



# Monitoring the Release of Black Iguana from a Management Unit into Two Ecosystems

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## Research Article

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## Abstract

Monitoring the release of a living being into a habitat is essential in order to generate information about the animal's adaptation to the natural environment. The aim of the present study was to monitor the behavior of the black iguana (*Ctenosaura pectinata*), undergoing the process of release into 1) tropical deciduous forests and 2) an agroforestry ecosystem. The study was carried out at the CECOREI-UMAR facilities, where 24 females in different states of the life cycle: hatchling, juvenile and adult were kept. The transportation and the release of the iguanas were carried out in four periods of 15 days. Behavioral variables were measured and evaluated through a completely randomized block design with a 2x3 factorial arrangement, with four time periods. There was an effect ( $P < 0.0001$ ) related to the age of the iguanas. The hatchlings and adults were seen to recognize predators and flee from them ( $P < 0.05$ ). In conclusion, the age of iguanas is a factor that can counteract the adaptation ability of the species when released into environments such as tropical deciduous forest or an agroforestry ecosystem.

**Keywords:** Reptiles; *Ctenosaura pectinata*; Release Iguana; Monitoring; Endangered Iguana

## Abbreviations

TDF: Tropical Deciduous Forest; AFE: Agroforestry Ecosystem.

## Introduction

Release is the action of setting an animal or group of animals free into a habitat, to prevent any existing link with humans [1]. Release and monitoring studies can support reintroduction, repopulation and translocation programs of animal species or subspecies within historical ranges and counteract extinction [2]. However, a major problem is the lack of scientific information on endangered species

that are released, because the expected results have not been provided [3]. Another important aspect is that confiscated specimens legally must be released, since they are the product of trade or illegal overexploitation [4,5]. The process of releasing wildlife specimens must meet several requirements: 1) prior evaluation of the specimens and the habitat, 2) structured plan with follow-up actions and indicators on the released specimens, in addition to other associated species and the habitat, 3) treatment of factors that affect the survival and sanitary control of the specimens to be released and 4) other protocols [6]. One of the most critical stages in this process must not be overlooked: before releasing the animals, a comprehensive assessment must be carried out, including behavioral patterns [7]. Because

animals under human care are in an unnatural environment, physical and biological factors in the life of the specimens can be affected [8,9]. The Wildlife Conservation Management Units (UMAs) are facilities which are formally registered with their government. They operate with an approved management plan that includes permanent monitoring of the specimens in captivity or in the wildlife. This has the objective of sustainable production and use of biodiversity [2]. Some UMAs like the one presented in this study are working to protect the black iguana (*Ctenosaura pectinata*) and green iguana (Iguana iguana) [10]. In the Mexican Republic, there are 10,228 UMAs, of which 11 UMAs on the Oaxaca coast manage iguanas and other species [11]. *Ctenosaura pectinata* is listed as threatened and is endemic to Mexico [12]. Furthermore, it is classified in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora [13,14]. Guerreroan Spiny-tailed Iguana *C. pectinata* has most recently been assessed for The IUCN Red List of Threatened Species: It is listed as Least Concern (14). The different types of native habitats or those modified by anthropogenic or natural activities alter the thermoregulation of reptiles because they are ectotherms. Ectothermy is an activity that is influenced by internal and external factors. The former consist of genetic variability, sex type and reproductive condition; the latter by the habitat composition, seasonality, time of day, competition for resources, and predators [15]. Another factor that directly affects ectotherms is the surface area to volume ratio, which has repercussions for capturing energy from the environment through radiation, convection and conduction [15-18]. Through these mechanisms, the black iguana can increase or decrease body temperature and adapt to different environments. It has been indicated that the release of wild animals after confiscation or human care is an alternative strategy to increase genetic diversity and strengthen endangered or at-risk populations [4]. However, there may also be some health and genetic risks to wild populations [7]. Therefore, it is necessary to further complement genetic and health studies of the species for release in any habitat [19,20]. In this way, iguanas of any age: hatchlings, juveniles and adults easily adapt to the environment, can develop immune capacity [21] and have the advantage of survival in the wild. Consequently, it is an important species that needs to be evaluated in the process of release into the environment. In this study, it is hypothesized that monitoring the release of black iguanas kept in management units will demonstrate the species' ability to adapt when exposed to two environments: tropical deciduous forest, and an agroforestry ecosystem. The objective of this study was to demonstrate the success of the release of the black iguana (*C. pectinata*) in a tropical deciduous forest and an agroforestry ecosystem.

