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Salt Excretion Rates in the Galápagos Marine Iguana (*Amblyrhynchus cristatus*): Is there a Relationship between Body Size and Salt Excretion Rate?

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Salt discharge frequency of marine iguanas relative to body size

Introduction

The Galápagos Archipelago is a place that is known throughout the world for the contributions Charles Darwin made to science by observing the wildlife on these isolated islands in the Pacific Ocean. Through his observation of wildlife, Darwin was the first person ever to suggest that observed geographical differences could cause speciation in animals and plants. The Galápagos Archipelago, and other isolated islands worldwide have been shown to allow evolution to occur through natural selection separate from the mainland (Gross 2006). An example of such isolated evolutionary processes is the island rule, which pertains to gigantism and dwarfism on isolated island areas (Gross 2006). The Island rule, in relation to gigantism, states that small animals, who lack predators on a specific island, will evolve into larger versions of themselves, outgrowing their relatives on the mainland (Gross 2006). On the other side of the island rule is dwarfism. Dwarfism occurs when large animals, namely mammals, enter an island archipelago, and the range in which they usually roam is decreased. These animals are often adapted for large roaming areas in which they can cover a lot of ground. These large areas are not present on small island ecosystems. Due to this decrease in area, these animals evolve to a smaller size, which gives them a better chance at survival on the smaller land area available on the islands (Gross 2006). The island rule is just one example of how evolution occurs in an isolated island system separate from the effects of the mainland.

Island Archipelagos are isolated from the nearest mainland by oceans. Due to this separation from the mainland, these Archipelagos are unaffected by mainland pressures. These pressures would include things like predators who are unable to survive in the island environment, diseases spread in mainland species groups, and natural events such as droughts that do not reach the islands. Without the presence of these evolutionary pressures, evolution can occur in a simpler ecosystem, which allows us to observe direct consequences of a change more easily in environmental pressures on the island or islands (Itescu et al. 2014). The Galápagos Islands are where Charles Darwin, who observed adaptations in the Galápagos finches on different islands, began to form his theory of evolution and the way that survival of the most fit within a species can affect phenotypes in the next generation. Finches in the Galápagos have been shown to adapt at a population level in response to their environment. An example of this is seen during years of drought. During such years, finches with thinner beaks struggle to find food because the plants with seeds small and soft enough to be eaten by finches with thinner beaks, have been already been eaten and the dry weather prevents a growth season, which means the soft seeds cannot be replaced. Therefore, larger, and harder seeds are the only types left at the end of the drought period, and only finches with thicker, stronger beaks have an advantage for cracking these seeds (Grant and Grant, 2014). The finches with thicker beaks survive at higher rates, and, since beak shape is heritable, these finches can pass on their genes to their offspring, who will have the same genetic advantage as their parents (Grant and Grant 2014). During the drought of 1973 in the Galápagos, the average beak depth increased by about 2 mm. This process is an example of adaptation. The Galápagos Islands are one

of the few places in which you can see the animals continuing to adapt to their environment, and scientists are able to observe and record these adaptations over time. The simpler ecological circumstances of the Galápagos Islands provides a unique glimpse into evolution on a broad scale.

One of the most recognizable animals in the Galápagos Archipelago is the Galápagos marine iguana, *Amblyrhynchus cristatus*. They are one of four species of iguana in the Archipelago. These four species include the Santa Fe land iguana, *Conolophus pallidus*, the Galápagos land iguana, *Conolophus subcristatus*, the Galápagos pink land iguana, *Conolophus marthae*, and the Galápagos marine iguana (CDRS, 2006; Bennett, 1975). Iguanas have been living in the Galápagos Archipelago for fifteen to twenty million years, which predates the current islands themselves (Rassman, 1997). It is believed that all the iguanas currently found in the Galápagos have a common ancestor from Central America and that originating species was a species of land iguana. The ancestral iguanid probably arrived in the islands via a raft of floating vegetation and colonized islands that are no longer above the ocean's surface. Scientists have found seamounts of the same volcanic origin as the current islands and these islands are now found as eroded underwater remnants to the north and east of the current islands (Grehan, 2001). Ancestral iguanas that lived on these islands were forced to adapt to their decreasing habitat area and swam to newly forming islands. Over millions of years, these organisms evolved into the marine and land iguanas we see on the islands today.

