

Multiple Threatened with Extinction Tropical Chelonians of the Western Ghats, India: Population, Ecology, and Moisture Sorption Characteristics and Mould Ecology of Trade Garri Sold in Southeastern Nigeria

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Abstract

The Cochin forest cane turtle (*Vijayachelys silvatica*) and the Travancore tortoise (*Indotestudo travancorica*) are two endemic terrestrial chelonians found only in the Western Ghats region of the Western Ghats-Sri Lanka hotspot. Estimates of the population as well as details on the scope and severity of the problems facing these Chelonians are mainly unavailable. This study makes an effort to fill in these gaps for two Western Ghats hill ranges. Thirty randomly selected quadrats in eight forest ranges were examined for chelonians and their carapaces, with those discovered during the search and during opportunistic surveys being recorded. This was followed by the discovery of 38 *I. travancorica* and three live *V. silvatica*, with total densities of 0.006 and 0.03 individuals per hectare, respectively. These chelonians were discovered in quadrats with lower light levels and warmer soil. Nine carapaces were discovered during the field investigations, with seven coming from human consumption, one from being caught in a pit, and one from being eaten by a wild animal. A *V. silvatica* and 38 *I. travancorica*, including a carapace, were found during household surveys conducted in 26 indigenous and nonindigenous human settlements in addition to field surveys. Two *I. travancorica* road kills were discovered as an examination of roads to determine the hazard they posed to chelonians. If chelonian conservation is to increase in the landscape, it is necessary to encourage more exchanges and conversations between management authorities and local communities.

Peeled, rinsed, mashed, fermented, and dewatered cassava roots are roasted until they become gelatinous and dry to yield garri, a creamy white or yellow starchy grit. In Central and West Africa, it is the most significant cassava product. Within 24 hours of sampling white and yellow garri from the market, the mean moisture content of each was 11.11 percent and 10.81 percent, respectively. After 3 months of storage at ambient temperature, the mean moisture content of each was 17.27 percent and 16.14 percent. After storage, the samples' initial water activity ranged from 0.587 to 0.934. Static gravimetric methods were used to measure the moisture sorption isotherms, which revealed temperature-dependent BET Sigmoidal type II behaviour that is typical of diets high in carbohydrates but was only very minimally influenced by the presence of palm oil. At constant water activity, equilibrium moisture content dropped as temperature rose. Ten distinct fungi from the genera *Mucor*, *Penicillium*, *Cephalosporium*, *Aspergillus*, *Scopulariopsis*, *Rhizopus*, and *Paecilomyces* were found, and their range widened as sample water activity increased.

Keywords: *Cephalosporium*; *Aspergillus*; Gelatinous

Introduction

A widely recognised region for the variety of endemic amphibian, reptile, and fish species is the Western Ghats (WG) region of India, which is a part of the Western Ghats-Sri Lanka Biodiversity Hotspot. The Travancore tortoise and the Cochin forest cane turtle, both of which are on the verge of extinction, are the two endemic chelonian genera represented in the WG. The IUCN Red List of Threatened Species classifies the cane turtle as "Endangered," but the Travancore tortoise is classed as "Vulnerable." It is known that Travancore tortoises are more common than cane turtles among these sympatric, cryptic species [1]. A variety of ecosystems, including evergreen, semievergreen, bamboo, *Lantana camara* and *Cromolarius glandulosum* bushes, as well as rubber and teak plantations, may be found over the southern WG in rocky hills at elevations of 100–1000 m a.s.l. On the other hand, the Cochin forest cane turtle, which is recognised as a habitat specialist associated with evergreen vegetation, has also been discovered in semievergreen, deciduous, and bamboo plant types at elevations of 180–800 m a.s.l. Despite having algal growth on their carapace, cane turtles are not attracted to perennial water sources. Even though there have been studies that have concentrated exclusively on one or both of the species, formal conservation evaluations and practical conservation measures are still missing. Using a combination of ecological and socioeconomic methodologies, we conducted a survey in the southern WG forest areas to better understand the two species in the context of

unreliable population estimates and systematic threat assessments [2].