## Material and Methods

### Location

The study was carried out at the facilities of the Center for Conservation and Reproduction of Iguanas of the University of the Sea (CECOREI-UMAR). It is located on the Federal Highway Pinotepa Nacional-Puerto Escondido at Kilometer 128.1, Bajos de Chila, San Pedro Mixtepec, Juquila Oaxaca, Mexico. It has a warm subhumid climate, with an average annual temperature greater than 22°C and an average temperature greater than 18°C in the coldest month. Average annual precipitation ranges between 500 and 2,500 mm [22].

The CECOREI-UMAR has eight hectares of land, where there are two types of vegetation: 1) tropical deciduous forest (TDF), which is delimited at 15° 55' 32" N and 97° 08' 57" W and 2) an agroforestry ecosystem (AFE), at 15° 55' 27" N and 97° 09' 00" W, both with an elevation of 9 masl [23]. The index code for the CECOREI-UMAR is: INE/CITES/DGVS-CR-IN-0668-OAX./00 and the Environmental Registration Number is: CCRSY2006711. Likewise, this study was endorsed by the Head of the Graduate Studies Division in the official document: OF/VRAC/POSG/553/2018.

The TDF is represented by plant species such as: *Acacia collinsii*, *Ceiba pentandra*, *Cochlospermum vitifolium*, *Cocos nucifera*, *Couratari guianensis*, *Enterolobium cyclocarpum*, *Gliricidia sepium*, *Leucaena leucocephala*, *Pithecellobium dulce*, *Swietenia humilis*, *Tabebuia rosea* and *Tamarindus indica* [24,25]. In addition, the area had a number of stumps, dry logs, roots, stones and branches. On the other hand, the AFE environment had only two cultivated species of tree: *T. rosea* and *E. cyclocarpum* and some observable leaf litter. In both areas, the flora consisted of dry grassland and herbaceous vegetation, with the presence of wild *Desmodium infortunatum*. Regarding fauna, there were wild animals: *Ortalis poliocephala*, *Colinus spp*, *Buteo spp*, *Columbina spp*, *Aratinga spp*, *Didelphis virginiana*, and unidentified snakes, among others species [26]. The domestic species found in this area were *Ovis aries* and *Bos taurus*; which were only present in the TDF.

### Iguanas used and Handling

Twenty-four female *Ctenosaura pectinata* were used in this study, Grouped into three categories by physiological status: hatchlings (6 months old), juveniles (12, 24 and 35 months old) adults (>36 months old). These iguanas were born at the beginning of the rainy season in the month of June [16,27]. The iguanas were selected from the respective breeding areas of CECOREI-UMAR with an average weight of 40.9 ± 4.7 g for

hatchlings,  $353.2 \pm 97.8$  g for juveniles and  $766.0 \pm 122.8$  g for adults. None of the iguanas had conformation defects, they were all in good visual health, free of parasites and received dignified treatment throughout the handling process [7].

In order to identify the iguanas released from the study, three methods of identification were used: 1) microchip (Trovan® Microchip in Sterile cannula: 2.6 x 40 mm), for which the numbering was confirmed by a manual reader (Trovan®), used only to release and recapture the iguanas so that they could be returned to the corresponding CECOREI-UMAR area, 2) beads, placed on the back of the tail and 3) an indelible red marker. The last two markings were used to recognize the iguanas released from a distance and to differentiate them from wild ones.

All iguanas were dewormed and given vitamins on two occasions: at thirty and fourteen days before the release process. The dewormers used were fenbendazole® (50 mg/kg orally) and praziquantel® (20 mg/kg orally). Vitamins were added to the drinking water: 1 g/L for 7 consecutive days (A 125,000 IU, D3 41,500 IU, B12 500 mcg, E 40 IU, riboflavin 90 mg, B1 100 mg, B6 50 mg, C 100 mg, K 100 mg, pantothenic acid 100 mg, nicotinamide 400 mg, folic acid 3 mg).

