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Paternal Care promoting offspring survival in Costa Rican Frogs

Costa Rica, famous for its biodiversity, is home to a highly dense population of anurans. Anurans are amphibians without tails such as toads and frogs. There are 133 known species of frogs in Costa Rica, and each can be sorted into one of six families; glass frogs, poison-dart frogs, tree frogs, leptodactylidae, true frogs, and narrow-mouthed frogs (Badger). While each of these families differ, they are similar to one another because they all undergo metamorphosis. Most frogs begin their lives as vulnerable eggs, hatch into squirmy tadpoles entirely dependent on water, and finally transform into four-legged adults who are well-suited for terrestrial life. This life cycle has many different renditions, especially in Costa Rica. This country's lush environment is home to the highest density of frog populations in the world. Frog species can be found on every continent except Antarctica, but even so they are undergoing a mass global extinction (Kriger). Frogs are extremely threatened due to habitat destruction and a disease epidemic caused by *Batrachochytriumdendrobatidis* (chytrid fungus). If frogs go extinct, then the natural and human world will both face the repercussions. Humans rely on frogs not only as a food source, but also for scientific research and medical remedies. Finding a way to preserve frog populations would benefit mankind and Mother Nature alike since frogs play such an integral role in the global ecosystem. The evolution of paternal care among different frog species

is helping to preserve frog populations by promoting offspring survival, especially in tropical environments such as Costa Rica.

Frogs are recognizable by their large eyes, sensitive skin, powerful back legs, and strong vocals. They are ectoderms which means they rely on external sources like the sun to power their metabolic functions (Wells). Their absorbent skin is especially permeable to water, oxygen, and carbon dioxide, and it makes them vulnerable to toxins in their environment. Frogs' skin is also what enables them to breathe in water; they diffuse oxygen directly into their bloodstream (Dugas). On land, they are able to breathe using buccal pumping similar to lungs (Wells). It is because of their fragility that they are considered an imperative indicator species- their health is representative of the entire ecosystem's health. They are also an integral part of the food web, playing the part of both predator and prey. The disappearance of frogs would be detrimental to the entire food chain (Kriger). They are carnivorous and keep insect populations under control. Most frog species have evolved some sort of protection mechanism to evade predators, the most prevalent being toxic skin secretions, camouflage, and escape maneuvers.

Frogs are significant not only to the environment, but also to humans. Their unique characteristics have led to many scientific and medical breakthroughs over the last century. Their skin secretions have been used to develop numerous medicines and are prized by scientific researchers. For example, the poison dart frog contains chemicals that have been used to develop a pain killer that is 200 times stronger than morphine (Kriger). These invaluable chemicals can only develop within frogs in their natural environment and are exceedingly difficult to reproduce in captivity. The waxy monkey frog is able to treat anti-biotic resistant staphylococcus. The Whites Tree frog contains a medicinal compound capable of stopping the transmission of HIV. The now-extinct gastric mouth brooding frog would have been an invaluable resource in

finding a cure for stomach ulcers because it had the unique ability to stop the production of its gastric fluids for long periods of time (Kriger).

The developmental processes of frogs have also been important for scientific discovery. Their transparent eggs are an invaluable tool for learning about genetics, cells, and fertilization because they are easy to manipulate and observe. Frogs were used in the development of the first pregnancy test. They have also been used in anatomical education and in the development of lifesaving medical procedures such as skin grafts (Badger). Frogs have the potential to save many human lives, but the global amphibian population is declining. As each new frog species becomes extinct, the possibility of using it to develop a new life saving medicine disappears with it.

Factors which contribute to the ongoing, global extinction of frogs include habitat destruction, pollution, invasive species, overharvesting, climate change, over-collection of specimens, and diseases. Chytridiomycosis, the most devastating disease among frog species, is caused by the Chytrid fungus. This epidemic affects frog populations all over the world, and only two species appear to be unaffected: the American bullfrog and the African clawed frog. These two species are also believed to be at fault for the fungus's spread worldwide. American bullfrogs are shipped worldwide regularly due to their high value in the food and pet industry, and the African clawed frog was once used by doctors to test for pregnancy (Batrachochytriumdendrobatidis). This primitive pregnancy test consisted of the injection of human urine into the back leg of a frog, which would cause it to produce eggs if pregnancy hormones were in fact present in the human sample (Kriger).

Research is being conducted to determine how these species are resistant to the chytrid fungus. The current belief is they either have genetic resistance, or they produce high levels of

antimicrobial peptides that suppress the growth of the chytrid fungi (Batrachochytriumdendrobatidis). More than 695 frog species in 71 counties have suffered the ill effects of this serious ailment (Kriger). There are rare occurrences of "persistent" populations who have survived despite the mass deaths among their species, but it is unknown if these isolated populations will be able to regain their original numbers (Chytrid).

