EVALUATING HEADSTARTING AS A CONSERVATION STRATEGY FOR ANEGADA ROCK IGUANAS (*CYCLURA PINGUIS*)

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ABSTRACT

EVALUATING HEADSTARTING AS A CONSERVATION STRATEGY FOR ANEGADA ROCK IGUANAS (*CYCLURA PINGUIS*)

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In an effort to combat the current extinction crisis, conservation biologists have developed a diverse set of tools, including conservation physiology. This scientific discipline applies physiological concepts and tools to generate conservation solutions for a broad range of species. The Anegada Rock Iguana (*Cyclura pinguis*) is a Critically Endangered species native to Anegada, British Virgin Islands. Feral cats depredate hatchlings within months of emergence, drastically reducing recruitment and population growth. By the 1990s, the population had declined by 80%. A headstart program was initiated in 1997 to combat this threat and increase the population. Wild collected hatchlings are reared in a captive environment and released to the wild once they reach a larger, less vulnerable size. Though headstart programs have been employed for a wide range of species, the method has been widely criticized for failing to assess the effects of captivity on the health and fitness of headstarted individuals, a lack of long-

term monitoring, and high failure rates. The goal of this research is to evaluate the efficacy of headstarting as a conservation mechanism to bolster reintroduction efforts for the Anegada Rock Iguana. To that end we employed conservation physiology to monitor growth, body condition index, and vitamin D and calcium metabolism in captive headstart and reintroduced iguanas on Anegada. The information gained in this study will be invaluable to the other existing headstart programs currently employed to save Caribbean Rock iguanas from extinction as well as future iguana recovery programs.

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DEDICATION

This is for my parents (my biggest fans), my husband Beau (my hero), and for Henry who started this whole mess!

CHAPTER 1

INTRODUCTION

As the rate of biodiversity loss accelerates, conservation biologists have developed a diverse set of tools to combat the extinction crisis, including conservation physiology. This scientific discipline utilizes physiological concepts and tools to provide conservation managers with solutions for a broad range of taxa (Wikelski & Cooke 2006, Cooke et al. 2013, Madliger et al. 2018). Conservation physiology methods can be applied to predict how organisms will respond to environmental change, to identify sources and consequences of stressors on organisms, and to evaluate and improve various conservation interventions (Cooke et al. 2013). In 2014, Tarszisz et al. introduced the concept of translocation physiology as a sub-discipline of conservation physiology. The authors encouraged translocation programs to examine a wide array of physiological parameters during all phases of release projects (e.g., pre-release phase through long-term post-release monitoring) to increase their probability of success. Translocation methods vary but include releasing animals to areas where they have been extirpated, areas where they still exist but in unsustainable numbers, or to novel areas outside the species' original range (IUCN 2013). Many times, translocation projects are coupled with headstart programs to bolster survival of the target species.

Headstarting is a conservation technique where juveniles are collected from the wild, or captive breeding programs, reared in a protected captive environment and released back to the wild once they reach a larger, less vulnerable size with increased survival potential (Knapp & Hudson 2004, Alberts 2010, Burke 2015). Though headstart programs have been initiated for a variety of species, the technique remains

controversial (Dodd and Seigel 1991; Reinert 1991; Germano and Bishop 2009; Seddon 2010; Perez et al. 2012). Many headstart programs have released hundreds or even thousands of individuals back to their native range or to novel landscapes. (Choudhury & Choudhury 1886, Bell et al. 2005, Butchart et al, 2006, Shaver & Caillouet 2015). However, critics claim these programs: are costly; do not sufficiently assess the effects of captivity on the health or fitness of released individuals; do not quantify the true long-term survivorship; do not address the actual cause of population decline (e.g., adult mortality); and rarely include long-term monitoring, especially for long-lived species (Woody 1990, Dodd & Seigle 1991, Frazer 1992, Heppell & Crowder 1998, Ricciardi & Simnerloff 2008). Despite the controversy, conservation managers continue to use headstart programs in their efforts to save species from extinction.

Caribbean Rock Iguanas (*Cyclura)* are the most endangered lizards in the world (Alberts 2000). The islands where these species occur have been heavily impacted by human development and the introduction of non-native species. Feral herbivore populations over browse tropical dry forest habitats, degrading the fragile ecosystems on which these species depend and often competing for resources. Feral predators populations (e.g., dogs, cats, rats, pigs and mongooses) prey on both adult and juvenile iguanas. By the early 1990's the situation for several *Cyclura* species became dire and headstarting was suggested as a mechanism to restore the Jamaican Rock Iguana (*Cyclura collie*), the Grand Cayman Blue Rock Iguana (*Cyclura lewisi*), and the Anegada Rock Iguana, (*Cyclura pinguis*) (Knapp & Hudson 2004).

The Anegada Rock Iguana is the most genetically and morphologically unique of the Caribbean Rock Iguanas (Malone et al. 2017). The species is considered Critically

Endangered according to IUCN Red List of Threatened Species (Bradley & Grant 2020). Its original range spanned across the Puerto Rican bank during the last glacial maximum, but rising sea levels and climate change are believed to have caused a contraction in the species' range to the remote island of Anegada (39 km^2) in the British Virgin Islands (BVI) (Pregill 1981, Perry & Gerber 2006). The Anegada Rock Iguana population has declined by 80% since the 1960's (Carrey 1975, Mitchell 1999, Perry & Gerber 2006). Human development and the introduction of non-native mammal species (e.g., rats, cattle, and goats) have all contributed to the decline of this species, but the chief threat is feral cats (*Felis catus*) that depredate most hatchlings within months of emerging, drastically reducing recruitment and population growth (Gerber 2000, Bradley unpublished data). A headstart program was initiated in 1997 by the National Parks Trust of the Virgin Islands (NPT VI) and the IUCN Iguana Specialist Group (ISG). The goal of the program was to offset the high rate of juvenile mortality to bolster wild population numbers until the problem of feral cats could be mitigated.

In the early days of the Rock Iguana headstart programs there was insufficient information concerning their success or their impact on the health of the animals in captivity or in the wild. Indeed, there were few studies investigating differences between captive and wild Rock Iguanas because there were limited wild populations remaining. In addition, the harshness of the remote localities where many species often occur precluded extensive capture and sampling or the use of sensitive field equipment needed to process blood chemistry samples. The Anegada Rock Iguana headstart program offered an opportunity to address critical information deficits concerning headstarting for this group of highly endangered species for several reasons. Anegada

is a small island, and most locations can be reached quickly, there is an existing infra structure (e.g., roads, housing, electricity, etc.), and the NPT VI offered signific logistical support. Thus, we investigated the efficacy of implementing headstarting as a conservation mechanism for Caribbean Rock Iguanas, using the Anegada Rock Iguana program as a model; examining three key physiological factors: growth, body condition, and vitamin D/calcium metabolism.

Growth rate has important fitness consequences as it can affect survival, competitive ability, and fecundity (Ferguson et al. 1982; Olsson and Shine 1996; Iraeta et al. 2006). In headstart programs, hatchling iguanas are in captivity when they undergo their fastest growth rates in years 1–4 (Bjorndal et al. 2012). Therefore, it is crucial to determine if captivity negatively affects hatchlings' growth rates while in captivity and after release. Headstarted iguanas must make the transition from being fed captive diets, often fresh produce, to foraging on native food plants. Examining post-release growth rates will indicate how well iguanas are making the transition and if additional steps are needed to prepare iguanas for foraging on native food plants, such as offering an assortment of native food plant before release, or conducting a soft release, where animals are in a semi free ranging enclosure but are still supplemented with food as needed until the animal is ready to be independent.

Body condition is often examined to ascertain an individuals' health, fitness, or nutritional state at a single point in time (Stevenson & Woods 2006). Condition has been used to indicate past foraging success, predict survival and reproductive outputs, and determine habitat quality (Jacob et al. 1996, Stevenson & Woods 2006). Body condition indexes (BCI) generate a score to quantify the mass of an individual and its associated

energy reserves after correcting for body size (e.g., SVL for iguanas) (Schulte-Hostedde et al. 2005). Examining BCI of captive iguanas will provide a quantitative measure to evaluate captive husbandry and procedures employed at the headstart facility, when compared to wild values. Analysis of BCI of released iguanas can determine post-release health and performance, the suitability of different habitats, and provide critical data to improve headstart release methods.

An additional concern for captive iguanas is that they commonly suffer from nutritional secondary hyperparathyroidism (NSH) (Mader, 2006). This metabolic bone disease is caused by a disruption in calcium and phosphorous homeostasis resulting from poor diet and low levels of vitamin D (Jacobson 1981, Frye 1997). Most vertebrates, including iguanas, need vitamin D to be able to absorb and utilize calcium (Jacobson, 1981, Frye 1997). When phosphorous levels become too high, the parathyroid gland releases parathyroid hormone (PTH) to mobilize calcium from bones to rebalance calcium phosphorus levels (Clark & Laverty 1985, Mader 2006, Rivera & Lock 2008). If this process is not corrected, bones become rubbery and prone to fractures (Mader 2006). Many basking lizards synthesize vitamin D when their skin is exposed to ultraviolet B radiation (UVB) from sunlight (Bernard 1991, Allen et al. 1994, Allen et al. 1996, Gehrmann 1997, Ullrey & Bernard 1999). Unfortunately, materials used to construct iguana enclosures (e.g., hardware mesh) often reduces UVB and thus vitamin D synthesis potential by 22% (Burger et al., 2007).

Though this disease is common in captivity, it is rarely observed in wild, free-ranging iguanas (Ullrey & Bernard 1999). When headstart programs were first initiated for Rock iguanas there was very little known about vitamin D and calcium metabolism,

or the other biochemistry elements (e.g., PTH, ionized calcium, total calcium, and phosphorus). However, an understanding of theses baseline health values is critical to validate the health of reintroduced individuals. To ensure the health of the headstarted iguanas and to increase the probability of a successful release program, we assessed vitamin D (25-OH-D3), parathyroid hormone (PTH), ionized calcium, total calcium, and phosphorus levels in captive and free ranging Anegada Rock Iguanas.

The following chapters provide insight into translocation physiology, demonstrating the efficacy of headstarting as a conservation strategy for the Critically Endangered Anegada Rock Iguana. By examining the physiological factors of growth, body condition, and vitamin D/calcium metabolism, critical problems resulting from captive husbandry can be identified and solutions can be developed to ensure a high probability of success for this conservation program. In turn, the information gained in this study will be invaluable to the other existing headstart programs currently employed to save Caribbean Rock iguanas from extinction as well as future iguana recovery programs.

CO-AUTHOR CONTRIBUTIONS

For Chapter 2 (Growth and body condition of headstarted rock iguanas, *Cyclura pinguis*, in captivity and post release on Anegada, British Virgin Islands), Kibby Treiber aided in the statistical analysis for the project. Glenn P. Gerber collected some of the early morphometric data and aided in the conceptualization of the study. For Chapter 3 (Comparison of Vitamin D and other biochemistry concentrations in captive and wild Anegada Rock Iguanas (*Cyclura pinguis*), Gary W. Ferguson aided in the development of the project design based on his experience with similar projects with reptiles and

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CHAPTER 2

Growth and body condition of headstarted rock iguanas, *Cyclura pinguis*, in captivity and post release on Anegada, British Virgin Islands

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ABSTRACT

Hatchling Anegada Rock Iguanas were collected from the wild and headstarted by captive rearing before being released once they were larger and less vulnerable to predation by feral cats. From 2003–2005, 72 headstarted iguanas were released at two ecologically distinct sites on Anegada: Middle Cay, with karst substrate and dry woodland/thicket habitat, and the Faulkner site, with sand substrate and coastal scrub. Growth rates for mass and snout-vent-length (SVL), and body condition indices (BCI) were compared across age categories for headstarted iguanas in captivity and after release to assess the headstart program and habitat quality on Anegada. In captivity, females and young males gained 15 g/mo with males increasing in mass gain to 33 g/mo after 3 y. Captive iguanas grew approximately 5 mm/mo for the first 2 years, after which they decreased to 2.5 mm/mo. Iguanas released at the Faulkner site grew 15 g/mo and 1 mm/mo, whereas iguanas released at Middle Cay grew 45 g/mo and 3 mm/mo, in the 24 months following release. However, BCI of released iguanas was similar at both sites and was higher than that of animals in captivity. Iguanas released at the Faulkner site maintained similar growth and body mass proportions and thus appear equally healthy. Seventy–six percent of released iguanas, regardless of release site, gained weight during the 60 d post release transition period. Our results demonstrate that iguanas headstarted and released on Anegada can grow and maintain body condition in two distinct habitats and that the population is not critically food limited despite habitat degradation from feral livestock.