### Evaluated Groups and Release

There were six groups evaluated, which were obtained by the combination of two main factors, ecosystem type, and the stages of development: 1) Physiological stage of the females: hatchling (C), juvenile (J) and adult (A) and 2) types of ecosystems: TDF and AFE. In the months of February to May 2019, the hard release of *C. pectinata* was carried out in the designated areas [28]. This implied that there was no prior preparation of the species for the release process and adaptation to the environment. These months are considered critical in the release, adaptation and survival of female iguanas to the environment. During this period there is little vegetation, scarcity of food, high risk of predators capturing the iguanas and little availability of drinking water [22]. The above factors are important to evaluate success in the release process.

The release was carried out by two observers. Each of them was previously trained for 20 days to monitor and record the variables to be evaluated. Each observer was in charge of a group of three animals: a hatchling, a juvenile and an adult, which were released at the same time in the respective areas. Four time periods were used, each period consisted of 15 days, where the iguanas remained free in the wild, during this time each group of iguanas was monitored.

The release of the iguanas took place within the CECOREI-UMAR facilities. The iguanas were transported to

the TDF and AFE areas in individual cages measuring 60 x 45 x 45 cm in length, width and height. The comfort of the females was ensured so that they did not suffer from stress at any time. Later, in each area and at the same time (09:00 am), the cages were opened and the observers withdrew fifteen meters away. Due to the hard release process, water and food were not offered to the released animals [28].

At first, the observers did not intervene with the iguanas to get them to leave the housing; however, when the females remained motionless for more than four hours, they were placed on top of the cage to stimulate their daily activities. After opening the cage, the specimens were monitored with the help of telescopic binoculars (LabG brand, model RT506). Every day, the iguana's activities were recorded from sunrise to sunset. Observers were able to see the three iguanas at the same time, considering that at the time when the research was carried out there was no abundant vegetation and that the radius of movement of the iguanas was a few meters. However, each iguana was dedicated two hours a day of observation, alternating viewing hours, to record daily activities over the 15-day period and thereby obtain activity data in a daily activity cycle.

At the end of each observation period, the iguanas were recaptured and verified to be the ones released with the help of the microchip reader (Trovan® ID-162B), apparent visual health status was recorded and they were returned to their areas within the CECOREI-UMAR. The observers rested for a week and then began another observation period of 15 consecutive days [7,9,29].

### Variables

The positioning of the iguana within the cage was observed and recorded (wall, floor or ceiling). The following variables were observed and recorded: The positioning of the iguana within the cage (wall, floor or ceiling); The order in which it left the cage (first, second or variable); state of alert when leaving the cage (yes, no or both); observations of the iguana when leaving the cage (vigilant, restless or indifferent). Further observation was also made regarding the following: type of movement outside the cage (ran, walked and did not move); Orientation of the iguana (north, south, east, west) or direction when exiting the cage (taken by means of a pocket magnetic brass compass, Overseas Trading Corp brand, model BR 48852A); time (days) to locate the first shelter (1st, 2nd, 3rd, did not exit, did not find); type of movement (climbed, hid on the ground, stayed in the cage); type of shelter (tree, leaf litter, climbed a tree and dry log, couldn't climb tree, did not search); mobility on ground (ground-tree-ground and ground-tree-log); recognition of predators (yes, no, both); reaction against predators (run or hide, vigilant, motionless). Recognition

of humans as predators (yes, no or both). Behavior of the iguanas regarding food consumption was observed (Licking, walked, did not see and combination), as well as the time (min) it took the iguana to leave the cage (using a digital clock, HUAWEI brand, model JAT-X3). Displacement (m) was evaluated by means of the search for shelter.

### Statistical Analysis

A randomized complete block design with a 2x3 factorial arrangement was used, which generated six experimental groups, with four periods or blocks. One factor was the type of habitat (tropical deciduous forest and agroforest ecosystem) and the other factor was the age of the iguanas (hatchlings, juveniles and adults). Categorical variables were analyzed using Kruskal-Wallis and Chi-square. The numerical variables were evaluated with an analysis of variance and where there were differences, the Tukey test was used [30].