Costa Rica is one of the countries affected by the chytrid fungus, which poses a significant threat to frog conservationists because of the country's high density of anuran species. There are multiple organizations in Costa Rica and Panama working to create an "ark" of amphibians to ensure their survival. Frogs in Central America are especially susceptible, because wet, highland rainforests with temperatures held constant around 77º F are ideal for the growth of the chytrid fungus (Batrachochytriumdendrobatidis). The disease spreads rapidly through the fungus's swimming zoospores, and is transmitted easily by mediums of water as well as by direct contact with infected moist materials such as soil, leaf matter, or another infected animal.

The fungus affects the keratinized epidermis by disrupting the normal functioning of the frogs' permeable skin. It is believed the fungus releases harmful enzymes which prevent the uptake of important electrolytes, leading to insufficient nutrient and oxygen uptake (Batrachochytriumdendrobatidis). The skin experiences excessive cell death, sloughing off, hyperkeratosis, and color change (Peisser). Behavioral changes associated with the disease include loss of energy, slowed reflexes, seizures, and the transition from nocturnal to diurnal activity (Chytrid). The fungus targets the keratinized epidermis primarily located on the digits, ventral body and underbelly of adult frogs. Even premature life stages can be affected by the fungi as they develop; tadpoles typically develop keratinized skin first around their mouth parts (Chytrid). Once the frog is infected, death can occur in as little as 21 days.

There are many species of terrestrial, arboreal frogs in the ecosystems of Costa Rica. A portion of these terrestrial frogs have acquired behavioral traits that benefit the survival of their species by caring for their offspring. With declining numbers of adult frogs, it is crucial that offspring reach sexual maturity in order to keep current populations afloat. Paternal care is an energetically expensive behavior that appears to have evolved in anurans from an ancestral state of no parental care or from maternal care (Wells). Predominately, female frogs invest more time and energy into offspring care than their male counterparts because of their respective definitions of reproductive success. Female reproductive success is based on egg production. Male reproductive success is based upon sperm production and quantity of female mates (Wells). The transition to paternal care emerged from an accumulation of necessary and favorable traits. The majority of species exhibiting paternal care are territorial, arboreal species. Frogs exhibiting behaviors of paternal care are concentrated in the tropical rainforests and help to increase the egg survival of offspring from predation and desiccation. Paternal care depends on paternity assurance, the energetic costs of egg attendance, and availability of successive mating opportunities. Common parental behaviors include egg guarding, hygienic filial cannibalism, egg fanning, egg brushing, egg brooding, provisioning and preparation of the oviposition site.

In order for paternal care behaviors to have evolved in these terrestial species, similar prerequisites most likely occurred. The ancestral state of frogs consisted of no parental care until there was a transition of sex position from inguinal to amplexus (Wells). Amplexus is when the male firmly grasps the female and fertilizes the eggs as they emerge, providing paternal assurance. Males are more likely to sacrifice future copulation and feeding opportunities for offspring that they know is their own. All anurans utilize external fertilization except the Puerto Rican Coqui frog and the North American tailed frog, who have acquired body parts which make

internal fertilization possible (Wells). Another divergence from the ancestral state was the employment of terrestrial oviposition rather than aquatic oviposition. Nonaquatic oviposition is thought to have evolved from avoiding placing eggs in oxygen-poor environments such as warm, swampy pools or places with high predation (Wells). The evolution transition of becoming, terrestrial and arboreal species involved physical changes in eggs, too. Oxygen availability in terrestrial eggs depends on temperature, metabolic rate, and rate of diffusion. In oxygen-poor sites, small eggs are favored and the clutch size correlates with the mother's size. In oxygen-rich sites, large eggs are favored, but there is a tradeoff seeing as less eggs are able to be laid (Wells).

Males becoming territorial is a factor which influenced the development of paternal care because it led to egg guarding and brooding. Influences such as high egg death rate, slower male maturation, low survival of juvenile males, higher death rates along adult males, and female selectivity caused males to invest more in existing offspring rather than obtain more offspring (Wells). The Overlapping Broods Hypothesis suggests females find males more attractive if they are already guarding eggs because they exhibit potential to be a good father. Statistically, there is a lower chance that a female's offspring will be eaten by predators if larger numbers of offspring are raised together (Gilbert).

Filial cannibalism is another example of the fitness tradeoff a father endures during parental care. When an egg becomes infected with fungus or mold, the father sacrifices his own health for the sake of his future progeny. He will eat the infected egg(s) to prevent the harmful substance from spreading.

Egg brooding, similar to egg guarding, is a behavior exhibited by territorial frogs. The difference between the two is that egg brooding entails physically touching the eggs. Frogs are cold-blooded, so they do not incubate their eggs; they brood simply for protective reasons.