Marine iguanas have evolved the ability to swim and dive in ocean water for the purposes of feeding (Bennett et al, 1975). They are the only iguana in the world, that

can swim (Bennett et al, 1975). Marine iguanas feed on green algae that grows on the rocky shores of the Galápagos Islands and tend to feed at or around low tide (Bennett et al, 1975). This is because the only iguanas that can swim and dive sustainably in the strong currents of the Pacific Ocean are the large, developed adults. The larger iguanas can swim and dive because their surface to volume ratio allows them to withstand the cold temperature of the water, something the smaller iguanas cannot do. The smaller adolescents prefer to feed at low tide when the green algae is most exposed and accessible away from the water on the shoreline (Bennett et al, 1975). This is because the smaller iguanas cannot handle the cold ocean water, which pulls heat from their body at a high rate. Marine Iguanas ability to swim, dive and eat algae is due to the mechanism of natural selection, resulting in various adaptations that allow them to spend part of their life as an aquatic organism. handle harsh conditions of the Pacific Ocean.

One additional adaptation that the Galápagos marine iguana has is a salt gland in their head with tubes connected to their noses for evacuation. Salt glands are necessary for reptiles and birds that spend time searching for food in saltwater (Figure one). Reptiles are not capable of creating liquid urine more concentrated than their body fluids and are therefore limited in their urinary electrolyte excretion. Like birds, many reptiles excrete nitrogenous waste as insoluble uric acid or urate salts (Hazard, 2007). The three ions that are secreted from this gland are sodium, chloride, and potassium (Dunson, 1969). Mostly Potassium (cation) and some sodium (cation) ions can be bound as insoluble urate salts; however, chloride (anion) ions must be excreted in solution (Hazard, 2007). Reptiles with salty diets may take in excess ions. This is

especially true if freshwater availability is limited, as is often the case in desert habitats such as the littoral and arid zone on some of the islands in the Galápagos. These glands allow the organisms to excrete excess salt to balance out the ions in their bodies, because they are unable to secrete a large enough concentration through their urine (Dunson, 1969). The salt glands in birds and lizards are both located above the eyes down to the front of the nasal area (Dunson, 1969). All marine iguanas feed on algae that is either below the ocean's surface or revealed at low tide. The Galápagos marine iguana's ability to use this gland has allowed them to become the only known lizard that has adapted to feed in a salty environment.

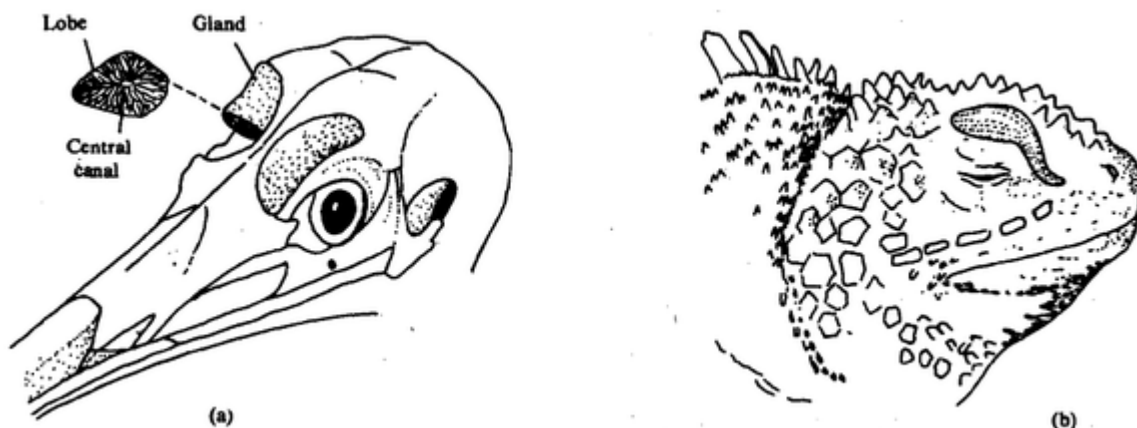


Figure One: A. An avian nasal salt gland. Image shows the location of the salt gland above the eye in the nasal area of the bird. B. A marine iguana salt gland. Image is a side view of the shaded gland above and in front of the eye on the head of the marine iguana (ExpertsMind.com, 2019).

