5-9-2018

Importance of Amphibians: A Synthesis of Their Environmental Functions, Benefits to Humans, and Need for Conservation

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Submitted in Partial Completion of the
Requirements for Commonwealth Honors in Biology

Bridgewater State University

May 9, 2018

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May 8th 2018

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Abstract

Amphibians are among the most threatened species in the world and are subjected to a substantial number of studies that have underscored their ecological and anthropocentric importance. Yet a synthesis of those aspects is in need. In this study I conducted a comprehensive literature review to investigate the importance of amphibians, including medical applications such as tissue regeneration, biomimicry of pharmaceutically useful compounds, direct socio-economic benefits, and overall ecosystem values. It is my intention to promote amphibian conservation by detailing various ecosystem services provided by amphibians and their uses to humans. Amphibians have tissue regenerative abilities, such as the ability to regrow entire limbs as adults as well as larvae, and heal cardiac, brain, spinal, and retina tissues. Study of these processes could allow the medical industry to restore sight and mobility, and to remedy neurological defects, along with many other medical discoveries that are currently under investigation. Findings have emerged detailing amphibian polypeptides that release insulin, and others that raise and lower blood pressure, showing the full scope of their pharmaceutical value is just beginning to be explored. The ecological importance of amphibians includes their association with both aquatic and terrestrial environments where matter and energy are circulated between aquatic and terrestrial habitats. Their movement cycles essential nutrients such as Phosphorus, Carbon, and Nitrogen improving the overall health and resilience of the ecosystem. In many northern forests and vernal pools, amphibians account for a greater biomass than birds, mammals, and reptiles combined. They are a central part of many food webs being both predators and prey, and being poikilotherms, they turn a greater portion of their calories into biomass compared to homeotherms. Amphibians provide many predators with a stable food and nutrient source. The large number of prey eaten daily by amphibians make them useful regulators of biomass in lower trophic levels, contributing to ecosystem stability, as well as biological control agents against pests such as mosquitos, biting flies, and crop-damaging arthropods. Their thin skin and superficial vasculature make them sensitive to environmental pollutants thereby making them useful indicator species as well. This review can help the understanding of the conservation importance of these unique animals highlighting their potential in general environmental functions as well as in the biomedical industry, which then can be used as an impetus to promote and encourage conservation.

Introduction

Systematics and Biogeography of Amphibians:

Amphibians represent a unique group of vertebrates containing over 7,140 described species worldwide that demonstrate an intrinsic aspect of evolution, niche and natural history (Frost et al. 2006). The evolutionary and phylogenetic history of amphibians goes approximately 365 million years back (Carroll 1992). Amphibians evolved from either the lobe-fin fishes (*Crossopterygii*) or the lungfishes (*Dipnoi*) in the early Devonian Period and represent a transition step in the evolution of tetrapods (Carroll et al. 1999). Since then, amphibians were shaped and reformed under multiple selective environmental pressures, radiating into distinct life styles and body forms (Wells 2007). Multiple extinction events occurred through the evolutionary process of amphibians in the Carboniferous, Permian, and early Jurassic Periods, ultimately leaving a handful of evolutionary relics and highly-derived amphibians (Carroll 2009). Modern-day amphibians have diverged into three orders with distinct anatomical features: Urodela (salamanders), Anura (frogs and toads) and Gymnophiona (caecilians, limbless amphibians). Among all amphibians, anurans have the widest distribution across many biogeographical realms with the highest diversity in the oriental, neotropical and afrotropical regions; diversity of urodelans is prominent in the nearctic and neotropical realms; caecilians are restricted to tropical wet biomes and mostly diverse in the oriental and neotropical regions (Duellman and Sweet 1999, Sweet and Pianka 2007).

Natural History of Amphibians:

Amphibians are dependent on moist conditions and high humidity. Therefore, amphibian diversity is highest in regions with high precipitation and/or lower evaporative water loss (Duellman and Trueb 1986). Many require freshwater habitats to breed and develop into adulthood. A few amphibian clades have independently evolved to breed in foam nests; some clades have completely lost their larval stages and live an entirely terrestrial mode of life (Wells 2007). Amphibians have radiated into terrestrial, aquatic (streams, cascades, and wetlands), scansorial (arboreal, phytotelms, rock outcrops) and fossorial (leaf litter, organic top soil) niches in both the Old and the New worlds; they are also found throughout the elevation gradients in both topical, subtropical and temperate biomes with considerable niche diversification at different ranges of altitude (Duellman and Trueb 1986, Duellman and Sweet 1999). Thirty-nine modes of reproduction and development have been recorded among amphibians, including parental care, viviparity, and terrestrial direct development (Wells 2007). Most amphibians are generalist insectivores although a few species are known to be specialist predators of gastropods, earthworms, ants, and termites (Duellman and Trueb 1986). For most non-tropical amphibians, prey selection is season dependent (Dodd and Dodd 1976, Dodd 2010). Being poikilotherms and having a biphasic lifecycle with an aquatic larval stage, they encounter a wide range of environments and habitats, each with different physiological constraints (Gibbs 1998, Beebee and Griffiths 2005, Semlitsch et al. 2009, Duellman and Trueb 1986).

Global Amphibian Decline

Amphibians are undergoing global-scale population declines from a variety of anthropogenic factors. Habitat destruction and fragmentation, overexploitation, environmental pollution, climate change, and introduction of invasive species have all contributed to catastrophic amphibian declines across many biomes; however, these threats are disproportionately high in biodiversity hotspots such as tropical humid ecosystems (Sodhi et al. 2008, Pounds 1999, Young et al. 2001, Stuart et al. 2004, Gallant et al. 2007, Becker and Loyola 2008, Lin et al. 2008). The most critical factor leading to amphibian population declines is habitat destruction and fragmentation, which is the common denominator among many threatened species. However, amphibian decline has been recorded in well protected areas, including those that are relatively pristine and not directly affected by human activities.

Pathogens— such as the chytrid fungus and parasitic trematode genus *Ribeiroia*— are the culprit for such amphibian decline; the former is considered the leading cause of enigmatic amphibian decline while the latter causes limb malformation leading to behavioral abnormalities, which then predispose the infected amphibians to a greater risk of predation (Johnson et al. 2002, Green and Dodd 2007). Chytrid fungus is also responsible for mass mortality of amphibians in Africa, Australia, and Latin America (Lips 1998, Olson et al. 2013). While these pathogens have co-existed with amphibians throughout their evolutionary history, current trends in global environmental change (climate change, acid precipitation, ozone depletion, increased UV-B exposure, and altered biogeochemistry) and unintentional anthropogenic introductions have rendered amphibians immunocompromised making them more vulnerable to diseases (Kiesecker et al. 2001, Johnson et al. 2002, Daszak et al. 2003).

Globally, approximately one-third of all amphibian species are threatened with extinction, and almost half are experiencing precipitous population declines (Blaustein et al. 1994, Stuart et al. 2004, Beebee and Griffiths 2005, Mendelson et al. 2006, McCallum 2007). In recent decades, the extinction rate of amphibians worldwide has exceeded the historic background extinction rate at least by 200 folds (Roelants et al. 2007). According to the global amphibian assessment, approximately 34 amphibian species have gone globally extinct and many of them were endemic to those particular regions they were found in (IUCN et al. 2012). According to the IUCN et al. (2012) report, 552 species are considered “Critically Endangered”, 869 are “Endangered”, 679 are “Vulnerable”, and 399 are “Near Threatened” (IUCN et al. 2018). At least 42% of all species are declining in population size, which suggests that in the future more species will either become extinct or enter higher Red List categories (Stuart et al. 2004, Beebee and Griffiths 2005, Sodhi et al. 2008). Amphibian decline is a key global environmental concern that requires immediate conservation attention (Blaustein et al. 1994).

The Global Amphibian Assessment, IUCN Red List, and the World Congress of Herpetology (1987-2010) provide evidence for this crisis (IUCN et al. 2012, IUCN et al. 2018). The greatest degrees of threat for amphibian fauna has been recorded from tropical regions with high levels of amphibian diversity and endemism such as from South and Central America, the Caribbean Islands, south and Southeast Asia (Blaustein et al. 1994, Mendelson et al. 2006, Sodhi et al. 2008). The amphibian assemblages occupying tropical rainforests are also ecologically unique and evolutionarily distinct, therefore they should be prioritized for conservation, as they hold radically different skin polypeptides that co-evolved to combat tropical diseases that will be migrating into much greater ranges due to climate change (Bickford et al. 2007). The current era of research into amphibian declines was initiated in the First World Congress of Herpetology in 1989, though long-term monitoring programs extending more than 25 years back into the past (Blaustein and Wake 1990, Blaustein et al. 1994, Pechmann and Wilbur 1994, Blaustein and

Wake 1995). However, the records on precipitous amphibian declines are much more recent where declining populations have been documented for more than 500 amphibian species was noted within 5-8 years all around the world.