Glass frogs are native to the montane zone in Costa Rican rainforests and exemplify paternal care among terrestrial species. The male protects the eggs from predation and desiccation by egg brooding at the terrestrial oviposition. Typically, glass frog eggs are laid by the female on the underside of a leaf above a body of water and are then protected by the male until they hatch. The tadpoles fall into the pool below afterwards. Glass frogs are opportunistic maters and breed throughout the year. Paternal care by male glass frogs can be observed especially during the dry season, when desiccation is more probable (Dugas). During the day, the father frequently urinates on the eggs to prevent them from drying out and supplements oxygen diffusion through egg fanning. The eggs diffuse oxygen much like the skin of a frog thanks to their spherical shape, lack of calcification, and jelly-like protective covering. The father glass frog also prevents predation of parasitic flies and wasps. Another important behavior is egg brushing, which helps prevent developmental abnormalities. By agitating or "brushing" the eggs regularly, developing embryos are prevented from sticking to the wall of the egg. This promotes normal growth (Wells).

A more extreme version of egg brooding occurs within the hemiphractidae family of frogs, which is considered marsupial. Costa Rica's South Caribbean slope is home to the Horned Marsupial frog. The father will either fertilize the eggs inside the mother's egg pouch or use his toe pads to place the eggs in the pouch after fertilization (Darwin's). The female carries nine eggs on her back until they mature into adults. The Australian marsupial frog has the father rather than the mother chauffeur the young in his hip pockets. The Chilean Darwin frog exhibits neomalia, a fascinating version of male brooding where the father ingests the young and raises them in his vocal sac. The male Darwin frog can accommodate up to 19 eggs as they undergo metamorphosis inside his vocal sac ("Darwin's). Currently, there are no known species who

exhibit Neomalia in Costa Rica, but new discoveries are made each day in the world of frog reproduction.

The strawberry poison dart frog is a diurnal species, indigenous to Costa Rican rainforests such as La Selva. When a male and female of the species wish to mate, the male will takes her to an optimal oviposition site on either a leaf or in moist leaf litter. The female typically only lays 3-4 eggs at a time (Ringler). While the eggs are developing into tadpoles, the father will stand guard to protect them from other male strawberry poison dart frogs. He will engage in other behaviors such as filial cannibalism, emptying bladder water onto the eggs, and egg brushing (Badger). Once the tadpoles have developed, the mother will return to transport them one at a time to either a pool of water or a bromeliad plant. This is necessary for the further development of the young. There have been reports in this species as well as other poison dart frog species that the attending male will sometimes transport the tadpoles to water instead of the female. This suggests that the parenting responsibilities are more flexible than originally believed and that biologists have more to learn about the strawberry poison dart frog's reproductive processes (Ringler).

Paternal behaviors in terrestrial frogs such as egg guarding, egg brooding, hygienic filial cannibalism, egg fanning, egg brushing, preparation of oviposition sites, and egg placement are promoting the survival of offspring. Habitat destruction and the *Batrachochytriumdendrobatidis* are the two major threats to the global frog populations. The Chytridiomycosis epidemic is spreading rapidly and leaving destruction among the anuran species in its wake. There is no known cure for wild populations infected by this fungi. Frogs in captivity can be disinfected and treated with antifungals, but there is no known cure to use on wild specimens (Chytrid). There is scientific research investigating the introduction of a symbiotic bacteria to wild frog populations

that would prevent the growth of the fungi in the keratinized skin. In this world now plagued with the chytrid fungus epidemic, it is imperative that as many frogs reach adulthood as possible (Chytrid). Aside from fish, birds, and some insects, paternal care is an evolutionary oddity (Gilbert). Mothers in the animal world are thought to be the more responsible sex when it comes to caring for offspring, especially because of the energetically expensive process of egg production. Necessary traits such as external fertilization in the amplexus sex positon, the evolution of an improved egg shape and size, terrestrial egg deposition, territorial behavior, and favorable environmental pressures have led to the evolution of paternal care in frog species. The behavior of paternal care has evolved over generations and is now prevalent in terrestrial and arboreal frog species, especially those in Costa Rica. With the devastating rate of disease and habitat destruction, there may not be enough time for frogs to adapt in a new world of threats. The glass frog, the strawberry poison dart frog, and the horned marsupial frog are species that have developed beneficial behavioral traits that better suit them to survive in this new world. The more offspring that reach adulthood, the greater chance the species has to mate and survive another generation.

The extinction of frogs would be devastating to the natural world and modern society. Organizations such as Save the Frogs, Amphibian Ark, and Frogs are Green are dedicated to spreading awareness and helping frog populations around the world. Everyone can help by reducing his or her personal consumption, thus lessening the need for resources which contribute to habitat destruction. Eating organic foods and eliminating pesticide usage will do further wonders for these endangered aquatic critters. Now that one third of all amphibians are on the brink of extinction, it is imperative to take action by spreading awareness and following these simple guidelines.

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