After observation of marine iguana behavior, we however have decided to look at the relationship between marine iguana size and the rates at which they secrete excess sodium ions. We were curious to see if excretion rates have a correlation between animal size, tide level and feeding time. We predicted that if the tide is low, but coming in, then larger marine iguanas will have a higher average discharge rate than medium and small marine iguanas. We believe that the larger iguanas will secrete more salt

because they spend more time feeding on the submerged algae as compared to the smaller iguanas. Small iguanas will have the lowest rate of excretion, because they are not yet good swimmers so they only feed algae far away from the waterline.

Methods

We observed Galápagos marine iguana salt discharge frequencies on San Cristóbal Island, Galápagos Islands, Ecuador. The Galápagos Islands are located about 1000 km west of Ecuador on the South American continent. Our location study site was north of the Nativo restaurant (on the ocean side of the Malecon), where the peninsula of lava rock meets the man-made stone wall (Figure two). This restaurant is in Puerto Baquierzo Moreno, San Cristóbal, Galápagos Islands, Ecuador. The study zone extended 9.1 meters from the peninsula/rock confluence point and extended out from the manmade wall for 5 meters. We stood atop the stone wall at different viewing points at the edge of the research zone.



Figure two. Image shows the observational zone utilized for observation north of the Nativo restaurant. The zone is 9.1 meters wide and 5 meters extended from the man-made wall pictured.

Two hours were spent researching here each day for a total of 7 research days. We recorded the number and size of marine iguanas that entered our zone for the whole or part of the study time of that day. We classified each individual iguana in our research zone as small, medium, or large. Small iguanas were a length of 12 inches or smaller. Medium iguanas were between 30 cm and 61 cm long. Large iguanas were 61 cm long or larger. The classifications were estimates based on comparing iguanas to shoe length and then this information was converted to a metric measurement. This is due to the iguanas being protected and our inability to measure them accurately from a distance. Once classified, iguanas were observed for the duration of time that they remained within our research zone, or until our research duration ended for that day. The time of each salt expelling event was recorded for each iguana. The iguanas were observed, and it was recorded every time that the iguanas would excrete salt through their nostrils. These excretion events were tallied and recorded under the size classification that the individual iguana fit into.

Results

We conducted our study of marine iguanas for a total of 7 days. In those seven days we saw a total of 168 marine iguanas pass through or inhabit our zone. There were 36 marine iguanas that we classified as small in our data collection. There were 102 medium sized marine iguanas and there were 30 large marine iguanas included in our final summed data. We saw the marine iguanas spit a total of 358 times. The entire

observed population of marine iguanas spit an average rate of 2.13 times per individual per hour.