INTRODUCTION

Caribbean Rock Iguanas, genus *Cyclura*, are considered one of the most endangered group of lizards (Alberts 2000). As island species, Caribbean Rock Iguanas are particularly vulnerable to extinction due to small ranges and rapidly changing environments including human development and invasive species (Paulay 1994; Foufopoulos and Ives 1999; Simberloff 2000; Ficetola and Padoa-Schioppa 2009; Anadon-Irizarry et al. 2012; Lemm and Alberts 2012).

Conservation biologists have employed various techniques to address population declines, including captive breeding, translocations, and headstarting/restocking (Seddon 2010). Many of these methods have been used in conservation efforts for rock iguanas (Alberts et al. 2004; Knapp and Malone 2003; Knapp and Hudson 2004; Perez-Buitrago et al. 2008; Echternacht et al. 2011; Wilson et al. 2016). Though these methods have produced some successes, their use has been criticized for lack of long-term monitoring, high program costs, and high failure rate (Dodd and Seigel 1991; Reinert 1991; Germano and Bishop 2009; Seddon 2010; Perez et al. 2012).

Long-term monitoring of captive and free-ranging animals is needed to determine the ultimate efficacy of headstart programs (Tarszisz et al. 2014). Examining growth rates and body condition indices (BCI) are two important non-invasive techniques used to assess health and fitness of captive and wild animals. Growth is a key element of natural history as it affects competitive ability, fecundity, and survival (Ferguson et al. 1982; Olsson and Shine 1996; Iraeta et al. 2006). Body condition index is defined as total body mass normalized for lean mass, with snout-vent-length (SVL) typically used as a measurement of lean body size for reptiles. Body condition indices are often used to quantify individual health at a point in time and provide predictive data about habitat quality and reproductive output (Stevenson and Woods 2006). This

index has been used to assess survivability of Marine Iguanas (*Amblyrhynchus cristatus*), habitat quality for Green Iguanas (*Iguana iguana*), and nutritional status of Mona Island Iguanas (*Cyclura stejnegeri*) in the wild (Romero and Wikelski 2001; Escobar et al. 2010; Perez–Buitrago et al. 2008).

Anegada Rock Iguanas, *Cyclura pinguis*, from the island of Anegada in the British Virgin Islands (BVI), are one of the most endangered species of *Cyclura*. Wild *C. pinguis* populations have declined steadily since the early 1800s (Schomburgk 1832; Barbour 1917; Carey 1975; Mitchell 1999; Perry and Gerber 2011). Today, this species is considered "Critically Endangered" according to IUCN Red List of Threatened Species™ criteria (Bradley and Grant 2020) with an estimated 200–400 Anegada iguanas surviving in the wild by the late 1990's (Garcia and Gerber 2016). Although the historical range of *C. pinguis* has been debated (Lazell 1997; Perry and Gerber 2011; Garcia and Gerber 2016), subfossil and climatic data suggest the species became restricted to Anegada at the end of the last period of deglaciation approximately 7000 years ago (Pregill 1981; Pregill and Olson 1981).

In addition to its historical range, the primary cause for population decline is also debated. Based on iguana body conditions observed in the 1980s, Mitchell (1999, 2000) claimed that the habitat on Anegada had been so severely degraded by feral livestock that it could no longer support a population of iguanas. In response, eight animals were translocated to the privately owned Guana Island, BVI between 1984 and 1986 (Goodyear and Lazell 1994). The population has successfully established itself and today numbers approximately 500 individuals (Mougey 2016). The Guana population was later used as the source for additional translocations to at least five other privately owned islands in the BVI. Although these translocated populations

act as a hedge against extinction, they may be genetically compromised, having all descended from eight founders (Perry et al. 2007; Perry and Gerber 2011; Mougey 2016).

Gerber (2004) proposed that feral cats were driving the population declines on Anegada because of intense predation pressure on recently-emerged hatchlings. He asserted that feral cats were reducing population recruitment to almost zero (Gerber 2004; Garcia and Gerber 2016; Bradley, unpubl. data). To offset high juvenile mortality and bolster recruitment on Anegada, the National Parks Trust of the Virgin Islands (NPTVI) and the International Union for Conservation of Nature Species Survival Commission (IUCN SSC) Iguana Specialist Group (ISG) initiated a headstart program in 1997, which is ongoing (Bradley and Gerber 2006). Wild hatchling iguanas are collected annually upon emergence in September and October and transferred to a captive facility on Anegada where they are reared in a protected environment by NPTVI staff. Animals are returned to the wild when they have reached a larger and presumably less vulnerable size.

Frazer (1992) warned that headstart programs are often a "halfway technology" because, as in the case of sea turtles, they "may serve only to release more turtles into a degraded environment in which their parents have already demonstrated they cannot flourish." If the Anegada population occupies a degraded habitat that is severely food limited, then headstarting may produce asymmetrical results by providing short-term relief from predatory feral cats, only to result in protracted harm by adding more consumers to a resource-limited environment. However, if iguanas are not severely food limited on Anegada, then released iguanas should augment the existing free-ranging populations and grow at rates like those seen in other species of *Cyclura*.

The continual release of headstarted *C. pinguis* provides a unique opportunity to evaluate the appropriateness and success of the headstart program and evaluate whether the habitat is sufficient to support an increased iguana population on Anegada. In this study, we analyzed growth rates and BCI of headstarted *C. pinguis* within different cohorts while in captivity and following their return to the wild in two different and distinct habitats on Anegada to assess captive management, the suitability of the different release sites (and habitats) on the island, and if the habitat can support more iguanas (e.g., the potential for success of the Anegada headstart program).

MATERIALS AND METHODS

Study site.—The island of Anegada (39 km^2) is located in the NE corner of the Puerto Rican Bank at 18°43' N, 64°19' W (The Settlement, Anegada) in the BVI. Two major geological formations divide the island: a large limestone plain encompassing the eastern two thirds of the island's interior and a series of sand dunes and beach ridges along the coast and comprising the western third of the island's interior. A large system of interconnected salt ponds dominates the western half of the island, covering over 5 km^2 (Kennaway et al. 2008). Anegada's harsh climate supports xerophytic vegetation communities with tropical dry woodland and scrub covering most of the island. In the current study, we released headstarted iguanas at two sites (Middle Cay and Faulkner) within the core iguana area (about 3 km^2), located in the northwestern part of the island, where the remaining wild population of Anegada iguanas is concentrated. Although the release sites are less than 1 km apart, they differ sharply in habitat.

The Middle Cay release site is located on a large peninsula of land in the western salt ponds of Anegada. The cay is part of the limestone plain and is filled with numerous solution holes that iguanas use as retreats. The site includes tropical dry limestone woodland and thicket

(McGowan et al. 2006) composed of mixed deciduous and evergreen trees, shrubs, and succulents (Clubbe et al. 2004). There is a true canopy on parts of the cay, but the understory is quite open, and wild adult iguanas persist despite the presence of feral cats, rats, and livestock (goats, cattle, and donkeys).

The Faulkner site is part of the sand dunes and beach ridges of Windlass Bight, located just north of Middle Cay but separated from it by salt ponds. Iguanas dig extensive burrows in the sand, and it is a key nesting area for the species. The vegetation is coastal scrub of intermediate height (Clubbe et al. 2004). This site has the same invasive species as Middle Cay and a similarly sized population of wild adult iguanas.

Study animals.—We collected newly emerged wild hatchling iguanas from Anegada between 1997 and 2002. Iguanas were collected emerging from previously located nests, surrounded by metal flashing for protection from feral mammals and to contain hatchlings, or opportunistically during or immediately following the period of hatchling emergence (mid–September through the end of October). A 12 mm passive integrated transponder (PIT) tag (Trovan Ltd.) was placed subcutaneously in the proximal dorsal left rear leg of each animal for permanent identification. All hatchlings were transferred to the Anegada captive facility where they were reared in groups of 5–20 similarly sized animals in large outdoor screen enclosures (2.89 L x 3.81 W x 2.43 H m). Iguanas were fed a diet consisting of fresh fruits and vegetables (romaine lettuce, carrots, yams, apples, grapes, etc.) shipped from Tortola, BVI supplemented with browse collected from the wild (e.g., *Portulaca oleracea*).

We selected 24 individuals for release in each of three consecutive years (2003–2005) based on mass and health status determined during annual health screenings according to the IUCN/ SSC Iguana Specialist Group reintroduction guidelines (Hudson 2000). Prior to release,

animals were fitted with internal or external transmitters. Fifty-six of the 72 released iguanas underwent surgery to implant radio-transmitters for post-release monitoring. The transmitters were surgically placed in the coelomic cavity of each animal prior to release (Ferral et.al. 2005, Goodman et al, 2009). Two transmitter models manufactured by Holohil Systems, Ltd. (Ontario, Canada) were used for implantation. Twenty-four animals were implanted with model SI-2, which weighed 9.3 grams and had a projected battery life of 12-14 months. Thirty-six animals were implanted with model AI-2T, which weighed 16 grams and had a projected battery life of 24-26 months. Sixteen of the larger animals (600-1000 grams) released in 2005 had an external transmitter (Holohils Systems LTD., Model RI-2CT) attached to the nuchal crest using plastic coated braided stainless-steel wire (Goodman et. al. 2009). All transmitters were less than 3% of the mass of the individual iguanas.

We released selected iguanas to the wild in October each year and radio-tracked individuals for up to two years. Each year we released 12 iguanas, 6 males and 6 females, matched by size, at both study sites (Middle Cay and Faulkner). The size range for mass of animals released in 2003, 2004, and 2005 were 750–2050 g, 600–1540 g, and 415–1055 g, respectively. For both study sites, animals were released at approximately the same location, time of day, and date each year.

Data collection.—We collected morphometric data upon capture as hatchlings, periodically while in captivity, upon release to the wild, after a 60 day post–release transition period, periodically while radio tracking (up to two years), and opportunistically after the transmitter batteries died. Approximately 79% of the study animals survived at least two years following release (Bradley, unpubl. data). Released iguanas were re–captured by hand, noose, or box trap. We measured SVL to the nearest mm by stretching animals along a Lufkin folding rule (Apex

Tool Group, Sparks, MD, USA), and body mass to the nearest g using the smallest capacity spring scale applicable (Pesola AG, Schindellegi, Switzerland) (Iverson et al. 2004). Individuals were sexed by cloacal probing (Dellinger and von Hagel 1990).

Statistical analysis.—Mass and SVL data for individual captive iguanas (n = 72; age 0–2192 d) were fit to broken–line models with breakpoints defined as 365, 730, and 1095 days (i.e., years) based on the range and appearance of the data and strength of fit (r–value) (Fig. 1). Hatchling iguanas were assumed to emerge on 15 October of their birth year if the exact emergence date was unknown. We assigned, hatchlings without collected morphometrics in the first 14 d of emergence (n=19), the median emergence mass and SVL of 49 g and 104 mm, respectively, to ensure a physiological fit of the models to the early data. The model for mass was constrained to an emergence mass >35 g (the minimum observed emergence mass) with the slopes of all lines constrained to >0 and increasing from 365 to 1095 d. Individual fits were assessed and only slopes defined by at least three data points within a designated age range or with two points spanning at least one–third of the age range were reported as $growth_{mass}$ or $growth_{SVL}$. For example, animals with data from 500 d to 1500 d of age would have growth rates reported for 365–730 d, 730–1095 d, and 1095–2200 d, but no growth rate for 0–365 d. When subjects had sufficient data within only one age range or two non-sequential age ranges (mass n=8, SVL n=18) we determined the growth rate for each age range independently by simple linear regression. We estimated hatch years for eleven iguanas from their earliest weight and known observations (n=4 Faulkner females, n=2 Middle Cay females, n=2 Faulkner males, n=3 Middle Cay males). All estimated mass and SVL curves for these animals fell within the range observed for animals with known hatch years.

The mass and SVL of free-ranging, headstarted iguanas were fit with a simple linear regression for all animals with at least two measurements spanning more than one year (mass n=42; SVL n=37; number of measurements per animal ranged from two to seven). Data points spanned 1095 to 5000 d of age. Initially a broken-line model with a breakpoint at 2200 d was applied, however, only one animal showed a SVL curve post release not well-described by linear regression and this may have been due to a single outlier point at 3900 d of age. Therefore, we used linear regression to describe growth rates of iguanas released after 1095 d of age (3 years). For individuals with measurements for 1095–2200 d of age in captivity and subsequently at release sites (mass n=26; SVL n=22) we compared captive and post release growth rates using the Wilcoxon matched-pairs signed rank test.

Body condition index (BCI) was calculated according to Romero and Wikelski (2001):

BCI= (masskg/〖SVLmm〗^3) × 〖10〗^6

Residuals were determined from the linear regression fit to log(mass) versus log(SVL) for captive iguanas (BCI residuals) (Fig. 2).

