assuming random mating. This was done for the entire sample and not for individual cohorts due to some cohorts' low number of samples (1995: 5 individuals; Figure 4). Studies show that estimations of effective population size can be sharply biased if the sample size is less than the true N_e (Waples, 2006). This estimation was calculated for the entire sample to reduce the chance of receiving a biased result.

Allele frequencies, F-statistics, Hardy Weinberg Equilibrium, and heterozygosities in each annual cohort of hatchlings and at each locus were also estimated with GenAlEx (version 6.5; Peakall & Smouse, 2012). Allele frequencies were used to calculate heterozygosity for each locus in each cohort. Average heterozygosities across all loci were then calculated for each cohort for a comparison between pre-headstart and post, to infer changes in levels of genetic variation over time. An Analysis of Molecular Variance (AMOVA) was used to test the statistical significance of these relationships for each cohort. This AMOVA compared the genetic variation between pre-headstart and post, and indicated the proportion of molecular variance found for pre-headstart and post-headstart groups, among individuals within years, and variance found among years.

CHAPTER III

RESULTS

Collection of Samples

Due to a small population size, early years of collections resulted in all

individuals being taken back to the zoo to be used in the headstart program (Table 1).

Table 1   Individuals collected for Headstarting

Hatch Year	Sent to Zoo for Headstarting	Released after tagging w/o Headstarting
1991	31	0*
1992	13	0*
1993	40	0*
1994	29	0*
1995	8	0*
1996	7	0*
1997	0	0
1998	10	0*
1999	5	96-100
2000	3	0*
2001	18	54-58
2002	19	56-61
2003	29	0*
2004	19	48
2005	20	123
2006	20	64
2007	39	24
2008	40	44
2009	29	99
2010	52	164
2011	43	161

Note: '*' Represents years when all captured individuals were Headstarted.

These samples were all collected from hatchlings; while blood from a few wild adults was collected a few years later. However, only samples from hatchlings were used in this study. In 1997, space for rearing headstart iguanas at the Hope Zoo became limited. This resulted in limited sampling of hatchlings between 1997 and 2001. From 1991 to 2011, it is estimated that between 1407 and 1420 individuals were captured. Blood samples for 882 unique hatchlings were available for use in this study, and DNA was successfully isolated from all of them (Figure 4). Three cohorts, 1998, 2000, and 2001 had samples sizes below 5 individuals and were not included in this analyses.
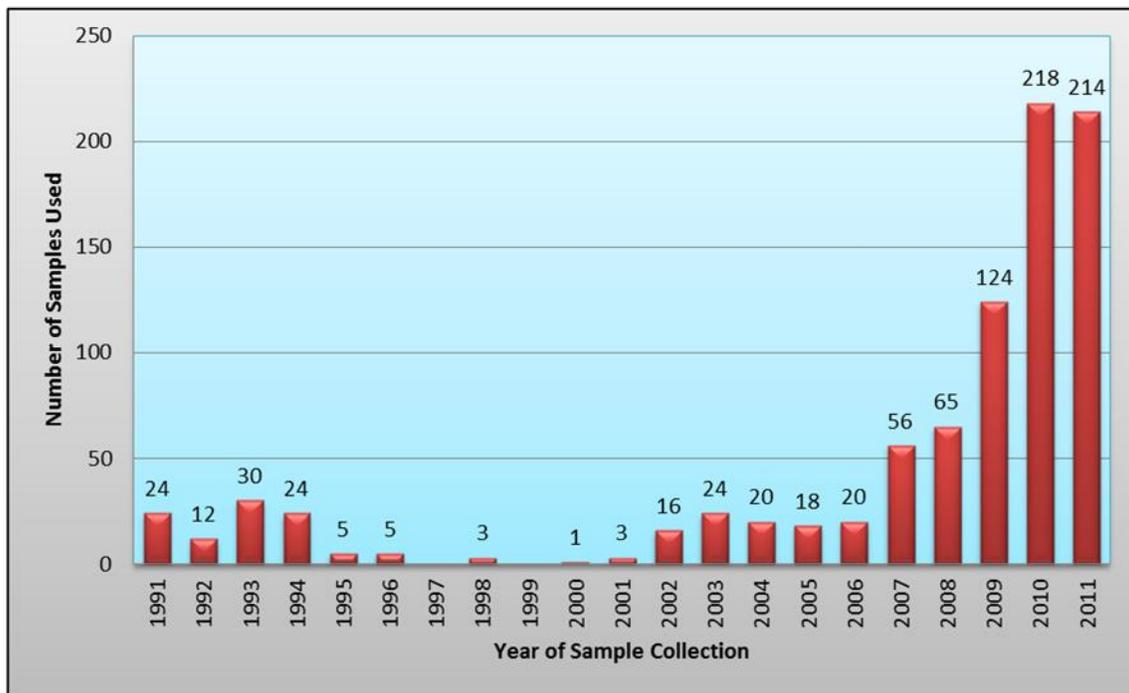