Maintenance of Ecosystem Stability and Resilience

Food Web and Energy Dynamics:

Most amphibians have a biphasic life cycle where different life-history stages are spent in different habitats, which includes aquatic, wetland, and terrestrial habitats (Wells 2007). In addition, different species of amphibians occupy different habitats given their evolutionary adaptations. Moreover, amphibian life-history and natural-history strategies diversify in terms of reproductive modes, oviposition modes, parental care, and feeding (Dodd 2003, Wells 2007, Dodd 2010). Therefore, a diverse array of amphibian niches make them an integral and critical part of food webs in different habitats and ecosystems (Duellman and Trueb 1986). The niche partitioning between the adult and juvenile stages of amphibians, particularly in terms of food selection leads to energy acquisition from different food sources, which enhances energy dynamics and energy flow in the ecosystem (Davic et al. 2004). Such niche diversification also prevents depletion of a particular resource as well as lowering intraspecific competition, which then leads to a greater availability of resources compared to organisms with equivalent biomass whose adults and juveniles feed on the same food source (Davic et al. 2004). Such aspects lead to ecosystem stability and ensure the continuity of all benefits amphibians provide to the environment. Anuran larvae are most often herbivores or planktivores, and thereby regulate the abundance and biomass of aquatic flora, including phytoplankton (Wells 2007). In contrast, adult anurans, caecilians, and urodelans are generalist carnivores primarily either insectivores or microcarnivores (Wells 2007). Their foraging activities regulate populations and abundance of invertebrate communities (Wells 2007).

Ecosystem stability is conferred because if a necessary food source becomes scarcer, this will only affect one life stage, while the others remain viable. Even if a food source becomes scarcer for a certain life-history stage, the other life stage would survive, which may help recuperate the population (Davic et al. 2004). For example if the larval stage of a salamander eats algae and the adult stage eats insects, if there is a sharp decline in the availability of insects and many of the adult salamanders die, there is a cushion time period while the aquatic larval salamanders are growing that gives the insect population time to recover, or even rebound from migration. Even if there are no insects present when the salamander fully metamorphoses, the stored energy from the algae will give them the ability to survive while they wait for an increase in insect populations. When insect populations rise again, the younger generation of salamanders can reproduce, and restock the population in that area. This extra time available for amphibians to wait for the improvement of environmental food supplies, makes them more resistant to fluctuations in food supplies compared to species of animal that depend on one type of food for their entire life. This allows amphibians to remain in an area and perform all their ecosystem functions and benefit and confer resilience to the entire ecosystem. In contrast, other species that only consumed one food source would die out when that food source was absent and instead of preventing ecosystem collapse, would contribute to and accelerate it by removing whatever ecosystem functions these non-amphibian species would have performed. This is of critical importance today, as humans are pushing ecosystems everywhere towards complete collapse from pollution, habitat destruction, and rapid temperature and precipitation changes due to climate change. The ecosystems of the world need the enhanced stability and resilience conferred by amphibians if they are to survive the damages humans are inflicting upon them. Conservation of amphibians also ensures protection of the other species in the ecosystem.

A multitude of predators consume amphibians using them as a reliable, nutrient rich, high calorie food source. Most pond-breeding amphibians are explosive breeders where all individuals in a given population or a subpopulation aggregate into vernal pools “en masse” in a short timeframe for reproduction. Such en masse gathering provides optimal foraging opportunities for predators such as northern water snakes, common garter snakes, and common snapping turtles in the United States Northeast (Dodd and Barichivich 2007). Similar aggregations of predators to amphibian populations breeding en mass occurs around the world (Dodd and Barichivich 2007). Amphibian eggs hatch synchronously in large numbers. In response several aquatic and semi-aquatic predators including predatory reptiles concentrate their foraging efforts in amphibian breeding habitats early in the growing season, gaining a massive nutrient intake that helps them survive the winter (Dodd and Barichivich 2007). Explosive reproductive rates enable amphibians to recover from and be resistant to this predation. Predatory species maintain balance between the different prey populations and prevent dominant species from depleting shared food sources, and thereby increasing the community stability and ecosystem resilience (Dodd and Barichivich 2007).

Amphibian production of a large number of eggs and offspring circulates a large volume of organic matter and nutrients per unit time, which fuels and sustain ecosystem functioning. If amphibians decline or became locally extirpated, the predators that depend on amphibians would also decline, or even disappear from that region. Without predators to keep the populations of prey species in check, there would be an overabundance of many prey populations, until the resources needed by those populations are exhausted, causing population and ecosystem collapse. This highlights the importance of conserving amphibians, as the predators that eat them are already at a heightened risk of extinction, and without amphibians many of these predators will be pushed to extinction.

The presence of amphibians also helps increase the diversity of invertebrate communities as the amphibian predation on dominant invertebrate species keeps their population sizes in check so that competitively superior species do not becomes overabundant and exhaust resources needed by subordinate species in the same community (Ives et al. 2005). Moreover, deposition of amphibian biomass, which happens to a significant degree seasonally in vernal pools in terms of unhatched eggs and larval morality, as well as their burrowing habitats, constant excretion and defecation, also add to enhanced nutrient and energy dynamics in their ecosystems (Colburn 2006).

Poikilothermy and Efficient Transformation of Energy into Biomass:

Amphibians are poikilotherms and therefore their internal body temperate is mediated mostly by behavioral adaptations as opposed to by metabolic processes (Duellman and Trueb 1986). This allows amphibians to use significantly less energy per unit body weight for thermoregulation in contrast to homeotherms such as mammals and birds (Pough 1983). Therefore, amphibians convert much more of the food they consume into organic biomass compared to homeotherms (Pough 1983). The chemical energy amphibians assimilate is much more conserved in the carbon cycle and the energy flow through wood webs, allowing higher productivity of the entire ecosystem as less energy is lost to thermoregulation and is instead available for access by predators and decomposers (Pough 1980). Amphibians unique trophic position as mid-level consumers make them both a predator of primary and secondary consumers and a prey-base for higher-order carnivores. In addition, as “low-energy systems”, amphibians can moderate their metabolic activities and survive extreme fluctuations in food, water availability, oxygen concentration, and other resources (Pough 1980). The ability of certain wetland-breeding amphibians to tolerate droughts, changes in the regional land-uses and changes in the natural land-cover have been well documented (Gibbons et al. 2006). Such ecological resilience and resistance of amphibians make them robust biological agents that can maintain critical ecosystem processes such as the transfer of biomass and energy transfer from isolated wetlands to surrounding terrestrial to a magnitude that cannot be replicated by other biota (Pough 1980, Gibbons et al. 2006).

Without having to devote as much energy to regulate their body temperature, amphibians can use the conserved energy to generate new tissues and reproduce. For example, the red-backed salamander, *Plethodon cinereus,* converts 39% of the energy from eaten food into new tissues and reproduction, compared to the marsh wren, *Telmatodytes palustris,* which only converts 0.35%, even though it also eats invertebrates (Pough 1980). Given this greater efficiency of biomass generation and energy transfer, woodland salamanders account for a far greater animal biomass than mammals and birds combined in northern temperate mixed-deciduous forest floors (Burton 1975, Burton and Likens 1975, 1976). For instance, salamanders also have the greatest wet-weight biomass of any vertebrate predator in New Hampshire forests, revealing their primary importance in the food webs of the area (Burton and Likens 1975). This finding was corroborated in the redwood forest/oak woodlands of California as well (Stebbins and Cohen 1995). The same has been observed in tropical rainforests, vernal pools in glaciated northeast, and southern Appalachian low-order streams. This is very important because free phosphorus can bind with sediments and become unavailable to organisms, therefore bioavailable phosphorous found in salamanders is very important to the ecosystem.

Salamanders are higher in protein content than birds and mammals and are a high-quality source of energy for predators (Burton and Likens 1975). In fact salamander-derived energy accounts for 20% of the energy used by birds and reptiles in New Hampshire forests (Burton and Likens 1975). Over 8% of the sodium from the freshly fallen leaf litter is retained in the biomass of the amphibians, providing an important source of this essential nutrient for predators (Burton and Likens 1975). Amphibians that have life histories that include aquatic and terrestrial environments cycle nutrients between the two (Davic et al. 2004). The nutrients present in aquatic habitats such as vernal pools and streams are assimilated by juvenile stages of amphibians. Later, upon metamorphosis these amphibians travel to land bringing those water-borne nutrients and carbon sources that are subsequently deposited when amphibians defecate, die, or are consumed by terrestrial predators or scavengers. Amphibians biphasic life cycle brings essential nutrients that are scarce on land , such as nitrogen, into the terrestrial environment from aquatic environments (Earl et al. 2011, Semlitsch et al. 2014). Similarly, when amphibians with terrestrial adult stages lay their eggs in water (such as in vernal pools and other wetlands), nutrients scarce in aquatic environments are added to the ecosystem when these eggs are eaten by predators, or the juveniles die and decompose, as well as when they defecate (Earl et al. 2011, Semlitsch et al. 2014). Even amphibians that remain in aquatic environments, during their adult stages feed on terrestrial invertebrates, such as caterpillars and flying insects, which bring in scarce terrestrial nutrients into the aquatic environments (Pough 1980, Gibbons et al. 2006). Tadpole fecal matter serves as an energy source for detritivores thus enhances the energy flow through detrivory-decomposition pathway, which is a critical part of the carbon cycle in wetlands and aquatic habitats (Verburg et al. 2007).