More than 500 million people in the tropics and subtropics eat cassava as their main source of energy, making it a crucial root crop and factor in the food security of producer nations. Of all the crops, it provides the maximum starch output per hectare. Cassava is the fourth most produced crop in the world, behind rice, wheat, and maize, with an annual production of more than 160 million tonnes. A factor lowering its attractiveness and value as food is its high cyanogenic glycoside concentration. After undergoing some sort of detoxification—often by fermentation, which causes linamarin to break down and be eliminated—cassava is typically utilised as food. Its postharvest life is often shorter than three days, making it the most perishable foodstuff.

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Garri, which is widely consumed throughout West and Central Africa and beyond, is a key of cassava processing [3]. Cassava roots that have been peeled, cleaned, and mashed are fermented to make garri, a creamy white or yellow starchy grit that is dewatered, sieved, and then dried and gelatinized. Garri is strong in fibre and provides a few vital vitamins. Because it can be kept for extended periods of time—up to a few months—and has a pleasant mild aroma unlike some other cassava products, it is the most extensively used and popular product made from cassava. It is well-liked in both rural and urban areas. It can be eaten dry (as a snack), soaked in cold water with a variety of accompaniments, or reconstituted with hot water to form dough that is eaten with soup, sauce, or stew as a main course [4]. There is no downstream crucial control point when garri is consumed as a ready-to-eat snack, as a plain dry product, or with cold water, coconut, palm kernel, peanut, beans, or other accompaniments; it needs to be a wholesome product.

Garri post-frying/processing handling for trade and consumption includes handling with bare hands, drying on bare cemented floor, mat, or basins, displaying in plain buckets, bowls, basins, and mats at points of sale, and transporting over extremely long distances in various kinds of typically non-moisture-proof sacks and bags. These procedures could increase the risk of food-borne illnesses and microbiological contamination [5]. Since fungi alter the chemical makeup of foods, the mycoflora of food is important for growers, processors, and consumers. In addition to discoloration, nutritional value loss, the generation of off-odors, and contamination with mycotoxins, fungi that infest stored goods are also to blame.

It's likely that the first technology used by humans to increase food stability was the control of moisture content during food preparation, which is an age-old method of food preservation. To keep meals chemically and microbiologically stable, water is either removed from them or bound to them. Microbial growth is frequently what shortens food's shelf life; hence several preservation techniques are designed to make food more resistant to microbial development. When comparing the vapour pressure of pure water at the same temperature to that of the food, the water activity of the food is calculated [6]. With water activity, the moisture content of the majority of foods rises curvilinearly. A moisture sorption isotherm, which provides details on the relationship between the food and water, is produced by the relationship between the total moisture content and water activity of food throughout a range of values when equilibrium conditions are met. Understanding the moisture sorption isotherms of dehydrated foods is helpful in solving engineering and food processing issues like predicting shelf life. This has been used to successfully preserve a variety of dried foods. To our knowledge, however, this has not been investigated in relation to garri and other dry food staples in the tropics, which are also the regions most affected by difficulties with food security.

The goal of this study was to add to the body of knowledge needed to extend the shelf life of this crucial security food by identifying the water activity and moisture sorption characteristics of garri and relating them to the product's mould ecology [7].

Materials and Method

Study Area

The Anamalai and Agasthyamalai Hills in the southern WG are where the study was carried out. The Anamalai Hills are where you may find the most shola grassland. The significant forest fragmentation in the Anamalais, which is home to various indigenous people, is of tree-felling for cardamom, coffee, and tea plantations during the imperial

period. Comparatively unaltered and adjacent to the Anamalais, the Agasthyamalai Hills are notable for their diverse plant life and distinctive habitats including *Myristica* marshes. They span 2112 km².

Household and Road Surveys

Indigenous and non-indigenous settlements were surveyed using a referral sample approach to identify households housing chelonians (local informants and respondents from parallel interview surveys). Visits to the referred homes were made while being accompanied by local informants, and the residents were asked to consent to the recording of the chelonians' morphometric data as well as their cloacal temperature. The procedure involved surveying 26 settlements in total [8]. Each of the eight forest ranges was examined once for any traffic fatalities involving chelonians by major thoroughfares and smaller roads that crossed across them.