Non-parametric statistical tests were used to reduce the influence of bias in curve fits due to varying population sizes and distributions of measurements. We performed gender and location comparisons using Kruskal-Wallis rank ANOVA with Dunn's multiple comparison for captive age categories within gender, gender within age category or release site, and released animals >1100 d to captive animals 1095–2200 d. Results are reported as median (range) or median (interquartile range, IQR). Box-and-whisker plots are median, IQR and 10th–90th

percentiles. Significance was defined as $p < 0.05$. Statistics and graphing were performed using used GraphPad Prism Version 6.03.

RESULTS

Captive growth and BCI.—Mean mass at emergence was 49 g (range 37 to 60) and 104 mm (range 93 to 112) for SVL. Results for broken-line models are shown in Table 1. The broken-line model was able to fit ($r>0.95$) 56 of 72 mass curves and 41 of 60 SVL curves.

Captive $growth_{SVL}$ varied between age classes and over time. $Growth_{SVL}$ was lower ($p<0.05$) for iguanas over 730 d than for younger iguanas. For October 2002 to October 2003, $growth_{SVL}$ began to drop in the 1095–2200 d age class and was lowest (essentially zero) from October 2003–October 2004 for iguanas in >730 d age classes (Fig. 3A). For October 2004–October 2005, $growth_{SVL}$ began increasing again. There were no significant differences in $growth_{SVL}$ ($p>0.05$) between sexes across any age category. $Growth_{mass}$ varied with age class, gender, and time. Eleven iguanas (30% of females and 38% of males) had decreased $growth_{mass}$ after 1095 d as compared to 730–1095 d. There were no significant differences in $growth_{mass}$ ($p>0.05$) for different age classes of captive females, but $growth_{mass}$ was greater ($p<0.05$) for males at 730 d, than for 0–365 d. Female $growth_{mass}$ at 1095–2200 d was lower ($p<0.05$) than $growth_{mass}$ of males of the same age. $Growth_{mass}$ was lowest ($p<0.05$) in October 2004.

Results for BCI of captive Anegada iguanas are shown in Fig. 4C. Captive BCI varied significantly between age classes and over time. Median BCI was 45 (range 27 to 87) and was lower for iguanas <730 days compared to iguanas 730–2200 d and was lowest for captive iguanas from October 2001 through October 2003. There were no significant differences in BCI ($p>0.05$) between sexes across any age category. The interquartile range for $BCI_{residuals}$ of captive iguanas was –0.087 to 0.053, or from about 80% to 113% of expected mass.

Post–release growth and BCI.—Just prior to release, all iguanas used in the study (1095–2200 d) had similar $growth_{mass}$ and $growth_{SVL}$ (Mann–Whitney test; $growth_{mass}$ $p=0.41$ for females and $p=0.55$ for males, and $growth_{SVL}$ $p=0.92$). Results for linear regression of mass and SVL measurements for released iguanas are shown in Fig. 4B. Iguanas showed a strong linear fit ($r>0.844$) for $growth_{mass}$ (38 of 42 iguanas) and $growth_{SVL}$ (36 of 37 iguanas).

$Growth_{mass}$ in the first 60 d post-release exceeded that of all captive groups (Fig. 6A). Nineteen of 25 iguanas had higher $growth_{mass}$ during the first 60 d of release than either their captive $growth_{mass}$ or their subsequent $growth_{mass}$ in the wild (Fig. 6A). $Growth_{mass}$ during the first 60 d after release was not correlated ($p=0.45$) to $growth_{SVL}$. While mass increased rapidly, $growth_{SVL}$ was not different ($p>0.05$) from zero during the first 60 d after release. Body condition was not different ($p>0.05$) 60 d post-release except for males released at Middle Cay where BCI increased ($p<0.05$).

After >60 days post release, there was no difference ($p>0.05$) in the $growth_{mass}$ between males and females within either location. Middle Cay iguanas grew faster ($p<0.05$) (1.5 g/d, IQR 1.24–1.68) than iguanas at Faulkner (0.42 g/d, IQR 0.025–0.79). When compared to similarly aged iguanas (>1095 d) of the same sex in captivity, females at Middle Cay had a higher ($p<0.05$) $growth_{mass}$, and males at Faulkner had a lower ($p<0.05$) $growth_{mass}$. $Growth_{SVL}$ did not differ between captive iguanas >1095 d and those released at the Middle Cay or Faulkner release sites. However, iguanas released at Middle Cay grew more quickly ($p<0.05$) in SVL than iguanas released at the Faulkner site.

For individual iguanas with growth rates in captivity and following release at Middle Cay, female $growth_{mass}$ increased by 1.0 g/d ($n=3$, significance not possible due to low sample size but individual growth rates increased 46, 58, and 70%, respectively), males maintained their

$growth_{mass}$ (n=7, p=0.68) with the four individuals with the lowest $growth_{mass}$ increasing and the three individuals with highest $growth_{mass}$ decreasing, and $growth_{SVL}$ of both sexes (n=8) showing no change (p=0.55) post release (Fig. 6A). For individual iguanas with growth rates in captivity and following release at the Faulkner site, female $growth_{mass}$ decreased 0.32 g/d (n=9, p=0.012, 8/9 decreased), male $growth_{mass}$ tended to decrease 0.71 g/d (n=8, p=0.078, 7/8 decreased), and $growth_{SVL}$ of both sexes (n=14) decreased (p=0.020) post release (Fig. 6B).

All released iguanas had higher BCI scores ($p<0.05$) compared to previous captive BCI scores, but there were no differences in post-release BCI scores between release sites or sexes (Fig. 4C). The Middle Cay median BCI was 58 (range 47 to 84) and the Faulkner median BCI was 59 (range 43 to 79). BCI for released iguanas plotted against the captive regression had higher residuals for Middle Cay (+0.096; IQR +0.074 to +0.152) and Faulkner (+0.103; IQR +0.066 to +0.145). These higher ($p<0.05$) $BCI_{residuals}$ indicate that released iguanas were approximately 25% higher in body condition than the total captive population.

DISCUSSION

This study compared growth rates and BCI of headstarted and released iguanas to evaluate whether Anegada habitats can sustain reintroduced iguanas. Captive growth rates and BCI for headstarted *Cyclura pinguis* on Anegada were comparable to rates reported for other *Cyclura* species. Growth rates and BCI of released headstarted animals support the ability of both release sites and habitat types to maintain increased iguana populations.

Captive.—$Growth_{mass}$ of captive iguanas from emergence to three years of age (1095 days) was well described by linear regression (i.e., a constant rate of growth), as has been suggested by other studies (Garcia and Gerber 2016). In this study, rate of increase in body mass for males increased with age, which was not noted in previous studies (Perez–Buitrago et al. 2008; Garcia

and Gerber 2016) except for the Jamaican Rock Iguana which reported higher growth rates (mass and SVL) at three years of age (Wilson et al. 2004). Higher growth rates of older males in captivity may have been the result of competition as was observed by Iverson (1979) where males were better able to monopolize limited food resources or may reflect ontogenetic changes associated with sexual dimorphism as adults.

$Growth_{SVL}$ of captive juvenile iguanas in the present study (~5 mm/mo) was similar to that observed in captive Mona Island Rock Iguanas (4.7 mm/mo) (Perez–Bitrago et al. 2008), higher than observed for Jamaican Rock Iguanas (3.7 mm/mo) (Wilson et al. 2004) and wild Turks and Caicos Rock Iguanas (1.6 mm/mo) (Iverson 1979), but lower than wild juvenile Cuban Rock Iguanas up to 22 months of age (8.4 mm/mo) (Alberts et al. 2004), Little Cayman Rock Iguanas (8.3 mm/mo) (Gerber unpubl. data), or Green Iguanas from Belize (6.6 mm/mo) (Henderson 1974). Differences in growth rates may reflect species differences or environmental differences. Higher growth rates might indicate more optimal environments with excess energy availability.

Mougey (2016) reported lower growth rates for captive *C. pinguis* in the Anegada headstart facility for $growth_{mass}$ (5.06 g/mo); and $growth_{SVL}$ (2.41 mm/mo) in hatchlings, and $growth_{mass}$ (9.76 g/mo) and $growth_{SVL}$ (2.92 mm/mo) in yearlings. Growth differences between these studies may reflect differences in captive husbandry or the difference in the quantity of data between Mougey 2016 (data collected annually for approximately three years between 2012 and 2014) and the present study (data collected multiple times annually for ten years between 1997 and 2007).

Body condition index of captive *C. pinguis* was constant from ages 0–2 years. There was an increase in BCI at three years that was maintained in the fourth year of captivity. This might

indicate that younger animals were less able to maintain muscle and fat tissue in the captive environment, perhaps due to higher levels of competition or increased stress. However, this difference could also indicate a bias in the BCI calculation due to ontogenetic changes in body proportions as Anegada iguanas age (e.g., long thin juveniles versus heavy–bodied adults).

Within the study population, a significant drop in BCI was noted between 2001 and 2004 when the majority of the captive population was 3 or 4 years of age. The decrease preceded (and therefore may have prompted) a cessation of $growth_{SVL}$ and a more gradual decline in $growth_{mass}$. This response is suspected to reflect the higher population density in the captive facility in 2001 to 2003, before releases began freeing space and relieving population pressure in the facility. A similar drop in $growth_{SVL}$ was observed in captive Mona Island Rock Iguanas when the headstart facility became overcrowded (Perez–Buitrago et al. 2008). Age may also affect iguana sensitivity to overcrowding due to increased stress and competition (Perez–Buitrago et al. 2008). Based on these observations, we recommend releasing iguanas before three years of age. For a hatchling Anegada Rock Iguana (49 g; 100mm SVL) to attain a minimum release size of 400 g and 200 mm SVL in three years, captive animals need to gain 9.76 g/mo and 2.77 mm/mo. Such growth should be easily achievable with adequate food availability, low cage densities, and good and consistent husbandry.

Transition Period (60 days post-release).—$Growth_{SVL}$ did not change significantly during the first 60 days post release, which is not surprising given the short time interval and potential variation in measurement. However, $growth_{mass}$ increased significantly during the first 60 days post release and was influenced by the release site. Seventy–six percent of released animals exhibited higher $growth_{mass}$ in the first 60d post release than previously in captivity or subsequently in the wild. Iguanas released at Middle Cay achieved the highest 60d post release

growth rates observed. This acceleration in mass gain during the first 60 days post release differs from other *Cyclura* release programs. Only six released Anegada Rock Iguanas lost weight (5 of 28 iguanas at Faulkner and 1 of 25 iguanas at Middle Cay). In contrast, Jamaican Rock Iguanas were observed to lose an average of 1.69 g per day during the first 3.5 months post release (Lewis et al. 2008). Likewise, both Mona Island Rock Iguanas (n=62) and Grand Cayman Rock Iguanas (n=23) showed an initial but temporary decrease in mass just after release (Burton 2005, Perez–Buitrago et al. 2008). The authors attributed these initial losses to changes in diet, costs of carrying a radio–transmitter, and increased energetic costs of foraging. The high moisture content of captive iguana diets has also been theorized to potentiate weight loss post-release due to gut water loss/dehydration on low moisture wild forage (Hudson 2000). Greater surface water on the rock substrate at the Middle Cay release site compared to the Faulkner site may have allowed for better-hydrated and therefore heavier animals during the first 60 days post release. Conversely, higher fiber forage is known in other herbivorous species to promote significant growth of digestive organs and increased gut capacity (Piersma and Lindstrom 1997; Munn et al. 2009). Such adaptation to the wild diet by Anegada iguanas would be reflected in higher weights and retention of greater gut–fill, particularly water-holding fiber. Such changes might explain the rapid weight gains observed in iguanas in this study. Another factor that likely contributed to the positive weight pattern in the iguanas in this study was the timing of their release. Anegada Rock Iguanas were released in early October, in the middle of the rainy season when the greatest variety and quantities of fruit are available. Other headstart programs (Jamaica, and Grand Cayman) released iguanas in late November to early December, during the dry season (Burton 2005; Lewis et al. 2008). The abundance of readily digestible, calorically–dense fruit (Knapp 1999) could have provided a rich resource that prevented mass loss during the

transition from captive to wild. Differences in plant species and diversity, and fruit availability between Middle Cay and Faulkner (Clubbe et al. 2004) could also account for difference in $growth_{mass}$ between release sites.

Post transition period (>60 days).—After 60 d acclimation to the wild, rates of $growth_{mass}$ and $growth_{SVL}$ were dependent on site. $Growth_{mass}$ and $growth_{SVL}$ for both sexes at Middle Cay decreased after 60 d but were similar to the highest captive growth rates observed. At the Faulkner site, $growth_{mass}$ decreased for males and returned to a rate similar to that observed in captivity for females, but $growth_{SVL}$ for both sexes slowed below their captive $growth_{SVL}$. In the late 1990's it was noted that the largest wild Anegada Rock Iguanas persisted on the interior cays (Gerber, unpubl. data). However, it could not be determined if this was because these areas produced larger animals, because larger animals preferred this habitat and excluded smaller conspecifics, or both. The present study released cohorts of equal number, size, and genetics in two different habitats with similar densities of resident iguanas, strongly suggesting that habitat influences differences in body size. This difference may be directly correlated with the higher diversity of plant species on Middle Cay than at the Faulkner site (Clubbe et al. 2004) or a higher total availability of food resources. Populations of Chuckwalla (*Sauromalus obesus*) were also shown to grow faster in habitats with more diverse food resources than in less varied environments (Case 1976).