Figure 4   Samples size of individuals used in analysis by annual cohort

Table 2   Twelve polymorphic microsatellite loci used in analysis

Locus	Species	FPC	AT (^0C)	# of alleles	F	H_o	H_e	HWE
C113	C. pinguis	0.2 µM	Td-52	3	0.114	0.551	0.495	***
C124	C. pinguis	0.2 µM	Td-57	12	0.086	0.616	0.674	***
C6	C. pinguis	0.2 µM	Td-52	5	0.056	0.182	0.173	ns
Ccste_01	C. cornuta	0.06 µM	Td-55	4	0.046	0.153	0.146	ns
Ccste_02	C. cornuta	0.04 µM	Td-55	2	0.225	0.609	0.497	***
Ccste_04	C. cornuta	0.06 µM	Td-57	10	0.173	0.770	0.656	***
Ccste_06	C. cornuta	0.08 µM	Td-55	3	0.237	0.505	0.409	***
Ccste_76	C. cornuta	0.06 µM	Td-52	11	0.022	0.592	0.605	***
D11	C. pinguis	0.2 µM	Td-52	4	0.041	0.608	0.584	ns
D137	C. pinguis	0.2 µM	Td-50	11	0.089	0.644	0.707	***
F436	C.cychlura	0.06 µM	Td-55	4	0.090	0.705	0.647	***
F519	C. cychlura	0.04 µM	Td-55	6	0.297	0.353	0.502	***

Note: FPC is the forward primer concentration, AT is Annealing Temperature, Td is Touchdown, F is the fixation index, H_o is observed heterozygosity, H_e is expected heterozygosity, and HWE identifies those loci with significant departures from Hardy-Weinberg equilibrium (*** = p<0.001)

Data Analysis

Twelve of 30 microsatellite loci analyzed amplified and were variable in *Cyclura collei* (Table 2). Of these twelve loci, five were originally characterized in the Anegada iguana, *Cyclura pinguis* (Lau et al., 2009), five were developed for the Mona Island iguana, *Cylcura cornuta stejnegeri* (Rosas et al., 2008), and two were developed for the North Bahamas rock iguana, *Cyclura cychlura* (Malone et al., 2003). The number of alleles per locus ranged from 2 to 12. All samples were genotyped via fragment analysis

for all variable loci. Results suggest that this population is not in Hardy-Weinberg

equilibrium due to 9 loci having significant departures from expected equilibrium

genotype frequencies. Averages of expected heterozygosity were used to assess change in

variation over time. Hatchlings collected before the release of headstarted iguanas (pre-

headstart, 1991-1996) showed an average heterozygosity of 0.495 ± 0.050 SE while those

in the post-headstart group (2002-2011) had an average of 0.476 ± 0.057 SE (Figure 5).

The inbreeding coefficient, F, for the pre-headstart group was -0.158 ± 0.094 and -0.098

± 0.065 for the post-headstart group.