Analysis

Every species that was found at a place had its population density estimated. The average of each species' population density across all the sites surveyed made up the total population density. The total number of individuals of a given species was divided by the total number of individuals of both species to determine the overall relative abundance of each species.

Independent samples, the Kruskal-Wallis or Mann-Whitney test Quadrats with and without chelonians were compared using the t-test or an analysis of variance (ANOVA). Chi-square and Fisher's exact tests were used if the frequencies among any group were fewer than five.

Additionally, morphometric variations between individuals found in settlements and the wild, as well as variations in habitat preferences between sexes, were discovered [9]. The Fisher's exact test and Shapiro-Wilk test were conducted using R.2.14.0, while all other statistical analyses were conducted using SPSS 11.5 instead.

Collection of Samples

Ikpa and Ogige main markets (Nsukka Local Council Area), Nkwo Ibagwa market (Igbo-Eze South Local Council Area), Obollo-Afor and Obollo-Eke markets (Udenu Local Council Area), all in Enugu State (a major garri producing belt), Nigeria, each had three samples of freshly processed (within 48 hours) white and yellow garri each. Within 12 hours, samples were taken to the lab for analysis in clean, dry cellophane bags of the highest quality.

Determination of the Initial Moisture Content of Garri Samples

By drying a weighed sample of garri to a constant weight in a mechanically ventilated oven at 105° C for 24 hours, the moisture contents (M.C.) of the samples were ascertained. The samples were dried and weighed several times to ensure a steady weight. On a dry weight basis, the percentage moisture content was calculated. Three replicates on average were found. In the laboratory, samples that were not immediately used for analyses were kept in insect-proof hessian bags at room temperature for up to three months before being reanalyzed for moisture content. Data on relative humidity for the research area and time periods were obtained from the relevant governmental organisations.

Determination of Water Activity of Samples

Using the techniques outlined by Landrock and Procter, the water activity of the samples was determined. At 20°C and 30°C, samples were

equilibrated with the proper saturated salt solutions. Both of these values were chosen as a guide to represent the low overnight temperatures that can be found during the cold months of November–January/July–September and the mean daily indoor temperatures, respectively, across much of Nigeria's savanna and rainforest. The product will often be stored at a temperature that falls between the two. The employed sample of garri (10 g) was insufficiently little to have any impact on the salt solution's saturation behaviour. Data on the amount of water lost or gained per gramme of the samples were recorded to measure their water activity. The amount of water that test samples keep at various equilibrium relative humidity levels might gain or lose. The sample's water activity is represented by the plot that crosses the line denoting zero moisture content. As equilibrating solutions, nine saturated salt solutions were chosen. The constant relative humidity settings they provide range from 20 to 97 percent and include CH₃COOK, MgCl₂, K₂CO₃, NaNO₃, NaBr, NaCl, KCl, BaCl₂, and K₂SO₄. To produce the requisite ranges of water activity, 200 mL volumes of each separate equilibrating solution with extra salt crystals were placed at the base of the desiccator in triplicates. The amount of salt solution was chosen so that the salt's saturation condition was not altered by the amount of moisture the sample absorbed or lost. The desiccators' environment and salts were allowed to acclimate to one another over the course of one night.

Preparation of Mould Isolation

According to the manufacturer's instructions, potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) (Oxoid) were made. Malt extract agar (MEA) and modified Czapek Dox agar (CDA) with 20–40% sucrose as moisture content modifiers were made in accordance with Pitt, Christian, and Smith's instructions. According to Medina, Magan, and Bekada, the water used to make the medium was changed to the necessary water activity level.