Mineral and Nutrient Dynamics:

Many amphibians feed frequently on a daily basis during their active period. Such a prolific feeding strategy leads to the concentration of essential minerals in their biomass. Amphibians are packed with essential and rare nutrients, such as Omega 3, phosphorus, carbon, calcium, nitrogen, magnesium, iron and sodium (Burton and Likens 1975). Their nutrient-rich biomass then serves as a nutrient source for other higher-order consumers. For instance, in New Hampshire forests salamanders have a higher concentration of calcium than any of the invertebrates amphibians consume making them an important source of calcium for mesopredators, such as birds and medium-sized mammals and snakes (Burton and Likens 1975). Amphibian feeding strategies also contribute to increasing the nutrient flow, thereby, increase the availability of nutrients to other organisms in the habitats. Many essential nutrients, including macronutrients, are trapped in the physical habitats or embedded in biomasses so that are in short supply for critical biological functions. Amphibian foraging increases the availability of critical nutrients and other energy substrates to organisms. For instance, grazing and filter-feeding tadpoles tend to increase nitrogen availability and increase nitrogen flux through wetlands by both defecation, and ecosystem function that can be attributed to fast gut passage in developing tadpoles. During their time in aquatic habitats, amphibians excrete ammonia, which is a great source of nitrogen for plants and phytoplankton. Phosphorus an essential biological nutrient, was found to be in a greater concentration in New Hampshire's salamanders (7.79 g/ha) than in small mammals (0.21-0.41 g/ha) and birds (4.27 g/ha) combined (Burton and Likens 1975). This is especially important on an ecosystem wide scale because freely available nutrients essential to life such as phosphorus often bind to sediments and become less biologically available. If amphibian biomass declines or disappears, some aquatic and terrestrial habitats will suffer nutrient limitations, which will lower the overall productivity and biodiversity of such habitats. The organisms that were depending on amphibians to transport an essential nutrient into their habitat, will be deprived of that nutrient, and will not be able to survive in that area. The increased levels of stress humans put on natural ecosystems is already pushing them towards collapse, the loss of even more biomass and biodiversity because of the scarcity of required minerals and nutrients will only worsen this trend. Therefore, by protecting amphibians, the nutrient and mineral availability of the ecosystems amphibians live in will be conserved, benefiting the entire ecosystem and the species within it.

Benefits of Burrowing Activity for Soil Quality and Aeration

Many species of amphibians have a burrowing mode of life. Amphibians tend to burrow to avoid adverse climatic conditions, such as high temperature, droughts, and moisture stress (Wells 2007). Some amphibians, particularly those that live in arid climates such as Spencer's burrowing frog *Opisthodon spenceri*, tend to lend a fossorial mode of life. In addition, many temperate amphibians— such as American toad *Anaxyrus americanus*, Fowler's toad *Anaxyrus fowleri*, Eastern Spadefoot toad *Scaphiopus holbrookii*, Boreal chorus frog *Pseudacris maculata*, Gopher frog *Rana capito*, and mole salamanders *Ambystoma talpoideum* — tend to spend a large proportion of their lives in burrows, particularly for aestivation and hibernation, and forage and breed on land or water (Wells 2007). Such movements between surface and subterranean fossorial habitats and alteration of underground burrow systems aerate the soil, add nutrients, organic matter, and moisture to the soil, and enhance soil productivity. This stability conference and the aeration and enrichment of the soil can have a profound effect on the composition of an ecosystem, indicating amphibians can be ecosystem engineers.

Regulation of Prey Populations:

Most salamanders, frogs, toads, and caecilians prey on invertebrates particularly insects, keeping their populations in check (Wells 2007). Amphibians are generalist predators exerting an equal amount of predation pressure on every species of prey invertebrate in the area, benefiting all of them in the process, as less interspecific and intraspecific competition results in a higher chance of survival for all species (Wells 2007). This included both larval and adult stages of biting flies and midges, sanguivorous mosquitoes, phytophagous insects, forest pathogens or vectors of forest pathogens and vectors of medically or veterinary important parasitic infections (Wells 2007). If amphibian populations decline, that would release herbivorous insects from predation pressure leading to an increase in population sizes of insects. This may lead to declining plant biomass and plant recruitment, which then can later change the community composition and canopy cover of forests. They could also be used to control disease vector insects like mosquitos as many species of amphibians eat large numbers of mosquitos and their larvae. Salamanders are the dominant predators in watersheds and headwater habitats, and are in nearly every other type of habitat present, being so common and having such a significant effect on prey populations that they can be considered ecosystem engineers (Murphy & Hall 1981, Petranka 1983, Resetarits 1997, Wilkins & Peterson 2000, Lowe & Bolger 2002).

In addition, amphibians could be used as an inexpensive and eco-friendly biological control agent. They consume significant amounts of insects, and could be used to control agricultural pests. Anthropogenic chemicals including pesticides, herbicides, and fungicides are extremely damaging to a wide variety of lifeforms. Synthetic pesticides that are lipid soluble can bioaccumulate in lower tropic levels, bioconcentrate over time during an organism's lifetime, and biomagnify along the food chain where the top predators may accumulate toxins beyond tolerable levels leading to mortality or other serious physiological and reproductive complications (Miller 2007). Some synthetic pesticides can adhere onto soil particles and detritus and persist in the environment (Miller 2007). Some additives found in commercial-grade pesticides, such as trace elements and heavy metals, are also highly toxic. Humans are known to suffer both acute and chronic physiological complications being exposed to pesticides, including renal failure, neurological disorders, cognitive impairment, and liver dysfunction (Relyea et al. 2005). Besides, use of synthetic peptides is extremely costly, and accounts for a major proportion of the budgetary investments in agroindustry. Additionally, many major forms of pesticides are rendered ineffective in each growing season when pests evolve resistance to those chemical agents (Jutsum et al. 1998). Using amphibians as a biological control agents would be significantly less expensive than using pesticides, as amphibians would restock their own populations every year through reproduction while pesticides have to be bought and applied every year. Unlike in the case of pesticides, the evolutionary arms race between amphibian predators and pests will keep co-evolving across multiple generation cycles. In the long-term, evolution of resistance is improbable. In the long run, using amphibians as biological control agents would also be more effective, and remain efficient over multiple growing seasons, particularly amidst evolution of insecticide-resistant arthropods, weeds, and fungi that pose a significant threat to the agroindustry (Jutsum et al. 1998).

Direct Value to Humans

In environmental economics, and conservation biology, there are five fundamental ways to evaluate biodiversity (Primack 1993, Groom et al. 2006, Hunter Jr and Gibbs 2006). First, consumptive use values based on direct uses of biodiversity at local-scale such as the market price paid upon extraction where species are harvested from the wild and sold or traded in the local market as food, hide, or for medicinal purposes. Second, productive use values where species are harvested from the wild but sold in a broader national or an international market for a wide variety of purposes including industrial agriculture and food manufacturing. The first two are considered direct use values. The third includes values of ecosystem services provided by different species including provisioning of goods and services, ecosystem productivity, maintaining energy and nutrient flow, and ecosystem engineering. The fourth entails non-consumptive use values, which includes recreation, aesthetics, cultural and spirituality-oriented amenity values. These later two types of values do not involve removal of the species for their ecosystems, for instances, as doing observations during ecotourism and supporting local community beliefs or rituals related to wildlife. A fifth type is the scientific and educational values; the former includes use of elements of biodiversity for developing educational programs for both informal outreach and formal education while the later involves exploring the potential of biodiversity and studying species as models to understand complexity of ecosystems. Given the current scientific understanding of amphibian biology, they can possess all the forms of values mentioned above.