These results were confirmed through the Analysis of Molecular Variance

(AMOVA, See Figure 6 & Table 3). The AMOVA showed that the proportion of genetic

variation explained by differences in allele frequencies between the pre-headstart and the

post-headstart group is 0.03 (p<0.001). This analysis also revealed that the proportion of

genetic variation attributable to differences in allele frequencies among cohorts within

groups was 0.02 (p<0.001). The remaining genetic variation, 95%, was found within

cohorts. NeEstiamtor (version 2.0; Do et al., 2014) produced an estimate for effective

population size in the Hellshire hills population of *C. collei* of 77.2 with a 95%

confidence interval of 70.5 – 84.4 using the software's linkage disequilibrium based

method. The simple analytical calculation, however, returned an effective population size

of 12.72. Using another analytical calculation based on neutral theory, we found that the

reduction in genetic variance in this population is consistent with a proportional loss of

0.075 from the original effective population size.
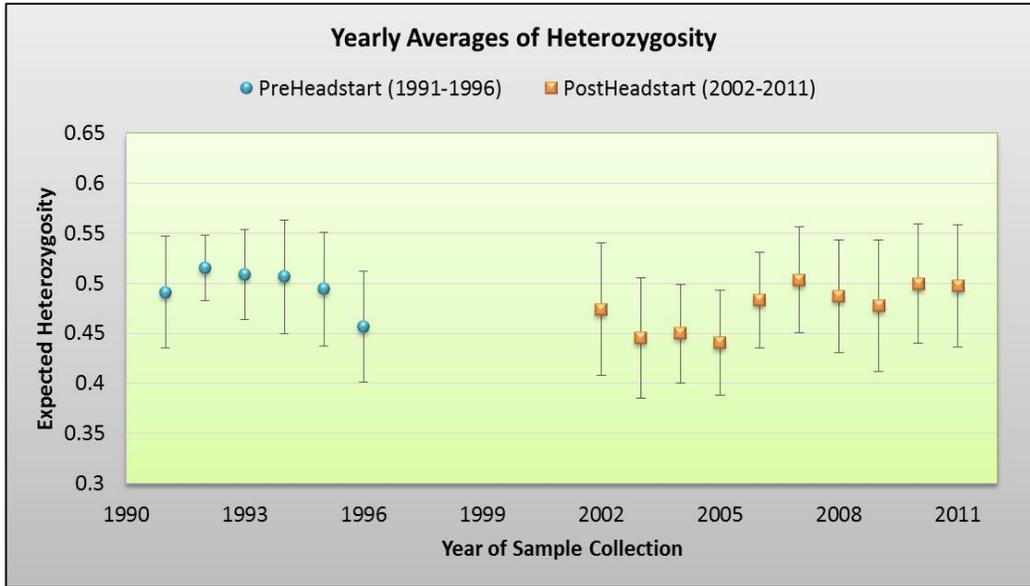
Figure 5 Heterozygosity comparison

Note: Comparison of individuals collected before and after the release of headstart iguanas. Standard error used to represent error bars
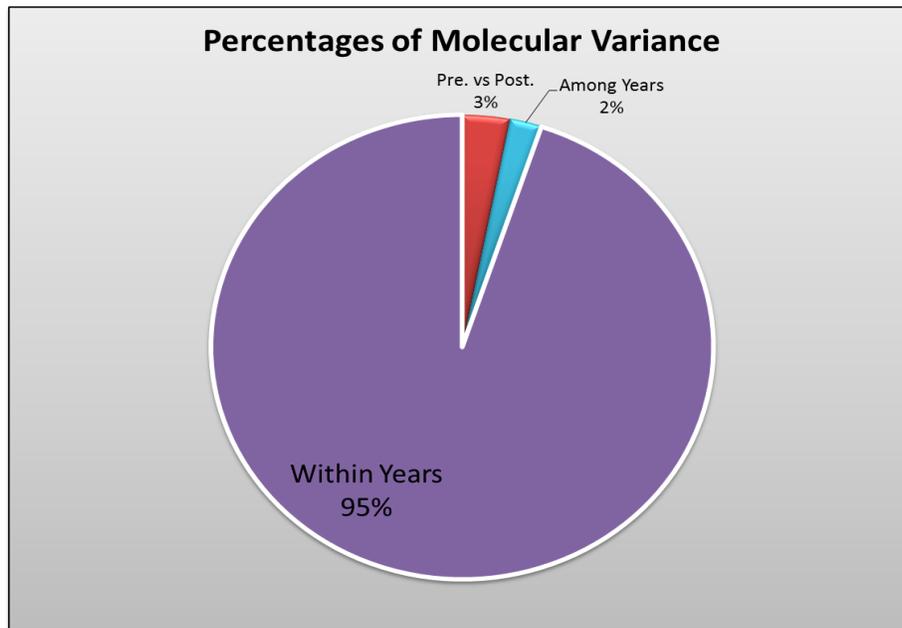
Figure 6 Analysis of Molecular Variance (AMOVA)

Note: Molecular Variance among hatchlings within years vs. among years and grouped by the years before and after the first set of headstart releases (p<0.001).