Isolation of Moulds

A sterile 500 mL beaker was used to aseptically weigh 10 grammes of each sample into 90 mL of 0.1 percent (w/v) sterile peptone water. The samples were then allowed to stand for 5 minutes while being occasionally stirred with a magnetic stirrer. After that, samples were diluted 10-fold in serial, and 0.1 mL of each dilution was plated on PDA with 50 g/mL of chloramphenicol added. Incubation of the inoculated plates took place in clear, airtight jars that also contained flasks of saturated salt solution with water activities that were roughly matched to those of the media and sample being incubated. According to the information and procedures provided by Robinson and Stokes, controlled water activity solutions were created. Along with the diluted samples, grains of garri samples were also directly dusted on media and incubated.

Identification of Mould Isolates

Based on cultural features found on SDA, CDA, and MEA, mould isolates were identified. After slide culture on SDA, CDA, and MEA, isolates were examined under a light microscope. Samples were stained as necessary with lactophenol cotton blue. Examination of the conidial heads, phialides, conidiophores, and the presence of foot cells or rhizoids allowed the fungal isolates to be identified. The rate of isolate growth was taken into consideration during identification and was measured as colony diameter after 3, 5, and 7 days on SDA, CDA, and MEA at the proper temperatures.

Water Sorption Characteristics and Mould Isolation

By exposing garri samples to atmospheres with known equilibrium

relative humidities, the water sorption isotherms were calculated gravimetrically. To allow the interior to equilibrate, the airtight glass desiccators were kept in an incubator at 20 or 30 C for 12 hours. On a plastic platform inside the desiccators, two parts of garri, each weighing 10 g, were placed in open petri dishes with a diameter of 5 cm. The samples were taken out and weighed every 72 hours until the mass remained constant for three consecutive measurements. By drying one portion to a constant weight in an oven at 105° C for 24 hours, the equilibrium moisture content of the samples was ascertained at the water activity point. Because the volume of the solution utilised was sufficient, moisture obtained or lost by the sample being condition did not change the makeup of the controlling solution [10]. After reaching equilibrium weight, one portion of 10 g was inoculated onto the proper plates and stored airtight for three months at room temperature in screw-capped sample bottles before being used to isolate the mould.

Results

Quadrat Surveys

A total of 240 quadrats were surveyed (150 at Agasthyamalai Hills and 90 at Anamalai Hills); 60 during the postmonsoon and 90 during monsoon, respectively. During the survey period, the elevation ranged from 54 to 1079 m a.s.l., the air and soil temperatures were respectively 20.4 and 36.8°C and 18.5 and 34.6°C, the humidity ranged from 36 to 90 percent, and the light intensity ranged from 0 to 1026 lux. Dry and wet deciduous, semievergreen, evergreen, and riparian forests, as well as grassland and plantations, were among the vegetation types sampled (including cashew, cocoa, coconut, rubber, and teak).

The two focal species occupied 18 of the 240 quadrats that were surveyed. Compared to *V. silvatica*, which was found in three quadrats at a density of 0.03 individuals per hectare, *Indotestudo travancorica* was found in 15 quadrats.

Additionally, thirteen live and nine carapaces of *I. travancorica* were discovered when travelling to the quadrats. Six adult charred carapaces were discovered on a hill in the Vazhachal range, one adult carapace was discovered next to a forest stream in the Kulathupuzha RF, and a third adult carapace was discovered in a pit that the Kerala State Electricity Board had dug and abandoned after using it to install cable posts in the Athirapilly range. In the Vazhachal range, a juvenile *I. travancorica* carapace was discovered with marks left behind by an unnamed carnivorous species.

Discussion

Population Estimates

Our estimations indicate low population densities for both species, which contrasts with earlier research where *V. silvatica* was discovered at densities between 0.1 and 0.6 individuals per hectare and *I. travancorica* was known to be more ubiquitous than *V. silvatica*. A different sample technique, the timing of the poll, observer bias, or sparse distribution could all have contributed to this. Additionally, it could have been caused by the collecting of chelonians for consumption by local communities and the impact of forest fires, as was the case in the Chalakudy Forest Division where three or four significant forest fires had occurred since previous research.