Bio Control Agents for Pests Destroying Agriculture and Spreading Diseases

Predation on Vectors of Human Disease:

Adult amphibians feed on mosquitos and biting flies, and tadpoles eat mosquito larvae reducing the vector population that is responsible for the spread of many human and veterinary diseases (DuRant and Hopkins 2008). Mosquitos and biting flies carry many diseases, many of which can be fatal to the host, including malaria, yellow fever, African sleeping sickness, eastern equine encephalitis , and dengue (brain-hemorrhagic) fever (DuRant and Hopkins 2008). Amphibians are efficient predators of medically-important pests, as multiple life-history stages of amphibians can prey on all life-history stages of vector pests across different habitats in many biomes (Durant and Hopkins 2008). Larval marbled salamander, *Ambystoma talpoideum* consumed 439 ±20 mosquitos of the species *Culex pipiens*, a day on average with the largest individual ingesting 902 mosquito larvae each day, in a laboratory setting (DuRant and Hopkins 2008). Given the large number of amphibian larvae and adults present in most aquatic ecosystems, up to 500,000 individuals per hectare, their predation removes a large quantity of mosquitos out of the environment in wetlands, ponds, streams, and rivers (Durant and Hopkins 2008). Using the numbers previously given for the average daily consumption of mosquito larvae by *A. talpoideum* and the average amphibian population densities in that area the total consumption of mosquitos by all amphibians in the area can be estimated to be 6,585,000,000 mosquitos eaten in a single hectare in a single month. Adult red spotted newts *Notophthakmus viridescens,* consume 316±35 *Culex pipiens* mosquitos a day, which equates to 4,740,000,000 mosquitos eaten each month in a hectare as the consumption by the other species is comparable to the red spotted newts (Durant and Hopkins 2008). Tadpoles of many different species have been shown to aggressively eat dengue mosquito (*Aedes aegypti*) eggs, which is important because mosquito eggs are protective, safe havens for deadly pathogens between mosquito breeding seasons during the dry season (Bowatte et al. 2013).

Because amphibians consume every life stage of mosquitoes from egg, to larvae to adult, they are excellent bio control agents against them and the estimates we calculated for the number of mosquitoes removed per hectare per month are significantly underestimated because they do not include all the mosquito eggs consumed. The monthly mosquito consumption per hectare estimates convey just how many mosquitoes both larval and adult amphibians consume. Tropical biomes such as those in South American, Central African, Western Africa, South Asia, and Southeastern Asia, have the highest rates of deadly and debilitating insect borne pathogens, and yet with the exceptionally high amphibian biodiversity and high biomass in these places, they also have a built in defense mechanism against the spread of these pathogens. If amphibians die out in an area, the intense predatory pressures they once exerted on disease vectors like mosquitoes will be lost, and the vector populations will increase. The mosquitos that would normally be consumed by amphibians instead bite humans, infecting many more people with pathogens, and go on to reproduce, further increasing their populations. The spread of mosquito borne diseases would be significantly worse if there were an extra 4,740,000,000 to 6,585,000,000 mosquitoes in many populated hectares near ponds, streams, wetlands, and rivers throughout the world.

Because of global warming and climate change, tropical mosquito borne diseases are migrating further away from the equator, and will be effecting more people every year as global temperatures continue to climb. The local amphibians in the tropical areas where these species of tropical pathogens and pathogen vectors normally live have co-evolved with them and through the process of evolution improved the efficiency that they consume these pathogen vectors. If tropical amphibians continue to decline there are no other predators that will be able to consume tropical disease vectors with the same efficiency. This highlights the extreme importance of conserving amphibians, especially the highly threatened tropical species, as the trends of global climate change and pathogen migration are likely only going to continue, and amphibians will be a useful tool in reducing the number of pathogen bearing insects. Explaining to the public that amphibians will help keep them safe and potentially reduce the chances of them getting a dangerous disease is a powerful way to make them care about amphibian conservation. The prospect of making human settled areas free of vector borne diseases is a universally desirable outcome that if articulated properly, will generate significant public and government support for amphibian conservation. I suggest that the federal government or state governments offer tax breaks, grants, and other incentives to towns and cities that delineate amphibian habitats as protected areas as a mosquito controlling measure. This would ease the initial financial burden of restoring these areas, or purchasing and setting them aside for this specific use. Data could then be gathered about reduced rates of mosquito borne diseases, lowered mosquito populations, and money saved from being spent on insecticides. These first few towns could serve as examples for others to follow, and as more data is generated regarding this techniques effectiveness, many town will see these bio control technique as the best option.

Predation on Agricultural Pests:

Amphibians feed constantly and consistently throughout their active period, and they consume food in large quantities per unit wet biomass (Toft 1980, Parmelee 1999). Frogs and toads are especially potent consumers, particularly those that have a relatively large body volume, and can eat a large quantity of pest invertebrates such as locusts, (Attademo et al. 2004, Table 1). Because they are generalist predators, they will eat indiscrimatingly with little prey selection, based on the availability (enounter rate) and profitablity of the prey (Table 1). In, cropfields their optimal forage will likely be invertebrates eating the crops (Attademo et al. 2004). This makes them extremely useful biocontrol agents for consuming herbivore invertebrates that are damaging a farmers crops (Attademo et al. 2004). The generalized predation strategy of frogs and toads also has led them to develop a generalized resistance to many of the toxic defense chemicals found in invertebrates, allowing them to eat pest herbivores that no other biocontrol agent can, including toxic invasive pests (Sloggett 2012). For example, in the Netherlands native frogs and toads are the only generalist native predator species that are unharmed by ingestion of the invasive and toxic lady bird beetles of the family Coccinellidae (Sloggett 2012). Coccinellidae create toxic alkynes, that allow them to eat crops and displace native species without the risk of being consumed by most predators in a novel biolgoical community that have not co-evolved to have resistance to the specific defensive chemicals they posses (Sloggett 2012).

Table 1: Invertebrate species consumed by three native species of frog Physalaemus albonotatus, Leptodactylus latinasus, Leptodactylus chaquensis and one species of toad Rhinella arenarum in two commercial soybean farms in Argentina, located in Córdoba and Entre Ríos. All species of amphibians predated on large numbers of herbivores that eat the soybean crops (Attademo et al. 2004).

Diet composition of amphibians in Córdoba (*B. arenarum* and *L. latinasus*) and Entre Ríos (*L. chaquensis* and *P. albonotatus*) soybean fields

Prey category	Córdoba soybean				Entre Ríos soybean			
	B. arenarum		*L. latinasus*		*L. chaquensis*		*P. albonotatus*	
	N	%FO	*N*	%FO	*N*	%FO	*N*	%FO
Coleoptera								
Elateridae								
Agriotes sp.[a]	9	20	12	33.3	2	13.3	4	13.3
Conoderus sp.[a]	1	6.7	–	–	4	26.6	–	–
Lagriidae								
Lagria villosa	67	73.3	1	6.7	9	13.3	–	–
Scaraboidae								
Diloboderus abderus[a]	26	26.7	1	6.7	1	6.7	–	–
Phanaeus splendidulus[a]	4	20	–	–	–	–	–	–
Anomala sp.[a]	4	13.3	3	20	2	13.3	–	–
Carabidae								
Oriozapylus sp.	24	40	–	–	6	33.3	–	–
Tenebrionidae								
Scotobius sp.	2	13.3	–	–	–	–	–	–
Curculionidae[a]								
Adult (n.i.)	3	6.7	–	–	–	–	–	–
Chrysomelidae								
Diabrotica speciosa[a]	1	6.7	1	6.7	–	–	–	–
Cicindelidae								
Adult (n.i.)	1	6.7	–	–	–	–	–	–
Dytiscidae								
Adult (n.i.)	–	–	1	6.7	–	–	–	–
Lepidoptera								
Arctiidae								
Spilosoma virginica[a]	2	6.7	–	–	13	40	2	13.3
Noctuidae								
Spodoptera sp.[a]	23	13.3	–	–	–	–	–	–
Peridroma saucia[a]	8	13.3	–	–	–	–	–	–
Anticarsia gemmatalis[a]	23	33.3	3	13.3	4	26.7	4	13.3
Rachiplusia nu[a]	–	–	2	6.7	3	20	–	–
Erebus sp.[a]	–	–	–	–	3	20	–	–
Ortoptera								
Acridiidae								
Schistocerca sp.[a]	4	20	–	–	3	20	–	–
Gryllidae								
Gryllus argentinus[a]	2	6.7	1	6.7	1	6.7	–	–
Anurogryllus muticus[a]	–	–	1	6.7	–	–	1	6.7
Gryllotalpidae								
Scapteriscus borelli[a]	–	–	3	20	–	–	–	–
Homoptera								
Delphacidae								
Delphacodes kuscheli[a]	2	6.7	2	6.7	–	–	–	–

Table 1 continued (1. 1): (Attademo et al. 2004).