Iguanas released at both sites (Middle Cay and Faulkner) exhibited increased BCI values compared to their pre–release BCI. This may further support the hypothesis of increased gut capacity or other lean tissue (muscle) required for survival in the wild. Further and most interestingly, although iguanas released at the Faulkner site generally exhibited the slowest $growth_{mass}$ and $growth_{SVL}$ recorded during this study, these animals maintained elevated BCI

values compared to the captive population of similarly-aged animals, and similar to that of animals released on Middle Cay, which exhibited significantly faster growth. This implies iguanas are adapted to maintain a given mass to length ratio under a range of environmental conditions. There is presumably an evolutionary advantage to balancing rapid growth rates with a reserve of energy stores (Lauri and Brown 1990). Conservation of BCI in Anegada iguanas could help ensure long-term survival of individuals despite temporal and spatial variability in resource availability (Lauri and Brown 1990).

Habitat quality on Anegada.—It has been suggested that food resources for iguanas on Anegada are inadequate due to the impact of feral livestock on habitat quality (Lazell 1997; Mitchell 1999; Mitchell 2000; Perry 2007; Mougey 2016). While it is apparent that livestock are causing habitat degradation on Anegada, growth rates of the released headstarted iguanas in this study, particularly at Middle Cay, and the conservation of a high BCI by iguanas at both release sites, indicates that the habitats selected for release of headstarted iguanas on Anegada are capable of supporting increased densities of iguanas.

Compared to wild iguanas measured on Anegada over the past 50 years (Carey 1975; Mitchell 1999; Perry et al. 2007), captive iguanas in the present study displayed similar ($p>0.05$; Kruskal–Wallis) BCI values (medians 44, 34, 49, and 51, respectively) (Fig. 7). However, iguanas released at Middle Cay and the Faulkner site had considerably higher BCI ($p<0.05$; Kruskal–Wallis) with medians of 59 and 58, respectively. Data from Carey (1975) were collected in 1968 and may be biased for lower BCI compared to the current study due the larger size (476-545 mm SVL) and therefore presumably greater age of iguanas in Carey's sample. However, the data from 1989 (Mitchell 1999) presents a weight range of animals overlapping with the present study but much lower in BCI. This suggests the environment in 1989 was less

conducive to maintaining high BCI, conceivably due to the severe drought in the area during Mitchell's study (Esther Georges, pers. comm.).

Growth rates and mean body size of hatchlings, juveniles, and adults have been reported for the introduced *C. pinguis* populations on Guana and Necker Islands (Perry et al. 2007; Mougey 2016). Reported growth rates for both of these populations are greater than those reported here for captive or post-release iguanas on Anegada. However, Mougey (2016) observed lower BCI values for the Necker Island population than for the Guana Island population, despite the presence of elevated food resources on Necker. She attributed this to the high density of individuals around the resort's feeding stations and the heavily-irrigated landscape. It is common for individuals in translocated populations of *Cyclura* to exhibit faster growth rates, reach sexual maturity earlier, and exhibit larger body sizes than individuals in their respective source populations (Knapp 2001; Carter and Hayes 2004; Gerber 2007; Iverson et al. 2016; Hayes et al. 2016). Lower initial population density in translocated populations results in less intraspecific competitions among founders (Iverson et al. 2016) and may explain these observations. This phenomenon has been experimentally induced in the lizard, *Eumeces okadae*. Researchers recorded faster juvenile growth rates and younger ages at first reproduction following population manipulation to lower its density (Hasegawa 1997).

Mitchell (1999) and Perry (2007) concluded that differences in growth rates and body size of assorted iguana age classes on Guana, relative to Anegada, are the result of Guana having superior habitat. However, it appears that population density also exerts a strong effect on growth, even in less diverse habitats. Gerber (2007) reported that despite lower plant diversities on recipient islands, juveniles in multiple translocated populations of *Cyclura carinata* in the Turks and Caicos Islands (TCI) grew two to three times as fast and were reproductively mature

three to four years earlier than individuals in their respective source populations. Both the Middle Cay and Faulkner release sites are in areas with the highest density of wild adult iguanas on the island, so there may have been enough intraspecific competition to reduce the growth rates below those recorded for Guana and Necker. Furthermore, Iverson et al. (2016) documented the reduction in mean body mass for a translocated population of *Cyclura rileyi nuchalis* on Bush Hill Cay, Bahamas over a 10-year period and suggested it was due to the population approaching carrying capacity. As Bush Hill Cay (3.3 ha) is much smaller than Guana (340 ha) or Necker (30 ha) it may take longer for either of these island populations to reach carrying capacity. As they do, we expect these populations to experience corresponding reductions in individual growth rates and body size and become more similar to the Anegada population.

Conclusions.—The *Cyclura pinguis* headstart program is intended to bolster recruitment and increase the size of the wild adult population on Anegada until the causes of population decline (e.g., feral cats) can be mitigated. We suggest the following recommendations for the *C. pinguis* headstarting program on Anegada: 1) provide adequate space and food resources in captivity to achieve a minimum growth rate of 2.77 mm/mo, 2) release animals at 3 y of age or less to avoid overcrowding and stress, 3) release animals during the rainy (i.e., fruiting) season to ensure access to abundant food resources during this critical time, and 4) expand the use of release sites for headstarted animals to include habitats with low resident iguana densities as these may result in increased iguana growth rates and population expansion. In addition, the reproductive success of released iguanas on Anegada should be measured to quantify the value of headstarting in maintaining the last remaining native population of *C. pinguis*. Finally, a meta–population

approach integrating the management of all *C. pinguis* island populations in the BVI is needed to conserve the species and promote population health.

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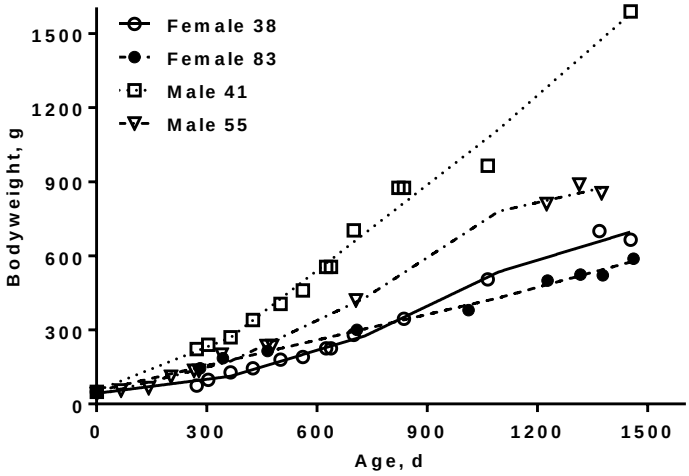

Fig. 1. Example of broken–line model fits to growth curves for four captive Anegada iguanas

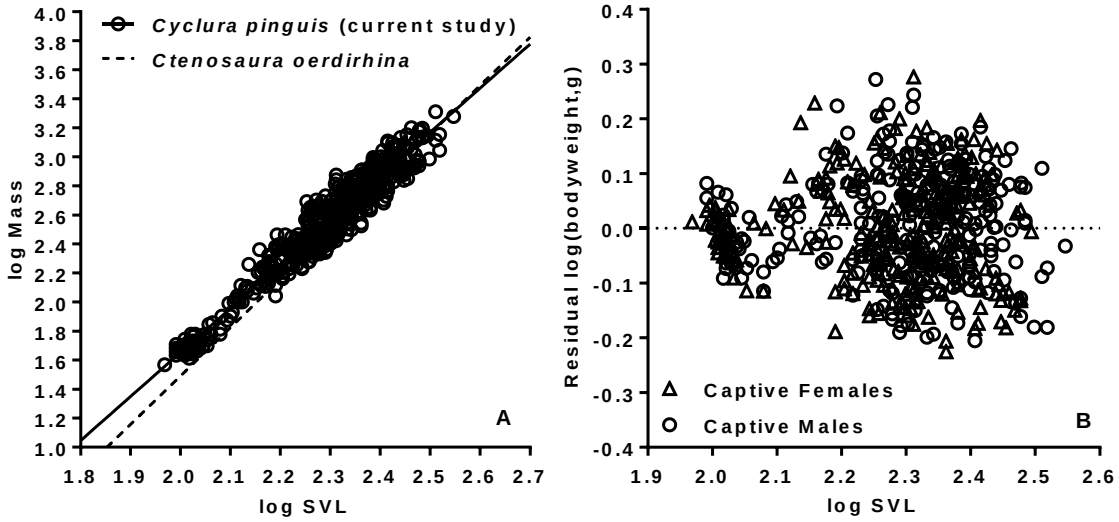

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Fig. 2. Log(mass) versus log(SVL) plot (A) of captive iguanas 0 to 2200 d, and corresponding Body Condition Index residuals (B). For (A) the regression for iguanas in the current study is shown and compared to the published regression for *Ctenosaura oerdirhina* (Pasachnik 2013)

Table 1. Growth rates and Body Condition Index (BCI) from broken-line models fit to 72 individual growth curves for headstarted iguanas in captivity at the Anegada headstart facility and following release at the Faulkner or Middle Cay sites. Superscripts[a,b] indicate a difference ($p<0.05$) in $growth_{mass}$, $growth_{SVL}$, or BCI for captive iguanas across age categories, within gender for $growth_{mass}$ and combined by gender for $growth_{SVL}$ and BCI. Superscripts[1,2] indicate a difference in $growth_{mass}$, $growth_{SVL}$, or BCI between captive iguanas greater than 1095 d and iguanas at each release site. Growth and BCI values are reported as median (interquartile range). Samples sizes (N) are reported combined by sex and for males (M) and females (F).

Age, d		**1 to 365 Captive**	**365 to 730 Captive**	**730 to 1095 Captive**	**1095 to 2200 Captive**	**>1100 Released Faulkner**	**>1100 Released Middle Cay**
Mass							
Growthmass, N	Total	29	34	44	47	23	19
	Sex Ratio	(13M/16F)	(15M/19F)	(22M/22F)	(22M/25F)	(10M/13F)	(12M/7F)
Growthmass Male g/d	Median	0.40^{a}	0.73^{ab}	0.72^{b}	$1.11^{b,1}$	0.352	1.571
	interquartile range	(0.33–0.43)	(0.47–0.89)	(0.57–0.96)	(0.89–1.49)	(0.26–0.72)	(1.24–1.87)
Growthmass Female g/d	Median	0.44	0.44	0.52	0.572	0.592	1.501
	interquartile range	(0.32–0.52)	(0.33–0.52)	(0.38–0.71)	(0.33–0.87)	(0.21–0.85)	(0.72–1.72)
SVL							
$Growth_{SVL}$, N	Total	22	27	44	42	21	16
	Sex Ratio	(9M/13F)	(14M/13F)	(21M/23F)	(18M/24F)	(9M/12F)	(9M/7F)
$Growth_{SVL}$, mm/mo	Median	5.7^{a}	4.4^{a}	2.5^{b}	$2.4^{b,12}$	1.02	3.01
	interquartile range	(4.9–6.6)	(3.4–5.8)	(-0.4–5.2)	(1.2–3.4)	(0.4–1.4)	(1.9–3.6)
BCI							
BCI, N	Total	130	92	158	197	64	78
	Sex Ratio	(72M/58F)	(52M/40F)	(77M/81F)	(89M/108F)	(31M/57F)	(40M/24F)
BCI, $(kg/cm^3)^6$	Median	42.8^{a}	39.9^{a}	47.4^{b}	$51.2^{b,1}$	59.22	58.22
	interquartile range	(39.5–47.3)	(34.9–46.6)	(40.2–55.5)	(41.5–58.2)	(54.7–65.0)	(55.4–64.7)