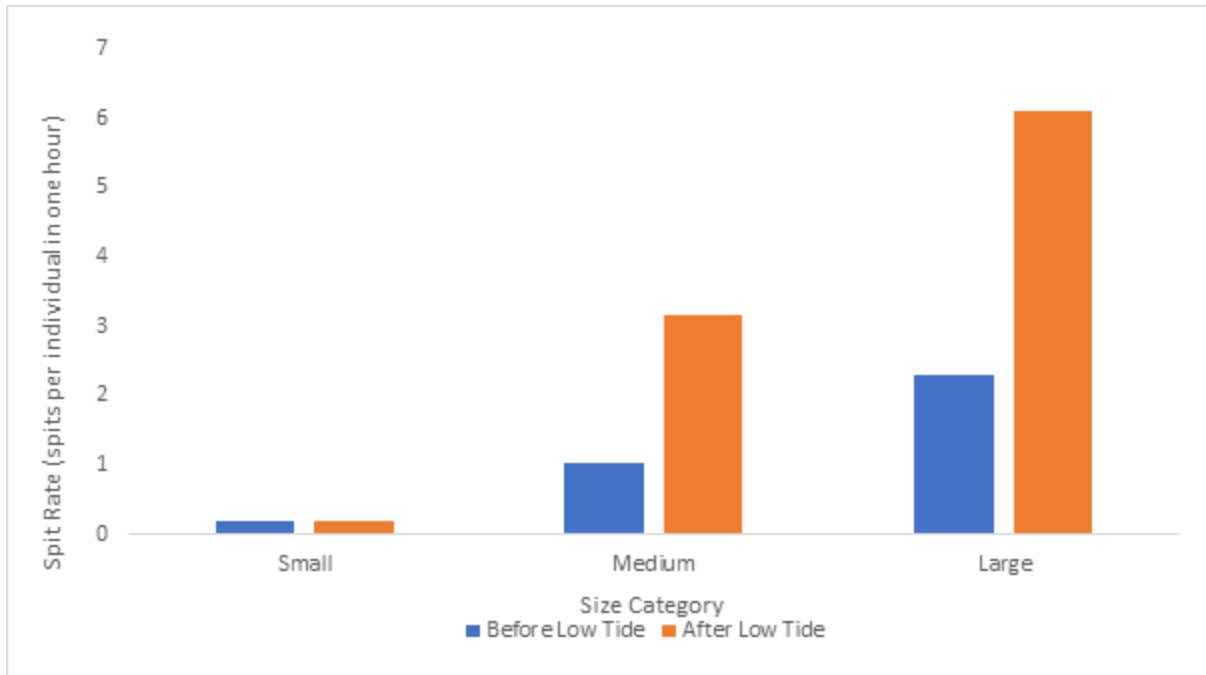


Figure three. Mean spitting rate per individual during average for the entire research period. Data collected north of native restaurant in Puerto Baquerizo Moreno, San Cristóbal, Galápagos Islands, Ecuador. (figure two)

The overall mean spitting rate for all sizes of marine iguanas during the entire study was 2.17 spits per hour (Figure three). According to our average data, small iguanas spit at a rate of 0.19 spits per hour before low tide and 0.2 spits per hour after low tide. The medium iguana spit at a rate of 1.04 spits per hour before low tide and at a rate of 3.17 spits per hour after low tide. The large iguanas spit at a rate of 2.31 spits per hour before low tide and spit at a rate of 6.12 spits per hour after low tide. Due to insufficient data caused by short research periods, limited research time, and small absolute values of spits, we are unable to run any statistical analysis on our collected data.

Discussion

We observed the number of salt excretion events from many marine iguanas. Unfortunately, we were unable to analyze our data (due to small sample size), but, when viewing the compiled data, two patterns seem to stand out. One pattern, that we referred to earlier in our raw data, is that marine iguanas will have a higher rate of salt excretion events after low tide than they do before low tide. We believe this is due to their feeding at low tide. The green algae being taken in, either submerged or not, is covered in saltwater, which increases the salt concentration in the iguana's bodies as they consume the algae. This higher concentration leads to the necessity of more salt excretion events to keep the iguanas salt levels in check.

Another pattern we saw was the effect size has on rates of salt excretion by the marine iguanas. The marine iguanas that fit into the large classification of our study spit at a rate twice as high as the medium classified marine iguanas did, and a rate at least six times higher than the marine iguanas classified as small. We believe that this is due to the difference in feeding practice that large iguanas have compared to the medium and small. The large marine iguanas willingly feed on algae while fully submerged in the water. We believe that this causes them to intake a higher level of salt than if they were feeding on algae that was not submerged. This higher rate of salt intake causes them to spit at a higher rate than the medium and small sized iguanas.