Prey category	Córdoba soybean				Entre Ríos soybean			
	B. arenarum		*L. latinasus*		*L. chaquensis*		*P. albonotatus*	
	N	%FO	*N*	%FO	*N*	%FO	*N*	%FO
Cicadellidae								
Empoasca fabae[a]	–	–	–	–	1	6.7	–	–
Cercopidae								
Zulia entrerriana	–	–	–	–	1	6.7	–	–
Hemiptera								
Pentatomidae								
Edessa meditabunda[a]	8	40	–	–	–	–	–	–
Nezara viridula[a]	4	20	–	–	–	–	–	–
Reduviidae								
Adult (n.i.)	1	6.7	–	–	–	–	–	–
Hymenoptera								
Formicidae								
Solenopsis sp.	12	6.7	–	–	9	6.7	–	–
Acromyrmex sp.[a]	28	20	–		–	–	–	–
Pheidole sp.	7	33.3	4	26.7	–	–	–	–
Ectatomma sp.	8	13.3	–	–	–	–	–	–
Camponotus sp.	4	13.3	–	–	–	–	–	–
Eciton (Labidus) praedator[a]	–	–	–	–	1	6.7	–	–
Crematogaster quadriformis	–	–	–	–	2	6.7	4	40
Atta sp.[a]	–	–	–	–	2	6.7	–	–
Wasmannia sp.	–	–	–	–	–	–	52	60
Vespidae								
Polistes sp.	–	–	–	–	6	13.3	–	–
Dermaptera								
Doru lineare	6	6.7	–	–	–	–	–	–
Blattaria								
Blattidae								
Blatta orientalis	1	6.7	–	–	–	–	–	–
Crustacea								
Isopoda								
Armadillium vulgare[a]	149	66.6	17	20	–	–	1	6.7
Animal parts (n.i.)	x	20	x	40	x	46.6	x	33.3
Plant parts (n.i.)	x	26.6	x	13.3	x	26.7	x	6.7
Diversity	1.09		1.1		1.25		0.53	
Average prey size (mm)	20		13		13.5		7.5	
Gastrointestinal tracts analyzed	15		15		15		15	

N, total number of organisms in the digestive tracts; FO, frequency of occurrence (%); x, no numerical value; n.i., not identified; (–) absent.
[a] Herbivore species.

Sustainable Source of Food for Humans

If properly harvested in a sustainable way from their natural environment or cultured, amphibians are an excellent source of food for human consumption. Containing more protein per pound than mammals or birds, amphibians are a high quality food source, full of essential vitamins and nutrients. As mentioned in the energy dynamics section, their ectothermic homeostasis strategy makes them extremely efficient at converting consumed energy into somatic growth. Their explosive growth and r-selected breeding strategies allow faster harvesting in large numbers compared to other animals such as mammals and birds with a slower reproduction cycle and slower growth rate. Amphibians, like many other anamniotes, have indeterministic growth where they can keep growing to larger sizes as they age provided that they receive sufficient caloric and nutritional intakes, and a lack of predation or environmental stressors. Thus, if reared under favorable environmental conditions, amphibians may produce a biomass comparable to edible fish, worth very large amounts of money when exported (Figure 1). Unlike traditional animal husbandry such as cattle or swine farming, herpetoculture (husbandry of amphibians and reptiles) requires less physical space, less resources, generates little waste, and their feed can be easily and locally obtained, thus the overall ecological footprint, particular the carbon footprint, might be very low (Chifundera 1996). If they are cultured under natural or semi-natural settings, a number of other native species can co-exist with the amphibians. In contrast, conventional farming practices, such as industrial livestock feedlots would exclude or destroy the native species and their habitats in the area, and further leads to environmental degradation, with the emission of greenhouse gasses, and pollution of the local environment (Gratwicke et al. 2010). In addition to eating frogs for subsistence, there is a booming export industry for frog legs being sent to Europe and the United States (Figure 1). Besides, frog legs are a delicacy in eastern Asia as well as southeastern Asia, as seen by the disproportionate import of frog legs by France, and the presence of massive numbers of frog farms in China and Indonesia (Figure 1).

From 1996 to 2006 the net value of frog legs imported globally from all sources was nearly half a billion United States dollars (Gratwicke et al. 2010). In addition the value of frog legs has not declined substantially in this entire time, suggesting the export of frog legs is a stable business venture that will not depreciate with time (Gratwicke et al. 2010). The export of frog legs, if conducted in a sustainable manner, could provide a source of income for developing countries, while also preserving the natural ecosystem and biodiversity (Gratwicke et al. 2010). A portion of the profits generated from the sale of frog legs could be used to further amphibian conservation either by purchasing and creating protected areas, or by donating to conservation groups that conduct research to try and better understand amphibians and how to conserve them thru husbandry and the housing infrastructure.

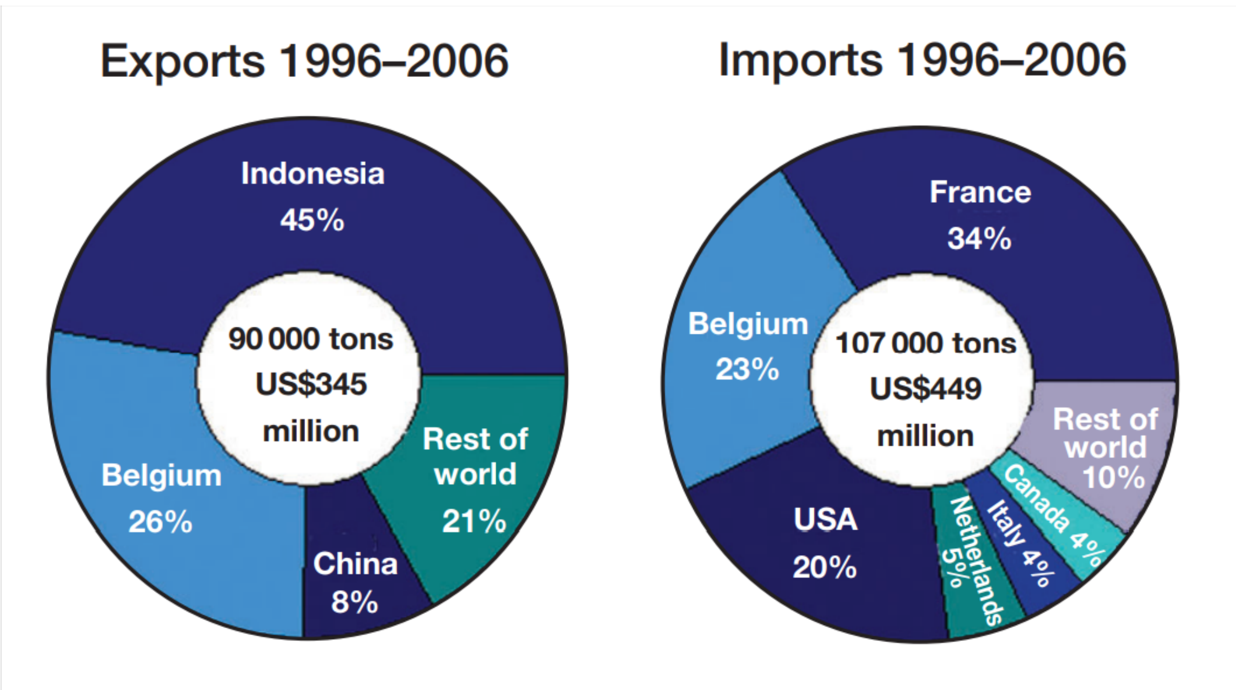

Figure 1: Global frog leg imports and exports 1996-2006. France remains a consistent importer of frogs, where frog legs are considered a delicacy (Gratwicke et al. 2010).

Food Source for Species with Hunting and Fishing, and Recreational Importance:

Hunting and Fishing are very culturally important for many countries across the world, and in some places make up an essential source of food for the people. In the United States hunting and fishing have a large cultural significance and are important recreational activities enjoyed by millions of American every year. Amphibians are extremely abundant and found in a wide variety of places, making them an important food source for animals of hunting and fishing importance to humans. For example in a characteristic kettle pond in Michigan's Upper Peninsula 12.3% of the biomass consumed by largemouth bass were amphibians (Hodgson et al. 2005). The frequently hunted waterfowl are the predatory merganser ducks that consume large numbers of amphibians (Mallory and Metz 1999). Many other large predatory fish and waterfowl of recreational or subsistence hunting and fishing use amphibians as a large portion of their diet. Thus, conservation of amphibians is also important to ensure protection of game species.

By explaining to the general public that many of the species they enjoy hunting and fishing depend on amphibians for food, the message that amphibians need to be conserved if these other species are going to remain abundant is directly conveyed. All the hunters and fisherman will begin to care about the health of amphibian populations, and want to help conserve them, to protect their sport or livelihood. By making conservation matter to people on a personal level by connecting it to something they are about, you can enlist their support for new legislation protecting amphibians, and even potentially generate donations to further their conservation.