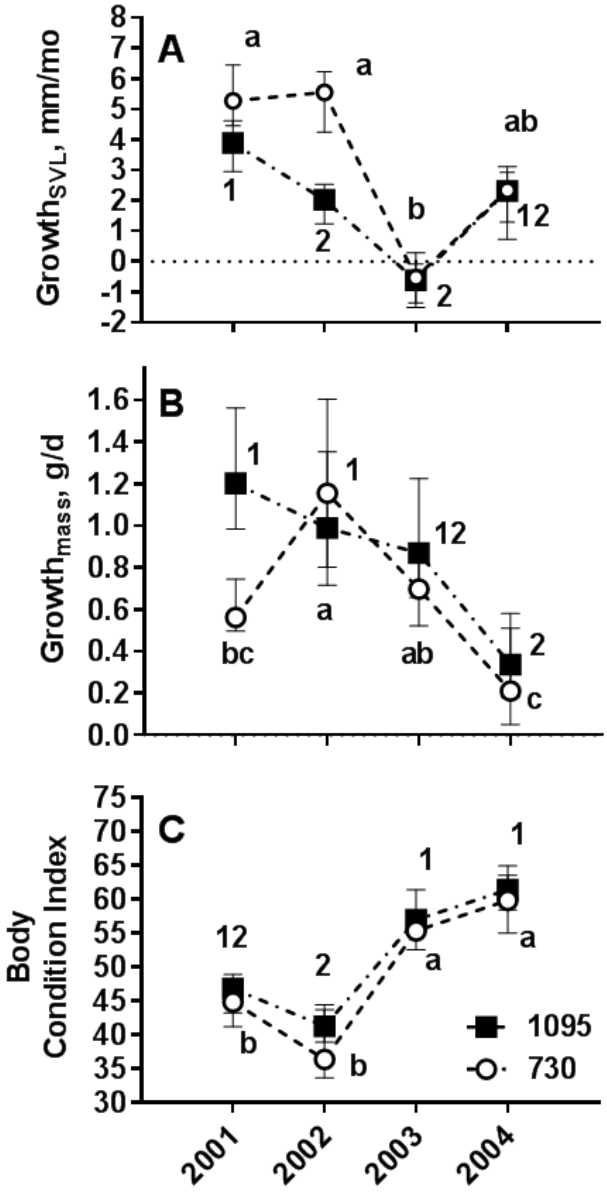

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Fig. 3. Growth$_{SVL}$(A), growth$_{mass}$ (B), and body condition index (BCI) (C) by year for captive headstarted iguanas 730–1095 d of age and captive headstarted iguanas 1095–2200 d of age. Bars are medians with interquartile range. Different letters (a, b) indicate a difference ($p<0.05$) between years for iguanas 730–1095 d of age. Different numbers (1, 2) indicate a difference ($p<0.05$) between years for iguanas >1095 d of age. Years are based on iguana age and begin in October and end in October of the following year.

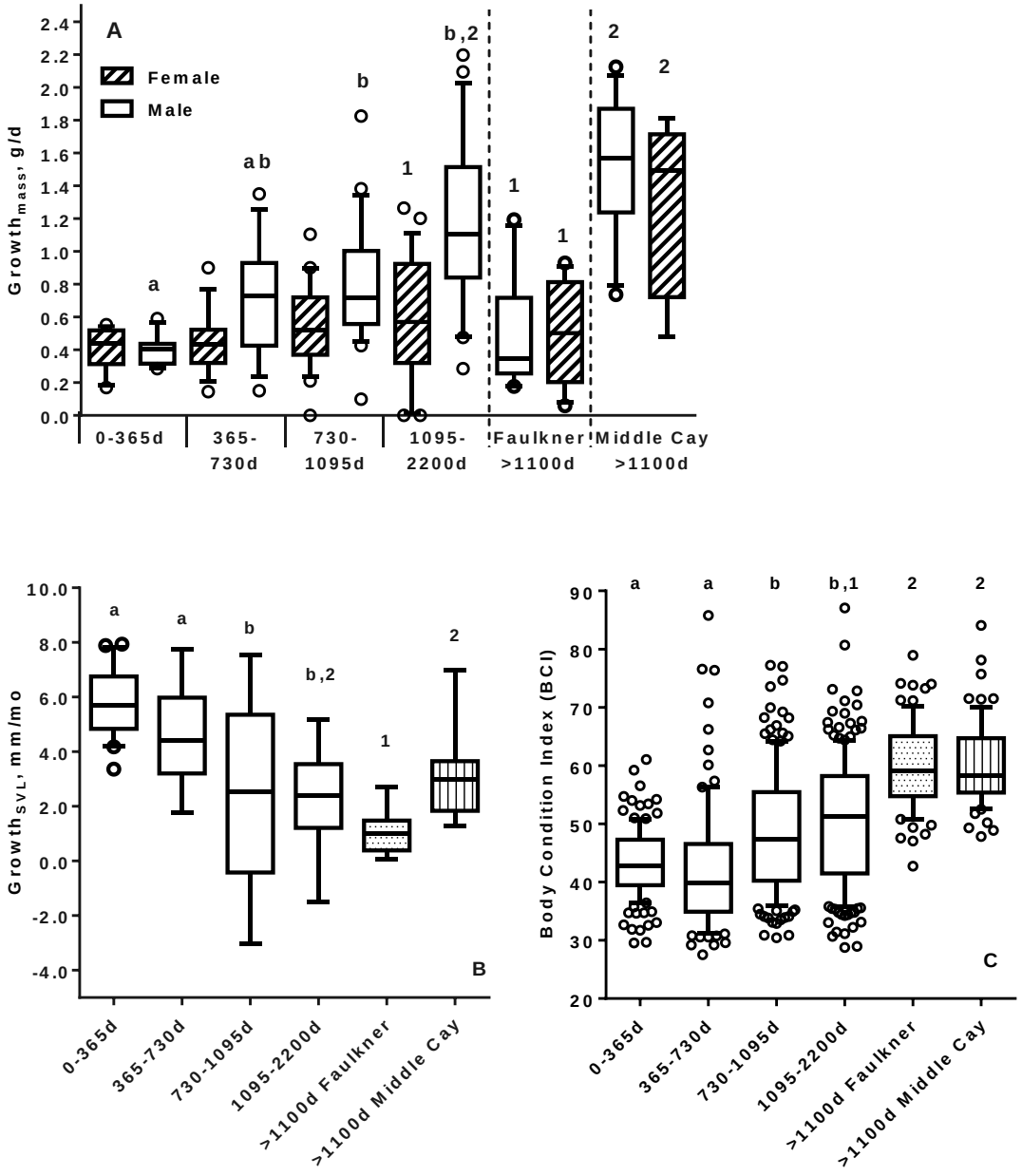

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Fig. 4. Growth$_{mass}$ (A), growth$_{SVL}$ (B), and body condition index (BCI)(C) of headstarted Anegada iguanas at different ages in captivity and after release at the Faulkner and Middle Cay sites. For graphs B and C, boxes for captive animals are white, stippled for Faulkner, and striped for Middle Cay. Different letters (a, b) indicate a difference ($p<0.05$) in growth$_{mass,}$ growth$_{SVL}$, or BCI for captive iguanas across age categories, within gender for growth$_{mass}$ and combined by

gender for $growth_{SVL}$ and BCI. Different numbers (1, 2) indicate a difference in $growth_{mass}$, $growth_{SVL}$, or BCI between captive iguanas greater than 1095 d and iguanas at each release site.

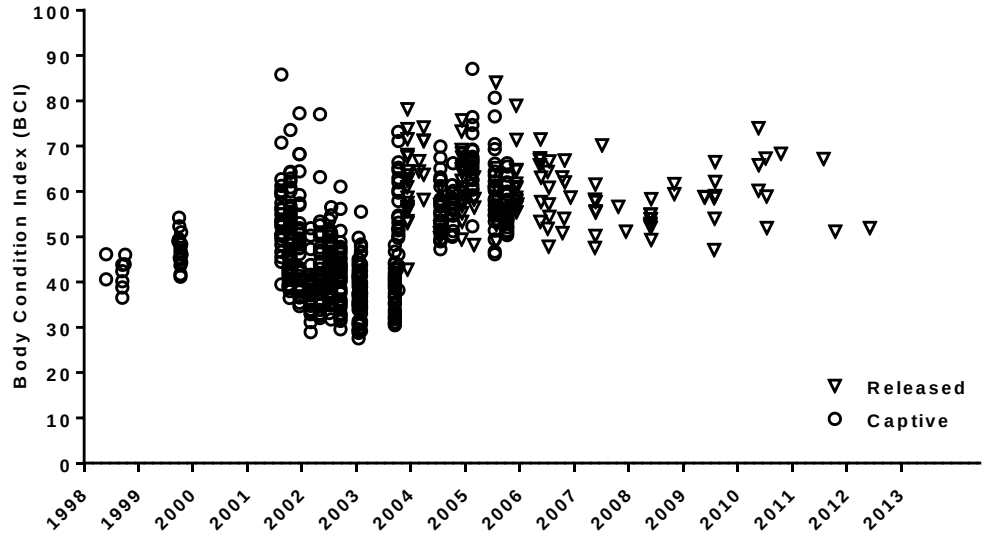

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Fig. 5. A chronological plot of Body Condition Index (BCI) for captive and released headstarted Anegada iguanas showing a drop in BCI for captive iguanas between 2001 and 2004. In comparison, released iguanas maintained a median BCI around 58 independent of release site.

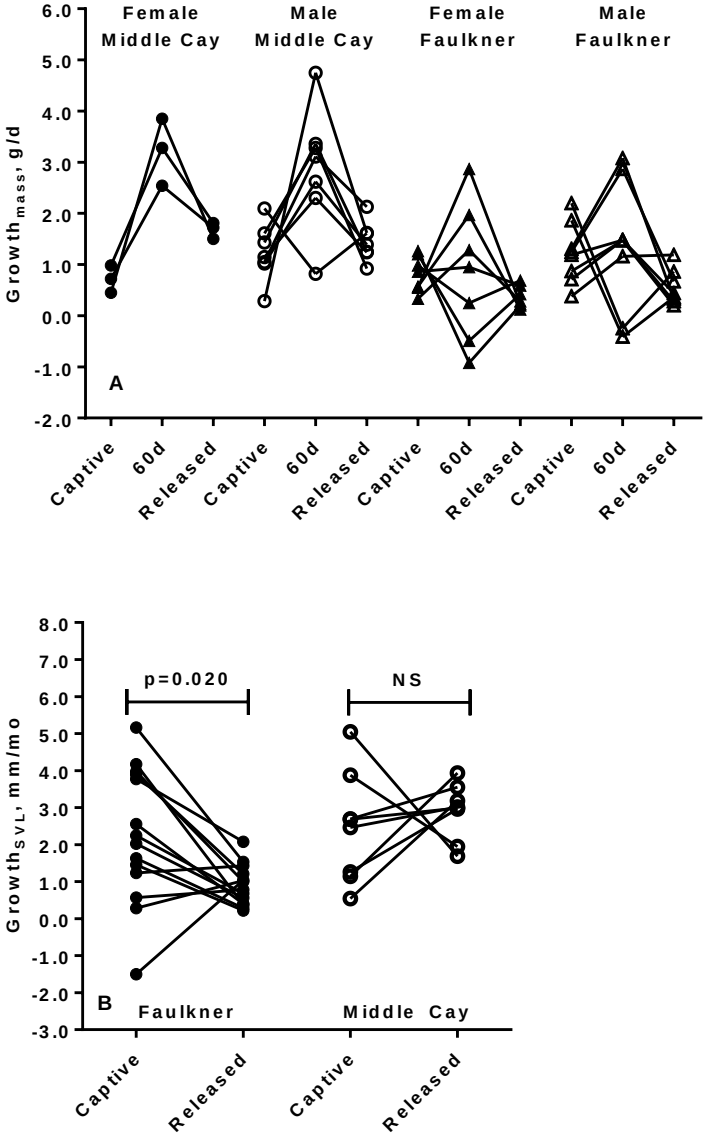

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Fig. 6. $Growth_{mass}$ (A) and $growth_{SVL}$ (B) of headstarted Anegada iguanas released at the Faulkner and Middle Cay sites while in captivity, 60 d after release ($Growth_{mass}$ only), and subsequently in the wild. There were no differences between genders for either release site.

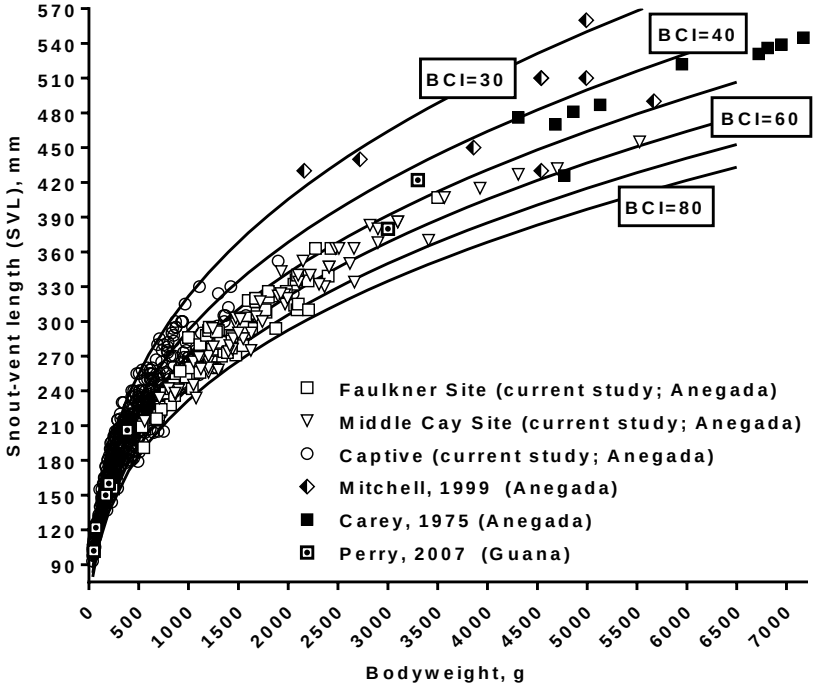

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Fig. 7. Plot of mass versus snout–vent length (SVL) for *C. pinguis* in the current study and previously published studies, with lines indicating body condition indexes (BCI) of 30 to 80 in increments of 10.