### Results

The age of the iguanas Table 1 shows different results in the behavior inside and outside the cage. The hatchlings preferred to stay on the wall of the cage ( $P<0.0001$ ), while the juveniles and adults preferred the floor. The order in which the iguanas exited the cage was different ( $P<0.001$ ) across the three ages, first all the hatchlings exited, then the juveniles, and finally the adults. The hatchlings and adults took a look ( $P<0.05$ ) outside the housing before or upon exit; half of the juveniles exited without looking around outside the housing. The variables, which refer to the behavior of the iguanas when exiting the cage were similar ( $P>0.05$ ) across the three age groups. However, hatchlings and adults hid ( $P<0.05$ ) from possible predators, while half of the juveniles did not hide from possible threats.

| Carácter                   | Age  |  |  |      |         |
|----------------------------|--|--|--|------|---------|
|                            | Hatchlings                                   | Juveniles                                      | Adults   | Chi2 | Prob    |
| <b>Inside cage</b>         |  |  |  |      |         |
| Positioning within cage    | Wall <sup>b</sup>                            | Floor <sup>a</sup>                             | Floor <sup>a</sup>   | 23   | <0.0001 |
| Order of exit              | First <sup>a</sup>                           | Second <sup>b</sup>                            | Variable <sup>c</sup>  | 14.2 | 0.008   |
| Observation                | Yes <sup>e</sup>                             | Both <sup>d</sup>                              | Yes <sup>e</sup>   | 6.57 | 0.037   |
| Action upon exit           | Vigilant, restless                           | Vigilant, restless and indifferent             |  | 1.92 | 0.38    |
| <b>Action outside cage</b> |  |  |  |      |         |
| Movement                   | Ran, walked                                  |  | Ran, walked, did not move  | 0.57 | 0.75    |
| Orientation                | NW, NE, W, S, SW                             | NE, W, SE, N                                   | NW, NE, N, E   | 2.45 | 0.29    |
| First shelter              | 1st, 2nd, 3rd                                | 1st, 3rd, did not exit                         | 1st, 2nd, 3rd, did not exit  | 2.17 | 0.34    |
| Type of movement           | Climbed, hid on the ground                   | Climbed, hid on the ground, stayed in the cage | Climbed, hid on the ground, stayed in the cage                               | 1.61 | 0.45    |
| Type of shelter            | Did not search, tree, leaf litter            | Did not search, tree, leaf litter, roots       | Did not search, leaf litter, climbed a tree and dry log, couldn't climb tree | 2.61 | 0.27    |
| Mobility on ground         | Ground-tree-ground                           |  | Ground-tree-ground and ground-tree-log                                       | 4.87 | 0.09    |
| Recognizes predators       | Yes <sup>e</sup>                             | Both <sup>d</sup>                              | Yes <sup>e</sup>   | 6.57 | 0.04    |
| Reaction to predators      | Ran, hid, vigilant, motionless               | Vigilant, motionless                           | Ran, hid, vigilant, motionless   | 3.39 | 0.18    |
| Human predators            | Both   | Both   | Both   | 2.7  | 0.26    |
| Actions of consumption     | Licking, walked, did not see and combination |  | Licking, walked, did not see   | 2.67 | 0.26    |

a,b,c Superscripts in the same row indicate significant difference at a level of 0.1% probability ( $P<0.001$ ). d,e Superscripts in the same row indicate significant difference at a 5% probability ( $P<0.05$ ).

**Table 1:** Main effect of age on categorical variables in the release process of *C. pectinata* into tropical deciduous forest and an agroforestry ecosystem.

The groups belonging to hatchlings, regardless of the type of habitat, preferred ( $P < 0.01$ ) to stay on the wall of the enclosure, while juvenile and adult iguanas preferred the floor in the TDF and AFE (Table 2). Hatchlings were the first to leave the cage ( $P < 0.05$ ) in relation to juveniles and adults

in both ecosystems. The direction taken by the iguanas was different ( $P < 0.05$ ) in all groups. The variables observation, action upon leaving the cage, movements made, first shelter found and the types of movements were similar ( $P > 0.05$ ) in the six groups evaluated.