Environmental Indicators of Ecosystem Health and Quality

Amphibians are considered accurate indicators of the environmental health and habitat quality, particularly to indicate pollution or the aftermath of habitat degradation (Quaranta et al. 2009). This is particularly true for stream ecosystems as well as wetland ecosystems. Nearly all amphibians respire through their integument, and generally have a highly thin, permeable skin, which readily permits absorption of oxygen. Amphibian skin is also highly vascularized to enable gas exchange, also enabling pollutants too quickly and easily enter the bloodstream (Quaranta et al. 2009). Thus, inevitably, amphibian skin will permit passage many chemicals, including toxins and pollutants such as synthetic pesticides and heavy metals that may either be lethal to them or induce serious physiological defects (Quaranta et al. 2009). However the ability to absorb minute environmental contaminants makes them fantastic bioindicators for determining what compounds have been present in an ecosystem. For instance, the common European anuran *Rana esculenta* absorbed 302 times as much of the herbicide atrazine compared to a domesticated pig (Quaranta et al. 2009). The dual life history of amphibians allows a survey of pollutants found both in the local terrestrial habitat as well as the local aquatic habitat (Van Meter 2018).

Moreover, many amphibians are highly philopatric, where they stay in one place in the stream, live a relatively long time, and have relatively stable populations (Welsh et al. 1998). For instance, tropical bush frogs of family Rachophoridae occupies a small extent of mature forests and woodlands where their activities (including breeding) are limited to the understory and the leaf litter (Stebbins and Cohen 1995). Although pond-breeding amphibians of the glaciated northeast tend to disperse far from their natal grounds, they migrate back to their natal pond for breeding over multiple reproductive cycles (Stebbins and Cohen 1995). Many southern Appalachian stream salamanders, particularly those that are large-bodied, tend to have a relatively conservative home range and have high fidelity to certain stream refugia (Stebbins and Cohen 1995). In addition, their seasonal migrations and dispersal and biphasic amphibious life cycle expose amphibians to different environments, environmental stressors and climate change. These attributes of their life cycle allow changes in their population sizes in a particular area to be accurately tracked, assessed, and damage to the ecosystem to be accurately quantified (Welsh et al. 1998).

Amphibians are cosmopolitan, and relatively easy to observe. This opens up the opportunity for members of the general public to become citizen scientists and survey amphibians, record their presence or abundance, and even recording their calls in different habitats. This is an excellent opportunity to engage the public in environmental monitoring, which not only generates valuable scientific data, but also educates the public about the importance of amphibians. By getting the public talking to scientists about the problems threatening amphibians and ecosystems in general, and also how important amphibians are to not only the environment but also to humans, a greater knowledge base and interest in conservation is created. This paves the way for greater public support of legislation that will protect threatened amphibians and the ecosystem in general. By interacting with and observing amphibians, the public will become more familiar with them, and get to see firsthand the variety of ecosystem functions they perform, including the consumption of mosquitos. The validity of the data collected can be ranked based on several factors, such as the similarity in appearance of a species of interest to other species, the consistency with other reports, and the experience level of the observer. Besides engaging and educating the public, this kind of citizen science may be a great way to identify clearly disturbed areas where professionally trained scientists can then be sent to investigate with more detail and accuracy.

Methods to Record Pollution Levels Using Amphibians:

The sensitivity of amphibian communities to deteriorating environmental health can be greater than that of the most advanced human-engineered monitoring devices and chemical analyses. Amphibians are constantly in the environment occupying a variety of habitats, thus assimilating and concentrating pollutants into their body tissues, including temporary and transitional pollutants that may diffuse and become diluted into undetectable concentrations over time and rendered undetectable by conventional environmental monitoring protocols (Miller 2007). In the heavy metal contaminated Yugansk Reserve of Russia the frequency of chromosome instability was measured by calculating the rates of aneuploidy and chromosome aberration frequency in the moor frog, *Rana arvalis*, and common toad, *Bufo bufo*, (Gileva and Schupak 2003). By comparing the aneuploidy levels of these animals to the levels in unpolluted areas, it was determined that there was no significant increase in chromosome instability in the Yugansk amphibians compared to those in uncontaminated regions. The amphibians in the Yugansk Reserve were all found to be contaminated with the known mutagen heavy metals copper, arsenic, cadmium, and lead (Gileva and Schupak 2003). This indicated the mutagenesis of these compounds is not significant at the moderate levels present in the reserve, and therefore this reserve is still capable of sustaining healthy populations of amphibians, and is worth further protection (Gileva and Schupak 2003). This real world impact of heavy metal pollution on living wild populations would have been nearly impossible to ascertain using synthetic monitoring devices. The previously mentioned Yugansk Reserve experiment, and others that use amphibians to understand how certain levels of specific pollutants actually effect living organism in the environment is important because the budgets of government conservation agencies and private conservation agencies are limited. It is therefore critical to collect data about what pollutants actually do to animals in the environment, so when conservation organizations have to decide which of two polluted areas to purchase and turn into a protected area, they can choose the one that will be the most viable in the long term, and contains the least damaging pollutants. This is of critical importance because many areas, even those considered relatively "pristine" far away from areas of high human density and activity have been found to contain pollutants (Gileva and Schupak 2003). By conserving amphibians, an irreplaceable tool for monitoring the health and pollution levels of an environment is being protected. No other tetrapods are as abundant, easily collected, and sensitive to environmental pollutants as amphibians are. If amphibians went extinct, it would become much more expensive, and much harder to determine if an ecosystem had been contaminated. Some essential characteristics, like how chronic exposure to a pollutant effects tetrapods in the wild, would be impossible to determine early on with low levels of pollutants in the ways that are possible with amphibians.

A widely-used technique that detects environmental contaminants using biological organisms is metabolomic analysis (systematic identification and quantification of the small molecule metabolic products of a biological system at a specific point in time) which involves identifying the unique metabolic profiles or fingerprints present in an amphibian after being exposed to a single or multiple pollutants (Van Meter 2018). These metabolite profiles produced via mass spectrometry and NMR spectroscopy can then be used as biomarkers in the future, to test environments for exposure to the pollutants that result in production of these metabolites (Miller 2007). For example in green frogs, exposure to the insecticide malathion can be measured with malathion's metabolite malaoxon in all body tissues using Agilent 6890 gas chromatography coupled to a 5973 mass selective detector utilizing Chemstation software (Van Meter 2018).

The availability of extremely accurate, and widely available techniques for ascertaining the exact levels and types of pollution present in amphibians and therefore present in the environment is very important for the protection of ecosystems from chemical pollutants. By finding out exactly what pollutants are present in an environment and what quantity they are present in, it is possible to attempt to find the source of the pollution and prosecute the polluter or pass legislation that makes it illegal to continue such practices. Without amphibians present in an ecosystem, and these accurate techniques to record the types of pollution present, it would be much harder if not impossible to detect low quantities of pollution in the environment. This would make it easier for companies and individuals to continue polluting an environment, and not be discovered, leading to increased rates of pollution and all the ecological damage, stress, and even collapse that accompanies the continued pollution.

Early Detection of Chemical Pollutants, Behaviors Valuable for a Bioindicator, and Physical Disturbance Monitoring:

Tracking sensitive indicator species like amphibians allows early detection of environmental disturbances and damage, which is critical for intervention to prevent a full ecosystem collapse (Rapport 1992, Rapport and Regier 1995). Early detection of environmental deterioration is crucial in effective environmental management since it is much more pragmatic and cost-effective to implement preventative and mitigatory measures than to reverse a major ecosystem-wide environmental catastrophe. In amphibian aquatic larval stages, they have extremely specialized use of either lotic or lentic microhabitats for feeding and cover, making them very sensitive to even minor changes to the environment (Blaustein et al. 1994 a, b, Stebbins and Cohen 1995). Lentic dwelling salamanders in the western North America, particularly salamanders in the western mountain ranges (pacific cascades, coastal range, northern Rockies, Sierra-Nevada) and those of southern Appalachian range are excellent habitat indicators for lotic water, while lentic inhabiting salamander densities in glaciated northeastern US are excellent indicators of lentic habitats (Stebbins and Cohen 1995).

For example when road construction in the redwood National Park Highway led to fine sediment contamination of streams in the Prairie Creek State Park in CA, in 1989, there was a significant drop in the number of tailed frogs (*Ascaphus truei*, larvae), Pacific giant salamanders (*Dicamptodon tenebrosus,* paedomorphs and larvae), and southern torrent salamanders (*Rhyacotriton variegatus*, adults and larvae, compared to neighboring uncontaminated streams (Welsh and Ollivier 1998). Aquatic Amphibians often share these microhabitats with anadromous and freshwater fishes, as well as many stream invertebrates, providing a more consistently abundant, spatially explicit, gauge for deleterious environmental problems in these locations that effects all the inhabitants.

If amphibian decline continues, there is no other indicator species that is as useful at picking up environmental contamination before it becomes too severe. The presence of damaging pollutants will likely not be detectable until the entire ecosystem is permanently harmed or even collapses. This will make environmental protection much harder, and accelerate the rate of ecosystem collapses and extinctions. Companies and individuals will not be able to be held accountable as easily, as the pollution released into the environment may not be as quickly detected, allowing time to pass and making it harder to connect the perpetrator to the event of pollution. Therefore by conserving amphibians, the ecosystems that can be saved by early detection of pollution and the intervention to halt the entrance of pollution will benefit.