CHAPTER 3.

Comparison of Blood 25-hydroxy-vitamin D, Calcium, Phosphorous, Parathyroid Hormone, and Ionized Calcium Concentrations in Captive and Wild Anegada Iguana (*Cyclura pinguis*)

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ABSTRACT

The Anegada Rock Iguana (*Cyclura pinguis*) is a Critically Endangered species native to Anegada, British Virgin Islands and is the subject of headstart program meant to offset high juvenile mortality. Blood samples were collected from 28 captive and 32 wild iguanas across three seasons to assess vitamin D (25-OH-D3), ionized calcium, parathyroid hormone (PTH), total calcium, and phosphorus levels. Captive iguanas had statistically significant higher levels of 25-OH-D3 than wild iguanas. There was also a statistically significant effect of 25-OH-D3 levels across seasons. May 25-OH-D3 levels for captive and wild iguanas were significantly lower than July and October levels which were not significantly different from each other. Parathyroid hormone and ionized calcium levels were not statistically different across captive and wild iguanas nor was there a difference across seasons. There were no statistically significant differences for total calcium and phosphorous levels across captive and wild iguanas. But there were statistically significant differences in calcium and phosphorous levels based on season. May calcium and phosphorous levels for captive and wild iguanas were significantly higher than July and October levels which were not significantly different from each other. This is the first study to systematically compare captive and wild populations, and seasonal variations in 25-OH-D3 for the Anegada Rock Iguana. The PTH and ionized calcium values reported here are the first for a *Cyclura* species. These biochemistry values are important because they are involved in the process of maintaining critical calcium concentrations needed to prevent metabolic bone disease. This work contributes important baseline health information for the management of the Anegada Rock Iguana and other members of the genus *Cyclura*.

INTRODUCTION

Conservation physiology utilizes physiological tools to understand conservation problems, develop conservation strategies, and evaluate conservation actions. (Wikelski & Cooke 2006, Cooke et al. 2013, Madliger et al. 2018). Reintroduction programs that examine vital physiological parameters prior, during, and after releases experience higher rates of success (Tarszisz et al. 2014). Captive reptiles slated for reintroduction should be tested for common metabolic bone diseases by examining the biochemistry involved in calcium homeostasis (e.g., vitamin D, parathyroid hormone (PTH), ionized calcium, total calcium, and phosphorous) (Clark & Laverty 1985, Frye 1997, Ullrey et al. 1999).

Vitamin D_3 (D_3) synthesis and calcium absorption are essential biological processes in all lizards so far studied (Allen et al. 1994, Webb & Holick 1998, Mader 2006). Most basking lizards, including iguanas, are thought to require exposure to ultraviolet (UV) light in order for these processes to occur normally (Bernard 1991, Allen et al. 1994, Allen et al. 1995, Gehrmann 1997, Ullrey et al. 1999). Ultraviolet B (UVB) from 290 - 315 nm triggers the cutaneous conversion of 7-dehydrocholesterol to previtamin D_3, which is then thermally isomerized to D_3 (Webb & Holick 1988, Bernard et al. 1997). After entering the blood, D_3 is metabolized by the liver to 25-OH-D3 (calcidiol), which is then further hydroxylated in the kidneys to 1,25-(OH)2-D3, the active form that promotes the absorption of calcium and regulates calcium and phosphorus homeostasis. Blood concentrations of 25-OH-D3 are considered the best indicator of vitamin D status in vertebrates, including iguanas (Haddad 1999, Holick 2004, 2007).

Parathyroid hormone is an important endocrine regulator of calcium and phosphorus concentrations, serving to maintain adequate concentrations of calcium by mobilizing calcium from bone, enhancing absorption of calcium from the small intestine, and suppressing the excretion of calcium by the kidneys (Clark & Laverty 1985, Mader 2006, Rivera & Lock 2008). Parathyroid hormone also enhances both the excretion of phosphate by the kidneys and its uptake by the cells, allowing more calcium to remain in solution rather than binding to available phosphate (Clark & Laverty 1985, Mader 2006, Rivera & Lock 2008). Documenting PTH concentrations is important in the understanding of the relationship between calcium, phosphorus, and D_3 in iguanas. Total serum calcium is composed of ionized calcium, the physiologically active form, plus calcium bound to albumin and other ligands. In healthy animals ionized calcium homeostasis is tightly controlled and may be a more accurate indicator of current calcium homeostasis (Dennis et al. 2001, Adkesson & Langan 2007, Stringer et al. 2010).

Calcium and phosphorus homeostasis is necessary to prevent diseases such as rickets, ostemalacia, and metabolic bone disease (Narbaitz and Tsang, 1989, Bernard et al. 1997, Frye 1997, Holick, 1999). Nutritional secondary hyperparathyroidism is the most common form of metabolic bone disease diagnosed in captive reptiles, especially herbivorous species such as iguanas (Mader 2006). It often occurs in captive iguanas who are fed diets low in calcium and high in phosphorus, accompanied by deficiencies in D_3 (Jacobson 1981, Frye 1997). In healthy reptile species, normal blood values for calcium and phosphorous typically occur in a ratio of 2:1 Ca:P (Frye 1997, Jacobson 2003, Mader 2006, Stringer et al. 2010). Captive Iguanas in reintroduction programs

must be monitored continually for signs of nutritional metabolic bone disease (Boyer 1996, Mader 2006).

The Anegada Rock Iguana (*Cyclura pinguis*) is among the most Critically Endangered and genetically unique of the West Indian Iguanas (Alberts 2000, Bradley & Grant, 2020). The only extant natural population of this species is found on Anegada, British Virgin Islands. Habitat loss is a major threat to the iguana's survival. However, the primary threat is feral cats (*Felis catus*) that depredate hatchlings within months of emergence, drastically reducing recruitment (Gerber 2000, Bradley unpublished data). By the 1990s, the population had declined by 80% making it one of the most endangered lizards in the world (Carrey 1975, Mitchell 1999, Perry & Gerber 2006). A headstart program was initiated in 1997 to combat this threat and bolster the wild population. Each fall newly emerged hatchlings are transferred to an on-island captive facility. The iguanas are reared in this protected environment and released back to the wild when they reach a larger, less vulnerable size, up to six years later.

Headstarting has been criticized for not determining if captivity negatively affects the health of the animals involved in the program (Mathews et al. 2006). Indeed, the Anegada headstart facility utilizes ¼" hardware cloth in the enclosures, which has been shown to reduce the amount of UVB irradiance transmitted and vitamin synthesis potential by 22% (Burger et al. 2007, Lung 2012). However, it is unclear if this lower UVB exposure results in significant differences in 25-OH-D3. A variety blood chemistry values, including of 25-OH-D3, calcium, and phosphorous have been reported for Rock Iguanas, genus *Cyclura* (Alberts et al. 1998, Ramer et al. 2005, James et al. 2006, Maria et al. 2007, Lung 2012, Kishbaugh et al. 2020, Rainwater et al. 2020). However, it

is unknown if seasonal levels of UVB irradiances impact 25-OH-D3 blood values. Ionized calcium levels have been reported for Common Green Iguanas (Dennis et al 2001), but there are no published reports for ionized calcium or PTH for Rock Iguanas. An understanding of the baseline health values is critical to validate the health of reintroduced individuals.

The goal of this study was to assess the health of captive iguanas and to expand the current knowledge of vitamin D and calcium metabolism for Anegada Rock Iguanas. A multi-seasonal investigation was conducted to assess vitamin D (25-OH-D3), PTH, ionized calcium, total calcium, and phosphorus levels in Anegada Rock Iguanas across wild and captive individuals. The results from this study will provide important information to guide health management actions for the headstart program.

MATERIALS AND METHODS

Study Site

Anegada is the second largest island (39 km^2) in the British Virgin Islands (BVI) and is located in the NE corner of the Puerto Rican Bank at 18°43’ N, 64°19’ W (The Settlement, Anegada). The island is composed entirely of sedimentary substrates (limestone and sand) and reaches only 8 m asl. Anegada hosts tropical dry forest, large mangrove forests, coastal shrubland, and dry salt flats, supporting a high number of endemic species. As such it is considered part of the Caribbean biodiversity hotspot and has been designated as a Tropical Important Plant Area (TIPAs) because it supports globally important concentrations of threatened plants (Sanchez et.al. 2021). The iguana’s distribution has been reduced to a small core area (3 km^2) located in the NW

part of the island. The headstart facility is located in The Settlement, on the SE part of the island.

Study Animals

Samples were collected from 60 Anegada Rock Iguanas, of which 28 were captive and 32 were wild. Blood samples were collected from each group (i.e., captive, wild) during three timeframes in May, July, and October, 2006. Seven captive iguanas were sampled twice, once in May and July. Two wild iguanas were sampled twice, once in May and July. All the remaining animals were only sampled once. Upon visual inspection, all study animals appeared in good health at the time they were sampled.

The 28 captive iguanas were collected as hatchlings and were being reared in the headstart facility on Anegada as part of the headstart program. The iguanas were housed in groups of 5-20 similarly sized animals in large outdoor enclosures (2.89 L x 3.81 W x 2.43 H m) consisting of a wood frame covered with 0.25” x 0.25” black PVC coated wire mesh. The age of the captive animals ranged from 3-5 years, and their mass ranged from 300-500 g. The precise sex of the smaller captive iguanas could not be determined consistently, therefore sex was not included in any analyses. Captive iguanas were fed a diet consisting of fresh fruits and vegetables (romaine lettuce, carrots, yams, apples, grapes, etc.) shipped from Tortola, BVI supplemented with browse collected from the wild (e.g., *Portulaca oleracea*). The captive animals did not receive any vitamin or mineral supplementation during the sampling period.

Thirty-two wild iguanas were sampled for comparison to the captive population. Three animals were wild adults. Twenty-nine individuals from were subadults who had previously resided in the captive headstart facility but had been living in the wild 1-3

years at the time they were sampled. The ages of the three wild adults were unknow, but they were large, fully mature adults whose mass ranged from 5.46-6.03 kg. The age of the sub-adult released iguanas ranged from 6-10 years, and their mass ranged 1.32-4.5 kg.

Blood Collection and Analysis

Whole blood (3.0 ml if possible, but no more than 1 ml per 100 g of body weight) was collected from the ventral coccygeal vein. Nonheparinized blood collection tubes were filled with blood to harvest serum for determination of 25-OH-D3, ionized calcium, and PTH concentrations. The remaining blood was placed in lithium heparin tubes to harvest plasma for chemistry analysis, including total calcium and phosphorous. The blood samples were placed on ice in a small cooler and centrifuged within four hours of collection to separate the serum or plasma from the red blood cells. The serum and plasma samples were transferred to pop-top tubes and frozen until they were exported to the USA, where they were temporarily stored at -70 C° until sent for further analysis.

Blood serum samples were divided. One portion was sent to the Vitamin D, Skin, and Bone Research Laboratory at Boston University School of Medicine (Boston, MA 02118, USA) for the determination of 25-OH-D3 concentrations (Chen et al. 1990); the remainder were sent to Michigan State University Endocrinology Laboratory (Lansing, MI 48909, USA) for the determination of PTH and ionized calcium concentrations. Blood plasma samples were sent to Antech Diagnostics (Irvine, CA 92614, USA) for determination of blood chemistry concentrations of total calcium, phosphorus, uric acid, total protein, SGOT, CPK, glucose, and cholesterol. This study was approved by the Institutional Animal Care and Use Committee of the Dallas Zoo.

Statistical Analysis

A two-way analysis of variance (*ANOVA*) was used to test for differences in 25-OH-D3 levels between captive and wild iguanas across seasons, as well as their interaction, using mass (log-transformed) as a covariate. Pairwise multiple comparisons (Holm-Sidak method) were used to determine difference between seasons. A regression analysis of 25-OH-D3 vs mass was completed. These tests were performed in SYSTAT version 3.5 (Systat Inc., San Jose, CA, USA).