Table 3   AMOVA Table

SOURCE	DF	SS	MS	EV	%
PRE. VS. POST.	1	45.889	45.889	0.105	3%
AMONG YEARS	14	144.550	10.325	0.071	2%
WITHIN YEARS	1734	5673.218	3.272	3.272	95%
TOTAL	1749	5863.657		3.448	100%

STAT	Value	P
F_{RT}	0.031	0.001
F_{SR}	0.021	0.001
F_{ST}	0.051	0.001

Note: DF is the degrees of freedom, SS is the sum of squares, MS is the mean square deviation, EV is the estimated variance, and P is the probability value estimate based on 999 permutations.

CHAPTER IV

DISCUSSION

Headstart Criticism

Although several headstart programs have been developed for other species of animals, the main brunt of criticism was directed at a few early programs focused on sea turtles. Previous headstart programs for sea turtle conservation continued to expand for several years before receiving heavy criticism (Fontaine & Shaver, 2005). The key concern was that these programs were not addressing the factors driving declines in sea turtle populations. Headstarting was effectively increasing egg viability and hatchling survival but was not increasing the chances of adult survivorship (Dodd & Seigel, 1991). High survivorship in adults and high mortality in juveniles and eggs was assumed to have been a life history strategy that evolved in turtles over the past hundred million years (Frazer, 1992). The new finding of high mortality rate in adults was found to be caused by several human mediated factors (Bowen et al., 1994; Ross et al, 1989). These factors included fishing for juveniles and adults, intentional killings of adults for meat, as well as unintentional killings of adults in fisheries by-catch. Frazer pointed out that the program was placing these headstarted individuals into an environment that their parents had already shown was inhospitable (Frazer, 1992).

One of the key criticisms of some sea turtle headstart programs was the lack of adequate post release monitoring to assess whether the project was succeeding in their

initial goal of enhancing recruitment rates (Dodd & Seigel, 1991). This was the central concern regarding the headstart program for Kemp's Ridley, *Lepidochelys kempii,* which lasted from 1978 until 1992 (Bowen et al., 1994). In the case of this species, hatchlings were experimentally imprinted on the beach they were born on to allow for recapture as they returned for nesting, but only 878 of the 22,596 individuals released were ever recovered, and of those several were deceased (Fontaine & Shaver, 2005). The authors concluded that the headstart program had some success, but it was for the best that it was discontinued in 1992.

Negative Impacts from Conservation Programs

Previous programs have shown that even good intentions by conservationists can have huge risks. In 1980 only about five Chatham Island black robins (*Petroica traversi*) were believed left in the wild (Cemmick & Veitch, 1985). Later in that year the population was reduced to a single breeding pair (Massaro et al., 2013) that was used as a base for a conservation program focused on the restoration of the species (Cemmick & Veitch, 1985). The program helped to increase the number of individuals found in the wild, but after the bottleneck, a few females were seen laying eggs at the rim of their nests. Eggs that were laid at the rim of nests always failed to hatch (Massaro et al., 2013). Conservationists pushed these eggs back into the nests and the eggs were incubated and hatched successfully (Massaro et al., 2013).

Other problems arose, as the population of robins increased, by 1989 over 50% of all females were found laying some of their eggs on the rim of nests (Massaro et al., 2013). The practice of pushing eggs was stopped immediately and helped to slow down the spreading of the behavior. If this practice had continued it would have been

detrimental for this species due to their survival being dependent on human interaction. As this practice was stopped and time went on, rim laying became less frequent and in 2011 only 9% of the population was still found exhibiting the trait (Massaro et al., 2013). This is a clear example of how delicately conservationist must tread when working with a population; one wrong step could lead the population closer to extinction.