Importance in Medicine and Pharmaceuticals

Antibacterial, Antiviral, Antifungal, and Anticancer Compounds:

Antibiotic resistance is a major problem in modern medicine. Soon there will be antibiotic resistant bacteria that cannot be eliminated by any known antibiotics, making even routine surgery much more dangerous than it currently is, returning to pre-industrial mortality rates. Each Amphibian species has an arsenal of small peptide antibiotics produced by their immune system that each target a specific pathogen, to create a broad range defense against a variety pathogens, and there is also some variation of antimicrobial polypeptides within a species (Amiche et al. 2000).

Amphibians have co-evolved with the pathogens in their local environments, creating peptide profiles that are especially effective against the local pathogen populations. In light of this, scientists trying to discover an antimicrobial agent against a specific pathogen could find a specialized arsenal of antimicrobial peptides in the amphibian skin secretions (Amiche et al. 2000). This is one reason why amphibian conservation is so critical, as the extinction of even one species of amphibian could permanently destroy a repertoire of life saving antimicrobials that exist nowhere else in the world (Amiche et Al. 2000). The antibacterial peptides are species specific with respect to specific pathogens. The uniqueness of amphibian defense peptides provides an immense selection of antibiotics that could be used to boost immunity of humans (Amiche et al. 2000). Antimicrobial peptides are promising; although they have only relatively recently been explored as a new type of antibiotic, and are fast acting, have a broad spectrum of efficacy, and have been shown to escape many of the mechanisms of drug resistance incorporated by bacteria (Govender et al. 2012).

Many pathogenic bacteria have evolved resistance to antibiotic compounds by simply pumping the toxic compounds out of their cells using efflux pumps (Govender et al. 2012). Other common methods of developing antibiotic resistance are (1) enzymatic degradation of antibacterial drugs, (2) alteration of bacterial proteins that are antimicrobial targets, and (3) changes in membrane permeability to antibiotics (Govender et al. 2012). The mode of action of many amphibian antibiotic peptides are multifarious so that such mechanisms are unlikely to be affected by pathogenic resistance. The amphibian skin polypeptide Gaegurin 4 is effective against pathogens. It is a strongly positively charged molecule that is attracted by the strong negative charge of both gram-positive and gram-negative bacteria (Won et al. 2009). Once pulled into the bacterial cell wall, Gaegurin 4 undergoes a physical conformational change that rips open a large hole through the bacterial cell membrane (Won et al. 2009). Neighboring Gaegurin 4 attack sites synergize together to make massive swaths of bacterial membrane float away, creating catastrophic loss of osmoregulation and homeostasis causing immediate cell death (Won et al. 2009). None of the previously mentioned methods of antibiotic resistance would help bacteria in any way against this attack on bacterial cell membranes (Won et al. 2009). Altered electric charge along the bacterial cell membrane results in a profound change so that several critical functions such as the proton motor force, are impaired (Won et al. 2009). Both gram negative and gram positive bacteria need a negatively charged membrane to keep the positively charged protons close to their membrane as they create an electrochemical potential to drive generate ATP. If mutation that lose the negative charge on outer cell membrane will interfere with the electrochemical gradient, thus, mutated bacteria would be unable to synthesize ATP.

Because Gaegurin 4 is attracted to bacterial by the negative charge on the cell membrane, the polypeptide targets both gram positive and gram negative bacteria (Won et al. 2009). The Gaegurin 4 uses the general intermolecular forces that generate hydrophobic and hydrophilic interactions to damage the lipid bilayer of bacterial cell membrane instead of cell-membrane proteins. The hydrophobic regions of Gaegurin 4 will always complex with the hydrophobic region of the bacterial cell membrane, regardless of other molecular constituents and molecular configurations of the hydrophobic region (Won et al. 2009). Likewise, the hydrophilic regions of GGN4 will also always complex with the hydrophilic region of the plasma membrane (Won et al. 2009). Because the exterior of the plasma membrane by definition has to be hydrophilic and the interior has to be hydrophobic, the probability of an effective countermeasure stemming from the bacteria is highly importable as any mutation changing basic cell-surface molecular interactions would lead to instability of the structural integrity of cell membranes, and thus are biologically non-viable.

Other antimicrobial polypeptides of amphibian origin besides Gaegurin 4 have also been found to be active against gram-negative and gram-positive bacteria, fungi, enveloped viruses, and cancer cells (Govender et al. 2012). These antibiotic peptides, once their amino acid and 3D structure has been mapped, can be modified to become more lethal against their target pathogen, or safer for humans to use with less toxicity to human tissue and fewer side effects. For instance, increasing the number of positively charged amino acids in Gaegurin 4 will have a stronger net positive charge, and become attracted to the negative cell wall of the bacteria from a further distance away, increasing the range of action and thereby, the effectiveness of Gaegurin 4.

Classification of Antimicrobials and Species with Most Promising Polypeptides:

The antibiotic amphibian peptides are classified into three overarching groups based off their sequence and tridimensional fold similarities: 1.) linear amphipathic helix-forming peptides like the magainins peptides found in the African clawed frog *Xenopus laevis*, and Gaegurin GGN4 from *Glandirana emeljanovi* 2.) six different groups of disulfide containing peptides from the family *Ranidae* 3.) the temporins which are cysteine free hydrophobic peptides isolated from *Rana temporaria*, made of only 10-13 residues (Amiche et al. 2000); And 4.) One promising family of antimicrobial peptides are the dermaseptins B family from the skin secretions of the South American frog *Phyllomedusa bicolor*. Dermaseptins B peptides are all broad-spectrum microbicides that destroy many bacteria, fungi, yeast, and protozoa, but have no negative effects on differentiated mammalian cells (Amiche et al. 2000).

Table 2: Chemical name, source of extracted chemical, and medicinal value. The thin skin of amphibians makes many of their skin chemicals diffuse into their bloodstream, leading to the evolution of relatively nontoxic, yet still effective compounds. This makes them safe for humans to use with few side effects.

Chemical extracted from aphibians	**Source species of the extracted chemical**	**Medical value**
Dermaseptins B	*Phyllomedusa bicolor*	Antibacterial peptide, mechanism of action makes evolution of resistance against it unlikely
Peptide DV-28 amide	*Bombina orientalis*	Prevents arterial smooth muscle relaxation: treats low blood pressure
Histamine-releasing pipinin-1	Rana pipiens	Releases insulin: treat diabetes/metabolic disorders
Gaegurin 4	*Glandirana emeljanovi* (formerly misclassified as *Rana rugosa*)	Antibacterial peptide, mechanism of action makes evolution of resistance against it unlikely

By informing the public on the pharmacological value of amphibians, a paradigm shift in perspective on amphibian conservation can be generated among both the public and pharmaceutical companies. When this happens the cause of conservation will gain many new supporters, and significant increases in economic, legal, and persuasive resources to bolster conservation dollars. Besides the moral and emotional power that stems from direct use values of amphibians, which alludes to self-preservation potential, the lucrative economic benefits of utilizing amphibian biodiversity are also a very compelling argument for conservation.

When amphibian-derived medications become available in the common market, and the profits begin to return to the investors, the entire world will become acutely aware of the value of amphibian conservation. Conservation will become a lucrative investment that companies compete for the right to take part in. The general public will see clear evidence that the tax money spent on amphibian conservation was well spent and produces a quantifiable economic return, and thus the support for conservation legislation and funding will increase.

Organ, Limb and Tissue Regeneration

Adult and larval salamanders are capable of scar free healing so that they can heal damaged tissues without fibrosis or wound contraction, with perfect regeneration as observed in axolotls and hellbenders (Brockes and Kumar 2008). Adult salamanders can regenerate whole limbs and tails and a range of critical organs and tissues including brain, spinal cord, heart, jaws, retina and lenses (Brockes and Kumar 2008). They are the only known class of vertebrates that are able to regenerate these tissues in adulthood. Many salamanders can also regenerate lost tails and many amphibians can repair every tissue before metamorphosis when they are in their tadpole juvenile stage (Brockes and Kumar 2008). By studying and understanding how amphibians do this, treatments could be developed for humans that could save or dramatically improve the lives of patients suffering from heart attacks, strokes, spinal cord damage, eye damage, brain damage, or anyone in need of organ drafting or prosthetics (Brockes and Kumar 2008).