The results for ionized calcium, PTH, total calcium, and phosphorus did not pass tests for normality or equal variance, so between group comparisons were performed with the non-parametric Kruskal-Wallis one-way analysis of variance on ranks. Nonparametric pairwise multiple comparisons (Wilcoxon method) were conducted to determine the differences between seasons. Mean and standard error were calculated for concentrations of uric acid, total protein, SGOT, CPK, glucose, and cholesterol. These analyses were performed in JMP version 16 (SAS Institute, Cary, NC, USA). A standard alpha of $P = 0.05$ was used for all statistical tests.

RESULTS

Table 1. shows the complete list of mean analyte levels for captive and wild Anegada Rock Iguanas. Sample sizes of the individual analytes vary because it was not always possible to collect enough blood to run all the tests. In such cases, the samples were prioritized for 25-OH-D3 first, calcium and phosphorous second (plus the remaining of the blood panel), any remaining blood was used for the PTH and ionized calcium. Antech Diagnostis includes tests for Cholesterol, glucose, CPK, SGOT, total protein, and uric acid as part of their standard plasma panel that was used to measured

total calcium and phosphorous. Though not the primary focus of this study, the results are important baseline health data, and this is the first multi-seasonal data set comparing captive and wild iguanas Anegada Rock Iguana for these analytes. The results were included in Table 1. so that they are available for future studies.

Captive iguanas had significantly higher levels of 25-OH-D3 than wild iguanas ($F(2,53 = 13.26$, $P = 0.001$) when adjusted for body size. There was also a statistically significant effect of 25-OH-D3 levels across seasons ($F(2,53 = 8.99$, $P < 0.001$) when adjusted for body size, however the interaction of these variables was not significant ($F(2,53 = 0.263$, $P = 0.770$). Iguana mass was not a significant effect ($F(1,53 = 2.90$, $P = 0.094$).

Pairwise multiple comparisons (Holm-Sidak method) were conducted to determine the difference in 25-OH-D3 levels between months. May levels were significantly lower than July and October levels which were not significantly different from each other (May vs July, $P = 0.011$; May vs October, $P < 0.001$; July vs October, $P = 0.108$). The result of the regression analysis of 25-OH-D3 and mass demonstrated that smaller animals had significantly higher levels of 25-OH-D3 (($F(1,58) = 8.715$, $p <.005$). However, the effect was too weak ($R^2 = 0.116$) to account for the other factor effects, as shown by the *ANOVA* with size as a covariate.

Nonparametric Kruskal-Wallis Tests were conducted to examine differences in PTH, ionized calcium, total calcium, and phosphorus levels between captive and wild Anegada Rock Iguanas. No significant differences between groups were found for these analytes (see Table 2.), so the groups were combined, and a second set of Kruskal-Wallis Tests were conducted to examine differences in PTH, ionized calcium, total

calcium, and phosphorus levels across seasons. Season was not a significant effect for PTH ($H(2) = 0.479$, $P = 0.786$) or ionized calcium ($H(2) = 0.450$, $P = 0.798$). But season was a significant effect for total calcium ($H(2) = 13.489$, $P = 0.001$), and phosphorous ($H(2) = 15.375$, $P < 0.001$).

Nonparametric pairwise multiple comparisons (Wilcoxon method) were conducted to determine the differences in total calcium and phosphorous levels across seasons. May total calcium levels were significantly higher than July and October which were not significantly different from each other (May vs July, $P = 0.039$; May vs October, $P = 0.001$; July vs October, $P = 0.414$). Likewise, May phosphorous levels were significantly higher than July and October which were not significantly different from each other (May vs July, $P = 0.023$; May vs October, $P < 0.001$; July vs October, $P = 0.173$).

DISCUSSION

Baseline blood chemistry values are important because they can be used to evaluate the health status of iguanas involved in conservation programs. Although many studies have reported baseline blood chemistry values, including of 25-OH-D3, calcium, and phosphorous for other *Cyclura* species (see Table 3.)(Alberts et al., 1998, Ramer et al. 2005, James et al. 2006, Lung 2012, Kishbaugh et al. 2020, Rainwater et al. 2020) none have systematically compared captive and wild populations, or seasonal variations in 25-OH-D3, or reported PTH and ionized calcium. These values are particularly important because they are involved in the process of maintaining critical calcium concentrations needed to prevent metabolic bone disease (Mader 2006). Herein we evaluate the health of the captive iguanas in the Anegada headstart program by

comparing them to iguanas in the wild to better understand the impact of headstarting on *Cyclura* iguanas. Captive animals had significantly higher values of 25-OH-D3 compared to wild animals and a season effect was demonstrated. Captivity did not have a significant impact on PTH, ionized calcium, total calcium, nor phosphorous, however there were seasonal differences in total calcium and phosphorus.

Previous studies have demonstrated that enclosures reduce the amount of available UVB irradiances (Burger et al. 2007, Lung 2012), however our results suggest this was not the case. One explanation is that dietary differences are the primary source of variation in 25-OH-D3 levels. Though many vertebrate species can obtain and utilize vitamin D through dietary means (Allen et al., 1995), there appears to be some variation in the importance of dietary versus UVB exposure as the source for vitamin D among different species. Several mammal species (e.g., cats, dogs, and polar bears) appear unable to produce vitamin D from UVB exposure, but their diets naturally have high levels of vitamin D (Howard et al.1994, Kenny 1999). However, many diurnal basking reptiles, with diets low in vitamin D, appear to rely on UVB exposure to maintain vitamin D levels. There is strong evidence that Common Green Iguanas are more dependent on UVB exposure for vitamin D than dietary sources (Bernard 1991, Allen et al. 1994, Allen et al. 1995, Gehrmann 1997, Ullrey et al. 1999, Ward & Dempsey 2006).

Typically, the iguanas in the facility are fed the same diet, so a consistent dietary source would not account for the seasonal changes in 25-OH-D3 levels observed in this study. An alternative possibility is that there is limited shade in the cages of the headstart facility, therefore the animals are forced to bask more. Wild animals may be

spending more time in the shade to forage in areas of thick vegetation, or perhaps they are under pressure to stay hidden because of territorial disputes with neighbors.

Several lizard species, including Hispaniolan Rhinoceros Iguanas (*Cyclura cornuta*), have been shown to regulate their vitamin D- status behaviorally by self-regulating their exposure to UVB (Ferguson et al. 2003, Karsten et al. 2009, Ferguson et al. 2013, 2015). Free-ranging iguanas would have ample opportunity to control their UVB exposure. The enclosures at the headstart facility may not have provide a suitable UVB gradient with enough UVB refuges. The captive animals were housed in groups of 5-20 animals. The dominate animals in the cage may have limited the opportunity for subordinate individuals to self-regulate UVB exposure by controlling access to UVB refuge. Analysis of all study animals (captive and wild) indicated smaller animals in this study had significantly higher levels of 25-OH-D3. Therefore, it may be that young animals require higher levels of 25-OH-D3 to maintain health and support growth. Further analysis of age-related differences in 25-OH-D3 levels could shed light on 25-OH-D3 requirements of different age classes.

The 25-OH-D3 results of this study are lower than what Lung (2012) reported for captive and wild Anegada iguanas in 2002-2003 (see Table 3). The values reported by Lung (2012) are the highest published 25-OH-D3 levels (319 ± 95.6 ng/ml) for any *Cyclura* species. This sampling period was before the first iguanas had been released from the facility so the number of animals per cage was at its highest. It may be that a greater number of individuals were unable to self-regulate their UVB exposure due to dominate cage mates. The wild 25-OH-D3 levels reported by Lung were similar to the

values recorded in July and October by this study, indicating they were most likely collected in the same season.

The findings of this study contradict what Ramer et al. (2005) found for 25-OH-D3 in Hispaniolan Rhinoceros Iguana, *Cyclura cornuta*, which showed no significant difference between captive and wild iguanas (P = 0.7). The wild and captive iguanas Ramer sampled both consisted of adults, not different age classes as in this study. This also supports the idea that younger animals naturally have higher levels. The mean 25-OH-D3 for wild Anegada Rock Iguanas was lower than wild Ricord's Iguana (*Cyclura ricordii*), wild Hispaniolan Rhinoceros Iguana (Ramer et al. 2005), and wild Jamaican Iguanas (*Cyclura collie*) (Lung 2012) but the captive Anegada Rock Iguanas were higher than the captive Rhino Iguanas and Jamaican Iguanas (see Table 3). These results may reflect physiologic or dietary differences between species, or that they were sampled in different seasons.

The change in 25-OH-D3 levels across the three-sampling period was comparable in both captive and wild Anegada Rock Iguanas. May had the lowest levels, but it is the coolest of the three months. Though July and October levels were not significantly different from each other, October was the hottest month sampled, and it had the highest values. Ramer et al. (2005) observed contrasting results for wild Ricord's Rock Iguana from the Dominican Republic (DR), which had the highest levels in February, the coolest month sampled in this study. Ramer suggests that iguanas are forced to bask more in order to achieve an optimal operating temperature. Though there are not many true deciduous trees in the DR, trees do leaf out and become fuller during the rainy season and could possibly reduce available UVB. The dry season for the DR

runs December through April. Ramer's February samples were collected three months into an average dry season. The higher levels may be a result of diminishing shade as trees adapt to dry conditions over time. These contrasting results illustrate there are differences across systems, and it is vital to consider the unique factors in individual systems. For example, the rainy season on Anegada starts in late August and continues to the end of October. Perhaps the high values for the October samples occurred because Anegada Rock Iguanas must bask more to counter the cooling effects of rain. Sampling more individuals and time periods throughout the year could identify an annual cycle of 25-OH-D3 levels for different *Cyclura* species.

There were no significant differences between group (e.g., captive, wild) or season for either PTH or ionized calcium. Parathyroid hormone values have been reported in the literature for very few species, but there are records for ostrich, parrots, and penguins (Yagil et al. 1987, Howard et al. 2004, Adkesson & Langan 2007). Stringer et al. (2010) recorded PTH levels in green sea turtles, *Chelonia mydas*, and compared PTH values of healthy wild turtles to sick/injured turtles undergoing rehabilitation at a captive rescue center. The Anegada Rock Iguana PTH values (1.29 nmol/L) were in between the values of the healthy (0.75 nmol/L) and sick (2.95 nmol/L) green sea turtles. Normal ionized calcium values have not been reported in the literature for any species of *Cyclura*. However, the ionized calcium levels reported for captive Common Green Iguanas (*Iguana iguana*) are very similar to what was observed in captive Anegada Rock Iguanas (*C. pinguis* = 1.53 mmol/L, I. iguana = 1.47 mmol/L) (Dennis et al, 2001). The values presented here for PTH and ionized calcium serve as references for Anegada Rock Iguanas and other species of *Cyclura*. These values are

important because both PTH and ionized calcium are tightly controlled, variations in this test result would suggest a calcium metabolism health issue for the animal sampled (Dennis et al, 2001, Adkesson & Langan 2007).

There were no significant differences in total calcium and phosphorous levels between captive and wild Anegada Rock Iguanas and the values observed in this study were similar to those recorded for other species of *Cyclura* (see table 4). But there was a significant difference between seasons, with both analytes higher in May than in July or October. May is the breeding season for Anegada Rock Iguanas (Gerber 2000, Bradley unpublished data). Calcium homeostasis is tightly controlled in iguana species (Dennis et al. 2001), but the reproductive season has been shown to increase total calcium, phosphorous, and cholesterol levels in female Common Green Iguanas due to vitellogenesis (Jacobson 2003, Knotkova et al. 2005). This explanation makes sense for the wild iguanas, but the captive animals did not appear to have reached sexual maturity at the time they were sampled. Perhaps females begin to cycle calcium levels prior to reaching full sexual maturity, or maybe the captive diet had been altered in a way that provided more calcium. Calcium and phosphorous metabolism are intricately related to each other. In healthy reptile species, normal blood values for calcium and phosphorous typically occur in a ratio of 2:1 Ca:P (Frye 1997, Jacobson 2003, Mader 2006, Stringer et al. 2010), so it is not unexpected that phosphorous increased when calcium levels rose as observed in this study.

This study is the first to investigate the seasonality of 25-OH-D3 levels in the Anegada Rock Iguana, and the first to report PTH and ionized calcium levels for a *Cyclura* species. Knowledge of baseline biochemistry values can be used to evaluate

husbandry practices and assess health status of both individuals and populations. Though higher 25-OH-D3 levels were recorded for captive Anegada Rock Iguanas than for wild animals, the captive animals are healthy and receiving proper nutrition and UVB exposure. Given there was minimal differences for the remaining analytes, the captive animals appear to be on target with healthy wild iguanas. This study determined that captivity for the first 3-6 years of age does not negatively impact the health of headstarted iguanas, and the program is producing healthy individuals for release to the wild. Headstarting the Anegada Rock Iguana and other members of the genus Cyclura is a valid method to augment wild population numbers and manage the recovery of this threatened species.

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Table 1. Mean and standard errror for all analytes sampled for wild and captive Anegda Rock Iguanas across three seasons.