Another issue that can arise with active management programs, and what we see in the headstarting of *C. collei*, involves negative impacts on the genetics of a fragile population. Ignoring genetic variability could result in reduced fitness for the entire population. This issue can be greatly exacerbated in a managed population if genetic variation is not properly monitored. One such case can be seen in the recent study focused on the Ryman-Laikre effect occurring in a wild population of steelhead trout (*Oncorhynchus mykiss*; Christie et al., 2012). The Ryman-Laikre effect refers to the result of an increase in census population size along with a reduction in effective population size due to restocking with captive bred hatchery fish (Christie et al., 2012). Researchers found a significant reduction in genetic variation and effective population size in the wild due to genetic swamping, flooding a population with genetic variation from a restricted source, because the source population used for captive breeding, while large in census size, was generated from a narrow gene pool that became relatively inbred (Christie et al., 2012). Results from this study of *Cyclura collei* reveals a significant yet modest loss of genetic variation consistent with that of the Ryman-Laikre effect. Since the initiation of the headstart program designed for *Cyclura collei* there has been a 3% loss in genetic variation and a 0.075 proportional loss in effective population size.

Potential for Success in Headstarting *C. collei*

With the Ryman-Laikre effect there are at least two separate populations and gene flow is unidirectional. That is, a captive bred population is used to supplement numbers in the wild. The number of individuals used to establish the captive hatchery population was genetically smaller than that of the original wild population (Christie et al., 2012). The subsequent overrepresentation of captive bred individuals and their descendants in the wild population resulted in the significant reduction in genetic variation in the wild (Christie et al., 2012). However, it is important to note the differences between supplementation with captive bred animals, and the ongoing headstart program in Jamaica. A key distinction between population supplementation with hatchery reared fish and population supplementation with headstarted *C. collei* is that there is only a single population involved. Individuals collected from the wild are offspring of parents who mated naturally and nested in the wild.

While headstarting avoids the pitfalls of stocking wild populations with captive bred animals, similar threats to the maintenance of genetic variation may arise. By headstarting, there may be a selective advantage given to few individuals in the natural population if the sampling of hatchlings for the headstart program is non-random. As a result of this dynamic, the loss of genetic variation occurring in conjunction with headstarting might even outweigh the initial benefits associated with increasing census size. Hence, it is conceivable that headstarting might lower the chances at species survival. In the case of *C. collei*, the benefits of increasing census population size are dramatic, and the loss of genetic variation appears relatively small implying the benefits seem to outweigh the risks. Studies have also shown that captive reared individuals may

not learn behaviors necessary for survival in the wild (Alberts, 2007; Veasey et al., 1996).

These are issues that all programs that use captive rearing or breeding methods must

consider, including the headstart program developed for *Cyclura collei*.

In her 2007 paper, Alberts did just that as she analyzed the effectiveness of using

a headstart program on Caribbean rock iguanas. Since the initiation of headstarting on *C.*

collei, hatchlings must spend a few years in an artificial environment at the Hope Zoo.

Alberts states that some of the learned behaviors an individual might gain from their

environment might never be acquired. She felt that captive rearing could result in naïve

behaviors in headstarted iguanas that would influence their response to potential

predators, foraging success, dispersal, and settlement (Alberts, 2007). After thorough

analysis of previous behavioral studies, she concluded that rock iguanas were in fact good

candidates for headstart programs. Most of their behaviors appeared to be unaffected by

captive rearing. Some of these include their natural wariness while in captivity, being

herbivores that do not require extensive training to obtain and process prey species, and

their ability to rapidly integrate into wild breeding populations (Alberts, 2007).

Captive rearing may only be appropriate in special circumstances. It might also be

detrimental, or minimally have drawbacks that limit its utility even when appropriate.

The critical detractor noted for the sea turtle headstart programs, was that they enhanced

survivorship for an early life history stage when adults were the ones experiencing

reduced survivorship. If the critical life history stage being impacted is reproductive

adults, headstarting is unlikely to have a positive influence on population viability.

Captive rearing may further be restricted for use when attrition is highest for the youngest

age classes, as one study pointed out that there should be a negative effect from headstart

programs when keeping individuals captive beyond their adolescence (Alberts, 2007). This helps to demonstrate that headstarting may be most efficient when used on a population that is experiencing reduced survival rates at an early life history stage, much like *Cyclura collei*.