According to the ecosystems they coexist in, our research indicates that *I. travancorica* is in fact more common and numerous than *V. silvatica*. Additionally, this is happening at the same time as parallel community interview surveys. In contrast to *Indotestudo travancorica*, which was mostly connected to medium canopy cover, *Vijayachelys*

silvatica was found at slightly higher humidity, lower light intensity, and more frequently in areas with high canopy cover. This suggests that *Indotestudo travancorica* may be more closely linked to water sources than *V. silvatica*. When compared to *I. travancorica*, *Vijayachelys silvatica* was more active on level ground.

Vijayachelys silvatica

Similar to earlier studies, the majority of the creatures observed were not near water sources and, because they were crepuscular, were inactive when found in leaf litter in the late afternoon. *V. silvatica* is reported to be a habitat specialist, occurring in both evergreen and semievergreen vegetation, despite the fact that we usually encountered it in semievergreen vegetation. All of the wild animals were discovered inside or adjacent to clumps of bamboo or cane. A few studies have suggested that bamboo and cane were present in the habitat of *V. silvatica*, but other research suggests that there may not have been a strong connection. It was discovered that the turtles lived in damp regions, which might encourage the growth of algae on their carapace. In contrast to other investigations, the wild individuals were also located in locations with little herb and shrub cover.

Indotestudo travancorica

Since the species is known to be crepuscular, it is possible that this is why the majority of the animals we observed during our studies were dormant. As the cloacal temperatures were greater than the air and soil temperatures during these times, it is also possible that the tortoises were more active in the evenings and during the monsoon season. gender differences exist in shell height but not in anal fork length since we did not measure it. Bridge length variations were also discovered. As previously reported, individuals were found to be predominantly using leaf litter, followed by leaf litter, bark, and rock/mud cavities to conceal themselves.

Threats and Chelonian Conservation

According to the study, younger *I. travancorica* individuals are gathered and raised until they are of a size that makes them appropriate for food, while larger individuals are eaten right away by local communities. Comparing *V. silvatica* and *I. travancorica*, it was discovered that human collection and associated consumption were far less common, which may be related to harder detection or smaller population sizes. The lack of forest produce in the area where it was found and local taboos of ill luck could possibly be to blame. This is similar to how people perceive the slim loris, *Loris lydekkerianus*, which discourages people from eating *V. silvatica*. Since larger individuals were not discovered throughout our surveys, *I. travancorica* ingestion from the wild may be having an impact on some populations. Field scans in Peppara and Neyyar WLS yielded either nil or a single detection in a study conducted two decades earlier, but similar surveys near human settlements yielded more detections. In particular, since *I. travancorica*'s natural growth and survival rate may not be high, efforts must be made to cut back on the gathering of adult females and boost current populations through ex situ programmes. The fact that road deaths are relatively infrequent right now relative to the volume of human consumption may also be the aim of insufficient surveys. However, in order to prevent incidents of chelonians and other species being killed on the road, vehicle traffic around the Athrapilly waterfalls should be planned ahead of time. Chelonian mortality from development operations provides justification for the need for future forest-related actions.

Conclusion

Chelonians have not completely disappeared from the landscape despite being used historically and currently by indigenous populations. The majority of the wild chelonians in the area were discovered far from human habitations, suggesting that current consumption rates may have contributed to local extirpation around habitations. To stop the continued decline of these species, local populations' use of the resource may need to be curtailed. Such a policy would need to be formulated through extensive consultations with local communities on the sustainable harvest and management of this natural resource.

Garri's moisture sorption isotherm displayed sigmoidal forms classified as type II, typical of dry foods high in carbohydrates. With increased water activity and reduced with rising temperature, garri's equilibrium moisture content rose. This investigation demonstrated the presence of several moulds, especially at greater moisture concentrations, in garri sold in the market. This is made worse by the unhygienic yet common practise of selling and distributing garri in Nigerian markets in open basins, trays, and mats. Given the range of ways the product is consumed and the requirement for a longer shelf life, these results underline the necessity for more attentive handling of the product to ensure its wholesomeness. Packaging is required, especially in modified environment or moisture-proof bags, especially in places with high humidity.

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Conflict of Interest

The author has no known conflict of interest associated with this paper.

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