Test	Captive			Wild		
	May	**July**	**Oct**	**May**	**July**	**Oct**
	(n) Mean ± SE	***(n)*** Mean ± SE	***(n)*** Mean ± SE	***(n)*** Mean ± SE	***(n)*** Mean ± SE	***(n)*** Mean ± SE
25 Hydroxy vitanin D (*ng/ml*)	(**7**) 130.43 ± 26.68	(**11**) 198.45 ± 21.28	(**10**) 239.30 ± 22.32	**12**) 81.33 ± 20.37	(**10**) 132.90 ± 22.31	(**10**) 164.20 ± 22.31
Parathyroid Hormone (*pmol/L)*	(**2**) 1.15 ± 0.18	(**6**) 1.43 ± 0.10	(**8**) 1.28 ± 0.09	(**9**) 1.37 ± 0.08	(**6**) 1.10 ± 0.10	(**5**) 1.23 ± 0.12
Ionized Calcium (*mg/dL*)	(**2**) 1.56 ± 0.12	(**5**) 1.55 ± 0.08	(**8**) 1.48 ± 0.062	(**7**) 1.50 ± 0.07	(**4**) 1.43 ± 0.09	(**6**) 1.68 ± 0.07
Total Calcium (*mg/dl*)	(**5**) 12.70 ±1.34	(**10**) 12.39 ± 0.95	(**10**) 11.47 ± 0.95	(**12**) 15.55 ± 0.87	(**11**) 11.35 ± 0.90	(**10**) 11.24 ± 0.95
Phosphorous (*mg/dL*)	(**5**) 6.64 ± 0.57	(**10**) 5.21 ± 0.40	(**10**) 5.32 ± 0.40	(**12**) 6.24 ± 0.37	(**11**) 4.84 ± 0.38	(**10**) 2.94 ± 0.40
Uric Acid (*mg/dL*)	(**5**) 3.78 ± 0.74	(**10**) 5.00 ± 0.52	(**10**) 4.08 ± 0.55	(**12**) 2.27 ± 0.48	(**11**) 3.71 ± 0.50	(**10**) 3.82 ± 0.52
Total Protein (mg/dL)	(**5**) 6.86 ± 0.36	(**10**) 7.17 ± 0.26	**10**) 5.98 ± 0.27	(**12**) 6.08 ± 0.23	(**11**) 5.66 0.24	(**10**) 5.45 ± 0.26
SGOT (mg/dL)	(**5**) 16.80 ± 5.34	(**10**) 13.90 ± 3.84	(**10**) 22.89 ± 4.05	(**12**) 17.42 ± 3.51	(**11**) 13.73 ± 3.66	(**10**) 16.80 ± 3.84
CPK (mg/dL)	(**5**) 954.20 ± 2406.57	(**10**) 1692 ± 1707.70	(**10**) 8535.67 ± 1793.75	(**12**) 3808.58 ±1553.43	(**11**) 945.09 ±1622.51	(**10**) 2915.70 ± 1701.70
Glucose (mg/dL)	(**5**) 211.80 ± 34.76	(**10**) 219.40 ± 24.58	(**10**) 206.00 ± 25.91	(**12**) 154.50 ± 22.44	(**11**) 206.00 ± 23.44	(**10**) 297.70 ± 24.58
Cholesterol	(**5**) 88.60 ± 11.33	(**10**) 49.50 ± 8.01	(**10**) 60.11 ± 8.44	(**12**) 69.58 ± 7.31	(**11**) 64.18 ± 7.64	(**10**) 74.30 ± 8.01

Table 2. Results of the nonparametric Kruskal-Wallis Tests for differences in PTH, ionized calcium, total calcium, and phosphorous between captive and wild Anegada Rock Iguanas. No significant differences were found for any of the analytes.

Analyte	*H(2)*	*P*	*df*
PTH	0.206	0.649	1
Ionized Calcium	0.856	0.354	1
Total Calcium	1.262	0.261	1
Phosphorus	2.370	0.123	1

Table 3. Comparison of 25-OH-D3 levels in captive and wild species of *Cyclura*.

Species	*n*	Captive	Wild	Citation
Anegada Rock Iguana	C = 28 W = 32	*189.39 ± 13.59 ng/ml*	*126.14 ± 12.52 ng/ml*	This study
Anegada Rock Iguana	C = 61 W = 10	319 ± 95.6 ng/ml	193.6 ± 35.4 ng/ml	Lung (2012)
Jamaican Rock Iguana *Cyclura collei*	C = 34 W = 14	145 ± 58 ng/ml	157 ± 137 ng/ml	Lung (2012)
Grand Cayman Blue Rock Iguana Cyclura lewisi	C = 34 W = 2	136 ± 30 ng/ml	215 ± 78 ng/ml	Rainwater et al. (2021), Lung (2012)
Hispaniolan Rhinoceros Iguana *Cyclura cornuta*	C = 13 W = 7	127.00 ± 33.00 ng/ml	133.19 ± 19.00 ng/ml	Ramer et al. (2005)
Mona Island Rock Iguana *Cyclura cornuta stejnegeri*	*C = 10*	142.4 ± 64.26 ng/ml		Lung (2012)
Ricord's Rock Iguana *Cyclura ricordii*	W = 22		*222.00 ± 11.00 ng/ml*	Ramer et al. (2005)
San Salvador Rock Iguana *Cyclura r. rileyi*	W = 21		132.82 ± 57.39 ng/ml	Kishbaugh et al. (2020)

Table 4. Plasma Calcium and phosphorous levels reported for captive and wild species of *Cyclura* and Iguana.

		Captive		Wild		
Species	***n***	***Calcium***	***Phosphorous***	***Calcium***	***Phosphorous***	***Citation***
Anegada Rock Iguana	C = 28 W = 32	12.18 ± 0.63 mg/dL	5.72 ± 0.28 mg/dL	12.71 ± 523 mg/dL	4.67 ± 0.22 mg/dL	This study
Anegada Rock Iguana	C = 60	11.64 ± 2.66 mg/dL	3.5 ± 1.30 mg/dL			Lung (2012)
Jamaican Rock Iguana *Cyclura collei*	*C = 99*	*11.59 ± 2.40 mg/dL*	6.01 ± 1.36 mg/dL			Lung (2012)
Allen Cays Rock iguana *Cyclura c. inornata*	*C = 99*			13.03 ± 9.20 mg/dL	5.31 ± 2.30 mg/dL	James et al. (2006)
Grand Cayman Blue Rock Iguana Cyclura lewisi	W = 37	*14.3 ± 3.7 mg/dL*	6.2 ± 2.7 mg/dL	15 ± 10.5 mg/dL	6.6 ± 3 mg/dL	Rainwater et al. (2021)
San Salvador Rock Iguana *Cyclura r. rileyi*	W = 21			12.3 ± 1.3 mg/dL	4.8 ± 1.8 mg/dL	Kishbaugh et al. (2020)
Ricord's Rock Iguana *Cyclura ricordii*	W = 23			12.42 mg/dL	5.57 mg/dL	Maria et al. (2007)
Cuban Rock Iguana *Cyclura nubila*	C = 19 W = 16	11.7 ± 0.2 mg/dL	5.4 ± 0.2 mg/dL	13.7 ± 1.2 mg/dL	5.8 ± 0.3 mg/dL	Alberts et al. (1998)
Mona Island Rock Iguana *Cyclura cornuta stejneri*	C = 31	13.23 ± 3.5 mg/dL	4.79 ± 1.59 mg/dL			Lung (2012)
Common Green Iguana *Iguana iguana*	*C = 39*	13.61 ± 0.93 mg/dL	4.69 ± 1.36 mg/dL			Grant et al (2009)

CHAPTER 4

GENERAL CONCLUSIONS

This research employed conservation physiology to evaluate the efficacy of headstarting as a conservation mechanism to bolster reintroduction efforts for the Critically Endangered Anegada Rock Iguana (*Cyclura pinguis*). Reintroduction programs that examine key physiological components at all stages of the projects (e.g., pre-release phase through long-term post-release monitoring) and use this information in an adapted framework exhibit higher rates of success. For this reason, we monitored growth, body condition index (BCI), and vitamin D and calcium metabolism in captive headstart and reintroduced iguanas on Anegada.

Analyses of growth rates and BCI of captive and released iguanas provided a broad assessment of potential negative impacts of captivity. Hatchlings in the headstart facility displayed constant growth rates (~5 mm/mo) for snout vent length (SVL) up to three years of age similar to findings for other *Cyclura* headstart programs (Alberts et al. 2004, Perez-Buitrago et al. 2008). At three years of age, males began to gain more mass than females, likely due to outcompeting females for resources in shared enclosures. Body condition index was constant for captive iguanas until densities became too high. This resulted in a significant drop in BCI and SVL growth ceased. To address these concerns, we suggest increasing the amount of food per cage, providing multiple feeding stations, and installing visual barriers, until the density within each cage can be reduced to one individual per cage.

Unlike other Cyclura headstart programs, animals released to the wild displayed a significant increase in mass during the first 60-day transition period (Burton 2005, Lewis et al. 2008, Perez-Buitrago et al. 2008). Animals were released during the rainy season when there was an abundance of food and water and quickly began foraging on native plants. Growth rates after the transition period decreased, but the rates were dependent on sex and release site, which is likely due to greater plant diversity occurring in the rocky woodland habitat. Despite these differences in growth rates across sites, there were no differences in BCI based on sex or site, implying that iguanas may be adapted to maintaining a minimum BCI, at the cost of reducing growth rates. Iguanas who maintain a reserve of energy stores are more likely to survive reduced resource availability resulting from natural events (e.g., droughts, hurricanes, etc.). Importantly, growth rates observe for release iguanas, particularly in the rocky woodland habitat and the elevated 60-day post release BCI at both sites, indicates that the release sites are capable of supporting increased densities of iguanas. Thus, we recommend the program continue to use these release sites and target releases during the rainy season.

To assess the potential impacts of captivity on iguana health in more detail we examined vitamin D (25-OH-D3), parathyroid hormone (PTH), ionized calcium, total calcium, and phosphorus. Captive animals had significantly higher levels of vitamin D (25-OH-D3) than wild animals, which may be due age or the captive environment preventing iguanas from self-regulating their UVB exposure. There were no significant differences in PTH, ionized calcium, total calcium, or phosphorus between captive and wild iguanas and no cases of nutritional secondary hyperparathyroidism (NSH) were

observed in the captive population. Seasonal differences in 25-OH-D3, total calcium, and phosphorous levels were observed in both captive and wild iguanas. Vitamin D increased throughout the course of the year, which may reflect seasonal differences in UVB radiation or seasonal changes in vitamin D requirements. In contrast, calcium and phosphorus levels decreased throughout the year which may be related to reproductive cycling with females needing increased calcium for vitellogenesis early in the year. However, it is unclear why the younger, captive animals who were not sexually mature showed the same pattern in calcium levels. Calcium and phosphorous metabolism are intricately related to each other, so phosphorous levels were expected to rise in healthy iguanas. Plasma PTH and ionized calcium levels are maintained within a narrow range in healthy iguanas, therefore values observed outside known reference ranges would suggest a health problem with vitamin D/calcium metabolism (Dennis et al. 200, Adkesson & Langan 2007, Stringer et al. 2010). We again recommend that captive density be minimized and additional shade and refugia provided to reduce competition and allow all individuals to self-regulate UVB exposure.

By examining the physiological factors of growth, body condition, and vitamin D and calcium metabolism, we have validated the efficacy of headstarting as a conservation mechanism for the Anegada Rock Iguana, made recommendations for improvements, and addressed several key criticisms of headstart programs. We have confirmed that 1) headstarting does not have a negative effect on growth and body condition of captive or released iguanas, 2) captivity does not prevent iguanas from foraging on native food plants after release, 3) multiple habitat types are capable of supporting higher numbers of iguanas, and 4) the headstart program is producing

healthy animals for reintroduction. Challenges resulting from captive husbandry were identified and solutions were offered to ensure a high probability of success for this conservation program. A strong criticism of headstart programs is that they do not address the true cause of decline (Woody 1991, Frazer 1992,). Feral cats are the cause of decline in this population, but full cat eradication is not possible at this time, though it is something we are working towards with our in-country partners the National Parks Trust of the Virgin Islands. However, the headstart program does provide protection to some individuals of the age class most affected by cats and has increased recruitment in the population annually since 2003 when the first releases began. The wild population has increased from 300 animals in 1997 to approximately 500 today (Bradley & Grant 2000).

In turn, the information gained from this study will be invaluable to existing headstart programs. Our applied research approach allowed us to design the release methods to answer specific questions that were critical in determining how effective headstarting is for this species. The physiological data provided a method to evaluate protocols and procedures during the process and make corrections as needed. This strategy will serve as a model for other programs to improve the method of headstarting for Caribbean Rock iguanas and other threatened species.

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