Conclusion

A previous reported that a significant loss in genetic variation had occurred in this population (Welch et al., 2012). That was the first complete assessment on the genetic impact of headstarting on this population. Those results were based on an analysis of 375 individuals at 6 variable microsatellite loci. The disparity in results that we see in our current analysis can be attributed to a significant increase in sample size to 875 individuals and a doubling of the number of variable loci to 12. Further, the vast majority of additional individuals used in this study were collected in 2010 and 2011. Those cohorts have relatively high levels of heterozygosity compared to other post headstart cohorts. While we do see a decline in heterozygosity, 0.495 ± 0.050 to 0.476 ± 0.057, between the early 1990's and more recent years of sampled hatchlings, it is important to note that the proportion of genetic variation attributable to allele frequency differences between the pre-headstart and post-headstart cohorts is a modest 0.03. We also see a proportional loss in effective population size of 0.075. Nonetheless, our results also show that heterozygosity in recent cohorts appears to be increasing (2010: 0.499 and 2011: 0.497), and this suggests that the population has begun to stabilize (Figure 4).

These results support the hypothesis that this population is experiencing a reduction in effective population in spite of an increasing census population size. These results also suggest that this population is likely to survive its recent severe population

bottleneck if it continues to grow in size as long as the factors that are driving population declines can be mitigated. Calculations of effective population size resulted in two values, 12.72, based on the difference in expected heterozygosity from pre-headstart to post, and 77.2, using the linkage disequilibrium method through the program NeEstimator, with a 95% confidence interval of 70.5–84.4. These values help to establish that this population in the Hellshire hills is a small population of sexually reproducing individuals. These low values also show that this population is susceptible to negative impacts to its genetic make-up (Hartl & Clark, 1997). However it is important to note that the results from NeEstimator are more than likely flawed. Our calculation using the difference in heterozygosity assumes that the change in expected heterozygosity was strictly due to genetic drift effecting this population. This is also an assumption of calculating N_e through linkage disequilibrium, however it is also assumed that the population is randomly mating. This is unlikely due to the survival advantage that headstarted individuals have over the wild population. Also, during its rediscovery it was assumed that this population had a census population size of less than 50 to 100 individuals (Vogel, 1994; Lemm & Alberts, 2011). The current census estimate has a range of about 100 to 200 individuals. In that time span from then till now it is highly unlikely that this population's effective population size has risen to the levels that we see from NeEstimator.

From our results we see that this population has a short-term risk of extinction, according to the 50/500 rule proposed by Franklin (1980). The 50/500 rule was proposed as a method for conservation biologist to assess the success of a conservation program. The rule states that managers should maintain an effective population size greater than 50

to help insure the short-term survival of a population and to help minimize the risk of inbreeding. This is due to the factors of inbreeding being able to quickly lead a small population into an extinction vortex (Harmon & Braude, 2010). The 50/500 rule also states that maintaining that same value above 500 is vital to ensuring the long-term survival of a population to maintain their ability to adapt to environmental changes (Franklin, 1980; Harmon & Braude, 2010). In a population with an effective population size below 500, genetic drift may lead to a significant loss of variation and could limit a population's ability to respond to environmental changes leading to loss in population size and potentially extinction (Franklin, 1980; Harmon & Braude, 2010). Our trusted calculation of effective population size for *C. collei* is below 50, and according to this rule, we can assume that while this population is still in great risk of imminent danger from extinction.

This rule has received some criticism, in the past. Some say that the tool is too broad for use on all species, while others suggest that the results from the calculations are too small (Harmon & Braude, 2010). Although this rule has received its fair share of criticism, it does provide a baseline for minimum viable population size that is well grounded in population genetic theory. These results suggest that this fragile population of iguanas is producing annual cohorts of hatchlings that are nearly as genetically variable as those upon the population's rediscovery. Hence, headstarting does not appear to be having a large negative impact on the species capacity to maintain diversity, and census sizes have risen dramatically. In the case of *Cyclura collei*, while there is no guarantee that headstarting will be a complete success, the evidence thus far indicates that the benefits of this active management practice far outweigh its detractors.

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