Thanks to research done on the number of electrolytes excreted during an iguana's discharge, information on ion excretion amounts per discharge for different sized marine iguanas was documented by Dunson (1969). According to the research's results, medium iguanas had a higher total loss of all three ions (chloride, sodium, and

potassium) per discharge compared to both the large and small iguanas, with small iguanas losing the least (Dunson, 1969). It is likely that large iguanas, due to discharging less ions per spit than the medium iguanas, must discharge more frequently to remove the excess electrolytes. This would support the data we gathered in our research. Continuing this logic, because the small iguanas only intake a small number of electrolytes and discharge a small amount per spit, then it is not necessary for them to spit frequently. Based on the information presented in the study and the trends we saw in our data; we believe that our conclusions on size's effect on secretion rates are supported.

Another study done on iguanas around the world has resulted in information on the control of salt excretion rates and what causes the rates to increase. Researchers have determined that the initiation of secretion is controlled by the parasympathetic nervous system (Hazard, 2006). The causes of excretion rate changes have been determined for the desert iguana. The desert iguana will increase its salt excretion rates in response to increased potassium and chloride intake, but not to sodium (Hazard, 2006). In comparison, the green sea turtle increases its salt excretion rate in response to an increased amount of sodium being taken in (Hazard, 2006). This research shows that environmental factors can affect the rate at which salt is excreted in several organisms that rely on salt excretion for ion balance. Sea turtles, living in a sodium rich environment, will increase their salt excretion rates when their sodium levels go up. If this is the same for the marine iguana, who are semi aquatic organisms that eat algae that has been submerged in water, then this information would support the pattern in our data. Our data showed that marine iguanas had more salt excretion events after low

tide (feeding time) than before. The marine iguanas are most likely taking in more sodium during their feeding times, and so in response to that, they increase their salt excretion rates to remain balanced.

Possible factors that could have affected our data include the weather. We noticed on the days that were misty and overcast, the iguanas seemed to excrete salt less frequently. We believe this may be due to them trying to conserve any energy currently in their system for the activity of feeding. It is also possible that their ability to absorb heat energy from sunlight helps them process the salt more easily, and without that sunlight, they were unable to excrete anything.

Other factors affecting our research may have included viewing angles. Due to being limited to staying behind the guardrail of our observational area we had limited viewing angles. This undoubtedly caused us to miss some discharges when the iguanas were behind rocks and out of our field of view. If this study were to be repeated, it would be beneficial to have a larger number of researchers involved for the same field of view. It would be ideal to have one researcher per iguana for the entire two-hour period, but even breaking it down to 2-3 iguanas per researcher would most likely increase the reliability of the data.

One study that would be beneficial in furthering our research would be a study on the weather's effects on the frequency of spits. This observational study would help us to gain further evidence on whether the presence of the sun's direct heat caused a shift in spit frequency. Along with this, time of day, and intensity of the sun at that time of day, could also be recorded as an even more specific measure. If this study were to be carried out, a larger number of researchers as well as study sites would be employed to

ensure the data we collect can be properly analyzed. If this study provides evidence of different spit frequencies based on the weather and sunlight, it might be evidence that marine iguanas prioritize energy conservation over ion balancing.

Our results showed two trends that are relevant to the overall research already done on marine iguanas. The trends were found show that large marine iguanas excrete salts at a higher rate than their smaller counterparts, and that all marine iguanas were seen to increase their rate of excretion immediately after low tide. We know that marine iguanas feed at low tide, and they use their excretion glands to maintain the balance of ions in their body. Based on this knowledge, and the trends that we can see in our data, we conclude that salt excretion occurs at the highest rate right after feeding. This is important to understand because it allows us to infer about the marine iguana's salt processing and the efficiency at which they process salts. Based on our data, we suggest that the iguanas begin to process the salts they intake as soon as they are able, and they efficiently move and excrete those salts as fast as they can so they may retain their body's salt balance. Marine iguanas are specially adapted for their specific environment and have found ways to electrolyte balance. Our results provide additional evidence for the adaptation and specialization of the marine iguanas to their environment.

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