| Grupo* | Behavior inside the cage |                                    |       |                                     | Actions outside the cage    |                            |                                      |                              |
|--------|--------------------------|------------------------------------|-------|-------------------------------------|-----------------------------|----------------------------|--------------------------------------|------------------------------|
|        | Position                 | Order of exit                      | Alert | Action upon exit                    | Movement                    | Heading                    | First to find shelter                | Type of movement             |
| HTDF   | Wall <sup>b</sup>        | 1st <sup>a</sup>                   | Yes   | Vigilant, restless                  | Ran, walked                 | NW, NE, W <sup>ef</sup>    | 1st, 2nd, did not find               | Climbed, followed the person |
| JTDF   | Floor <sup>a</sup>       | 2nd, did not exit <sup>b</sup>     | Both  | Vigilant, restless, and indifferent | Ran, walked                 | NE, W, SE <sup>de</sup>    | 1st, did not exit                    | Climbed, hid on the ground   |
| ATDF   | Floor <sup>a</sup>       | 2nd 3rd, did not exit <sup>b</sup> | Yes   | Vigilant, restless, and indifferent | Ran, walked or did not move | NW <sup>f</sup>            | 1st, 2nd, Did not find               | Climbed, stayed in the cage  |
| HAFE   | Wall <sup>b</sup>        | 1st <sup>a</sup>                   | Yes   | Vigilant, restless                  | walked                      | NE, W, S, SW <sup>cd</sup> | 1st                                  | Climbed, hid on the ground   |
| JAFE   | Floor <sup>a</sup>       | 2nd, did not exit <sup>b</sup>     | Both  | Vigilant, restless, and indifferent | Ran, walked                 | N <sup>c</sup>             | 1st, Did not find                    | Climbed, hid on the ground   |
| AAFE   | Floor <sup>a</sup>       | 2nd 3rd, did not exit <sup>b</sup> | Yes   | Vigilant, restless, and indifferent | Ran, walked                 | NE, N, E <sup>cd</sup>     | 1st, 2nd, Did not find, Did not exit | Climbe <sup>d</sup>          |
| Chi2   | 23                       | 14.55                              | 7.67  | 3.39                                | 5.54                        | 13.7                       | 4.63                                 | 5.75                         |
| Prob   | 0.0003                   | 0.01                               | 0.17  | 0.64                                | 0.35                        | 0.02                       | 0.46                                 | 0.33                         |

\*HTDF: hatchlings in tropical deciduous forest. JTDF: juveniles in tropical deciduous forest. ATDF: adults in tropical deciduous forest. HAFE: hatchlings in an agroforestry ecosystem. JAFE juveniles in an agroforestry ecosystem. AAFE: adults in an agroforestry ecosystem. a,b,c Superscripts in the same column indicate significant difference at a level of 1% probability ( $P \leq 0.01$ ).

**Table 2:** Effect of behavioral variables on the groups used in the process of releasing black iguana in the tropical deciduous forests and an agroforestry ecosystem.

Differences ( $P < 0.05$ ) were observed in the type of shelter used (tree, roots and dry log) in the groups of hatchlings, juveniles and adults released in the tropical deciduous forest, in relation to the sets of juveniles and adults from the agroforestry ecosystem (tree, leaf litter and dry log). The above is related to the type of shelters that can

be found in each type of habitat. There were also differences ( $P < 0.05$ ) between the groups in the pre-feeding behavior. However, there were no differences ( $P > 0.05$ ) in the variables of mobility in the substratum and in the recognition of predators (Table 3).

| Group | Type of shelter                         | Mobility in substratum              | Recognizes of predators | Reaction to predators             | Human predator | Actions of feeding                          |
|-------|---|-------------------------------------|-------------------------|-----------------------------------|----------------|---|
| HTDF  | Tree <sup>b</sup>                       | Ground-tree-ground                  | Yes                     | Run or hide, vigilant, motionless | Both           | Licking, walked <sup>d</sup>                |
| JTDF  | Tree, roots <sup>b</sup>                | Ground-tree-ground                  | Both                    | vigilant, motionless              | Both           | Licking, walked <sup>d</sup>                |
| ATDF  | Tree, dry log <sup>b</sup>              | Ground-tree-ground, Ground-tree-log | Yes                     | Run or hide, vigilant, motionless | Both           | Licking, walked <sup>d</sup>                |
| HAFE  | Tree, leaf litter, dry log <sup>a</sup> | Ground-tree-ground                  | Yes                     | Run or hide, vigilant, motionless | Both           | Combination <sup>c**</sup>                  |
| JAFE  | Tree, leaf litter <sup>a</sup>          | Ground-tree-ground                  | Both                    | vigilant, motionless              | Yes            | observation and combination <sup>cd**</sup> |
| AAFE  | Dry log, leaf litter <sup>a</sup>       | Ground                              | Yes                     | vigilant, motionless              | Both           | Licking, walke <sup>dd</sup>                |
| Chi2  | 13.87                                   | 7.98                                | 7.67                    | 9.93                              | 5.6            | 14.78                                       |
| Prob  | 0.02                                    | 0.16                                | 0.18                    | 0.08                              | 0.35           | 0.01  |

\*HTDF: hatchling in the tropical deciduous forests. JTDF: juveniles in the tropical deciduous forests. ATDF: adults in the tropical deciduous forests. HAFE: hatchling in agroforestry ecosystem. JAFE: juveniles in agroforestry ecosystem. AAFE: adults in agroforestry ecosystem. \*\*Combination: licking, observation, monitoring of movements. a,b Superscripts in the same column indicate significant difference at a 5% probability ( $P<0.05$ ). c,d Superscripts in the same column indicate significant difference at a 1% probability ( $P\leq 0.01$ ).

**Table 3:** Effect of behavioral variables on the groups used in the simulation process for the release of *C. pectinata* in the tropical deciduous forest and agroforestry ecosystem.

There was no effect ( $P>0.05$ ) of habitat, nor of habitat\*age interaction on the variables time taken to exit the cage and movement of the iguanas. However, there was an effect ( $P<0.0001$ ) of the age of the iguanas on the time taken to leave the cage (Table 1). The hatchlings left the cage faster ( $P<0.0001$ ) than the juvenile and adult iguanas. The group

of hatchlings released in the TDF were observed to leave the cage faster ( $P<0.05$ ) (65 min) than the adult iguanas in the agroforestry ecosystem (285 min). The movement towards a refuge or sheltered area for the iguanas was similar ( $P>0.05$ ) in the groups studied, with an average displacement of 6.85 m (Table 4).

| Main Factors              |                                   |   |         |                                   |                                  |
|---------------------------|-----------------------------------|---|---------|-----------------------------------|----------------------------------|
| Factor                    | Time took to leave the cage (min) | Displacement (m) From point of release to shelter | Groups* | Time took to leave the cage (min) | Displacement (m) towards shelter |
| Habitat (Probability)     | 0.14                              | 0.9   | HTDF    | 65e                               | 3.75                             |
| Age (Probability)         | 0.008                             | 0.84  | JTDF    | 180.0de                           | 10.75                            |
| Habitat*Age (Probability) | 0.78                              | 0.07  | ATDF    | 190.0de                           | 3.25                             |
| Fc 5,18                   | 3.11                              | 1.29  | HAFE    | 100.25 de                         | 4.75                             |
| Effect of Age             |                                   |   | JAFE    | 222.5 de                          | 6                                |
| Hatchlings                | 82.63b                            | 4.56  | AAFE    | 285d                              | 12.6                             |
| Juveniles                 | 201.25a                           | 4.94  | Media   | 173.79                            | 6.85                             |

|             |         |      |             |      |      |
|-------------|---------|------|-------------|------|------|
| Adults      | 237.50a | 5.88 | R2          | 0.46 | 0.25 |
| Probability | 0.0001  | 0.32 | Probability | 0.03 | 0.32 |

\*HTDF: hatchlings in tropical deciduous forest. JTDF: juveniles in tropical deciduous forest. ATDF: adults in tropical deciduous forest. HAFE: hatchlings in an agroforestry ecosystem. JAFE juveniles in an agroforestry ecosystem. AAFE: adults in an agroforestry ecosystem.

a,b Superscripts in the same column indicate significant difference at a level of 0.1% probability ( $P < 0.001$ ).

d,e Superscripts in the same column indicate significant difference at a 5% probability ( $P < 0.05$ ).

**Table 4:** Time and displacement of *Ctenosaura pectinata* in the release process, in tropical deciduous forest and an agroforestry ecosystem.

## Discussion

During the release process, the hatchling iguanas positioned themselves on the wall of the cages, where solar radiation allowed them to warm up, since they are ectothermic organisms [15]. This is because the maximum heat transfer in the iguana is by radiation and conduction [17,18]. However, in habitats with high ambient temperature (natural or artificial), convection plays an important role in the exchange of energy with the body of *C. pectinata* [16,18]. In the process of releasing the iguanas, we placed the cages in the shade, under the bushes, to prevent predators from directly reaching the specimens and to prevent the iguanas from overheating.

One adult iguana belonging to the TDF group did not move from the floor of the cage, possibly due to the lack of solar radiation, which would have caused it not to obtain ambient heat; therefore, it was necessary for it to rest and was placed on top of the housing. When carrying out this process, it was noted that the iguana had a cold body temperature by touch, which coincides with our understanding that iguanas in the wild maintain their body temperature below the ambient temperature [31]. Therefore, to maintain adequate metabolism, they require intermittent periods of sunbathing to carry out their daily activities.

Whether there is an increase or decrease in ambient temperature, the black iguana can maintain adequate body temperature by employing the strategy of entering a burrow, a shelter or simply looking for a place with adequate temperature. *Ctenosaura pectinata* heats or cools itself from different forms of heat transfer in the habitat such as wind, humidity, soil, rocks and direct solar radiation [18,32]. These sources help keep the iguana's body at an ideal temperature for its daily activities and well-being [15]. According to the above, the iguana hatchlings left the cages first, possibly because they reach optimal body temperature for metabolism and start activities faster [33,34]. Meanwhile, juvenile and adult iguanas need more exposure time to soak up solar radiation and warm their bodies [31], which may result from the greater surface area and volume ratio of the iguana hatchlings compared to the adults.

The ecosystems of the tropical deciduous forest and agroforestry ecosystem were made up of bushes or trees respectively, which are the preferred sites for iguanas to move around and seek shelter. However, a problem that was observed in 75% of the adult iguanas released in the tropical deciduous forest and in 25% released in the agroforestry ecosystem was that they initially could not climb. It is possible that this was because the iguanas came directly from their cage, at room temperature, which kept them slightly too cold for immediate strenuous activity. This delay in exposure to the sun could have prevented them from obtaining thermoregulation immediately upon release [35]. The ambient temperature drops puts the iguanas at greater risk from predators and is considered inappropriate. Therefore, it is suggested that the release of iguanas should be carried out in sunny places and when the ambient temperature is high.

On the other hand, the direction of movement of *C. pectinata* was random in hatchlings, juveniles and adults, and they were able to find shelter a few meters away from the release site in both ecosystems.

This coincides with the distance traveled by iguanas of the same species of 2.9 to 3.5 m from their shelters [36]. This means that the action radius of the black iguana is small and thus as a side note, seed dispersal by endozochory [37] may only make a small contribution.

In relation to the recognition of predators [35], the alert reaction in *C. pectinata* occurred when there were sounds, shadows of birds and trees, the approach of humans and the presence of other wild or domestic animals. Although the adult, juvenile and, to a lesser extent, hatchling iguanas had been subjected to human handling conditions, they retained the alertness to any noise or movement, and have the ability to recognize when an organism is invading their particular area and either flee or remain motionless. The territoriality of iguanas is limited by the hierarchy of the species with which they share the habitat.

Anti-predation behavior can function as a survival mechanism in complex habitats resulting in resilient ecosystems [38]. Anti-predator learning or awareness techniques to help animals detect danger in the wild has been used with wild vertebrates, born in captivity or captured from the wild [39]. However, in iguanas it is not necessary to perform these learning techniques for release [40], as signs of imprinting of iguanas under human care are not a limitation for release. The disadvantage mentioned in released animals is the lack of behavioral competence to survive and reproduce [41], though it is possible that *C. pectinata* has a high degree of recognition of predators.

Foraging observed in hatchlings, juveniles and adults is similar to other behavioral descriptions of the species [17,42,43]. In the TDF, the iguanas fed on dry and green leaves of *Enterolobium cyclocarpum*, *Leucaena leucocephala*, *Pithecellobium dulce*, and wild bean leaves; while in the AFE, individuals in the three developmental stages fed on wild bean leaves and grass leaves of different species. One hatchling iguana followed an ant, but did not consume it. It is important to note that the choice of food in both areas was based on what was found in each habitat.. According to the results obtained, none of the iguanas in the experiment suffered mortality, the health status of all of them was visually adequate and they were not found to have parasites.

## Conclusion

Hatchlings and adults of *Ctenosaura pectinata* which have been under human care and released into either tropical deciduous forest or into an agroforestry ecosystem have the same ability to adapt. In adult iguanas, the release process must take place in areas with abundant vegetation or shelters, and with the benefit of adequate solar radiation. The species does not require a period of adaptation to release; they retain the ability to recognize shelters and predators. However, the juvenile iguanas presented problems recognizing predators, but there was no mortality due to predation. Considering that the study was carried out in times of food scarcity, the iguanas did not lose the ability to adapt to food sources.

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## Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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