Amphibians with high tissue regeneration capacity use precise immune cell signaling to suppress inflammation and scar tissue formation. This is essential to their ability to regenerate tissue as scar tissue formation prevents any sort of tissue regeneration, as is the case in the human liver, which is able to regenerate lost tissue so long as no scar tissue is present. Amphibian scar free healing begins with the covering of the wound with a layer of epithelial cells known as an apical wound cap, followed by the innervation of the wound cap, as cut nerves dedifferentiate into pluripotent cells capable of elongation and division. These nerves send signals revert local differentiated cells into undifferentiated stem cells called mesenchymal cells and form a structure called a blastema (Brockes and Kumar 2008, Figure 2). Once the blastema is formed the limb, organ, or tissue reforms in a way similar to how it originally formed during development (Roy and Lévesque 2006). Essential to this regeneration is the presence of anti-inflammatory macrophages (Godwin and Rosenthal 2014). The immune system plays a significant role in this regeneration process. Immune cells regulate the development of the new tissue, reprograming differentiated cells into dedifferentiated cells using signaling chemicals that change gene expression and chromatin organization, as well as controlling the inflammation (Godwin and Rosenthal 2014).

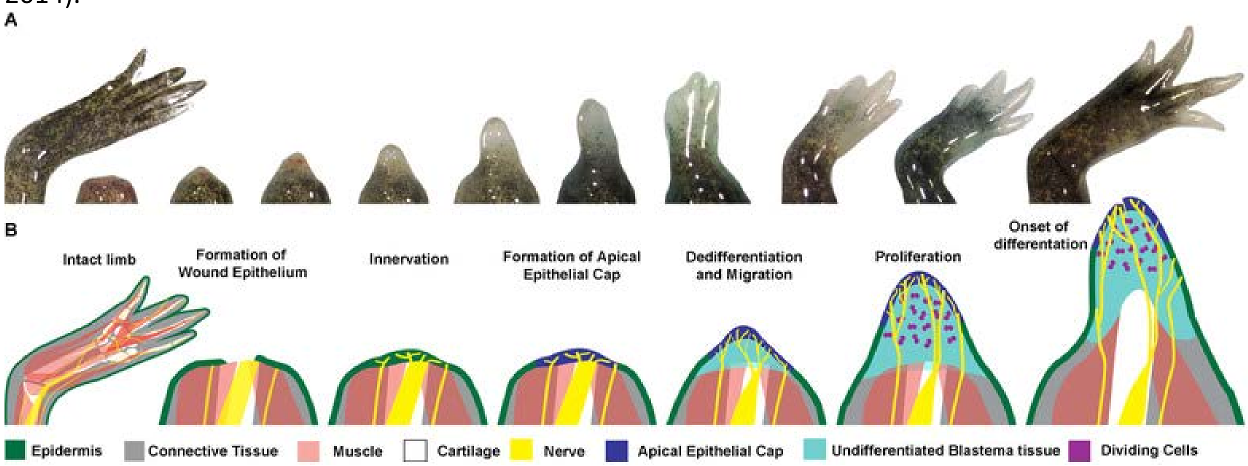

Figure 2: Regeneration of an axolotl limb. The photographs in section A were taken at day 1, 7, 9, 11, 13, 15, 17, 21, 25, and 31 days after amputation. The apical Epithelial cap dedifferentiates epidermal tissue into pluripotent cells, and attracts more cells to the location, acting as the signaling center that directs the division and growth of the dedifferentiated cells into the correct tissues and directing them to grow in the correct directions and into the right structures to generate a fully functional replacement limb. (Mccusker et al 2015).

<u>Application of Regeneration Pathways to Mammals:</u>

The most exciting part about all the discoveries about genesis gained from studying amphibians is that there is clear evidence that it can be applied to mammalian cells, including humans. In mammalian cells myotubules are multinucleated, committed, differentiated contracting muscle cells normally incapable of division or development into anything except larger muscle bundles (Andrés et al. 1996). However when a nuclear protein critical to urodele dedifferentiation called *msx1* was expressed in mouse myotubules, they regained their ability to divide into both mononucleated cells and multinucleated cells, and could be induced to re-differentiate into cells expressing chondrogenic, adipogenic, myogenic, and osteogenic markers (Odelberg et al.2000). This indicates that terminally differentiated mammalian cells can be dedifferentiated into pluripotent stem cells with the help of msx1 just like amphibian cells (Odelberg et al. 2000). This discovery implies that the intercellular signals that govern the reprograming and development of differentiated cells can be applied to mammals, including humans. This is extremely promising because it suggests if researchers continue to map out amphibian regeneration pathways, we can apply the same set of cellular signals and precise modification of gene expression to human cells to allow damaged patients to undergo the same kind of incredible regeneration amphibians are capable of.

Modern medicine cannot completely fix many medical complications, such as, strokes, heart attacks, or spinal cord damage, and the costs of the lingering damage, emotionally, financially, and otherwise are immense. The prospect that research on amphibian regenerative pathways could one day provide treatments for humans that can repair and reverse these currently untreatable conditions is a powerful one. The prospects of such biomedical potential has created support for the further study of amphibians and a greater appreciation of their abilities. Because many species of amphibian have not been studied, conservation becomes a practical investment into potential new treatments. If the general public is properly informed about how conservation and the preservation of biodiversity opens new doors into understanding how to cure and treat diseases, currently untreatable, large amounts of support and funding for conservation can be generated. I suggest integrating what we already know about amphibian regeneration into public school education, and creating informational television shows, radio programs, and interactive exhibits at aquariums, zoos, and wildlife centers. This will spread the message to many more people and increase the general public's interest in and appreciation for the natural world. With an increased knowledge base about the unique potential amphibians have to teach humanity about new pathways for regeneration, there will be a large increase in support for legislation protecting amphibians, and in the funding available for amphibian conservation. The paradigm shift to seeing nature as a valuable resource that humanity is only beginning to understand and take advantage of, will generate an increase in public desire for and interest in conservation.

Undiscovered Biomedical Value and Importance of Conservation:

With the large number of amphibian species spread throughout the earth, few of which have been extensively studied, an immense potential for new uses and discoveries may exist. With 7,844 species of amphibians, every single species has unique polypeptides in their skin secretions that are completely different from those of other species and even other phenotypes within the same species. With the plethora of potential that these skin polypeptides create, humanity can harvest medically and pharmaceutically useful agents, which will also yield sizable financial incentives. The emergence of antibiotic resistant bacteria and killer "superbug" strains like methicillin-resistant Staphylococcus aureus (MERSA) has displayed the urgent need for the development of new antibiotics especially ones that are effective against gram negative bacteria, and have a mechanism of action that attacks an essential component of bacteria physiology, like Gaegurin 4 does when it attacks the general negative charge of the cell membrane that is essential for bacterial energy production through the electron transport chain. Therefore evolution of resistance mechanisms are highly unlikely. Without new medicines derived from amphibian skin polypeptides, as antibiotic resistant bacteria evolve and spread, humanity will likely return to pre-antibiotic mortality rates from routine operations like getting ones wisdom teeth out, and small cuts and burns.

Some of the profits generated from the sale of medicine created from amphibian compounds can be directed to the conservation of amphibians, especially threatened tropical species. Seeing amphibians as a valuable resource that can be converted into significant economic dividends is a very compelling argument for why humans should spend the time and money necessary to conserve amphibians. In the face of hard economic times and worldwide recessions and uncertainty about the economic future, explaining to people that the conservation of amphibians and amphibian biodiversity is a lucrative investment is an extremely compelling argument.

Indirect Values to Humans

Improvement of Ecotourism:

Colorful and unique amphibians add to ecotourism experience, and most tourists interviewed in Namaqua National park, South Africa mentioned they would have stayed longer in an area, or would be more likely to visit an area if amphibians could be observed and if information was provided about them during the tour and in information centers (Loubser et al. 2001). Amphibians improve the attraction of protected areas to tourists and thereby significantly improve the success of protected areas and all the benefits to humans and the ecosphere these areas provide (Fig 3-6).

Ecotourism is a growing industry, with the number of visits to protected natural areas exceeding 8 billion visits per year, as of 2015 (Balmford et al. 2015). In addition to receiving more visits than the total human population of Earth each year, visits to protected areas generated $600 billion in direct spending in US dollars per year as of 2015 (Balmford et al. 2015). This is exceptional considering the spending on maintenance and protection of these areas was only $10 billion US dollars (Balmford et al. 2015). Tourists' trips to protected areas create a long term monetary incentive for locals to maintain the protected area rather than destroy it with short term, unsustainable practices like farming, mining, logging, and drilling for oil (Hausmann et al. 2016). In addition to the incentive to protect the area for locals, these areas also generate interest in conserving the charismatic species observed, and conservation in general for both the tourists who are visiting the area, as well as the natives who have become tour guides, or come to appreciate the long term value of these areas. The presence of these areas allows for species richness, prevents environmental degradation, and combats climate change when the present plants convert the greenhouse gas CO_2 into 0_2 (Melillo et al. 2016). Despite only consisting of 7.8% of the earth's landmass, protected land areas are responsible for one 20% of all carbon fixation occurring each year (Melillo et al. 2016). Symbolizing amphibians as a focus on ecotourism will enhance the revenue generated and create further support to acquire more conservation lands for ecotourism.

Because amphibians are so colorful, interesting, and eye catching they are excellent symbols for ecotourism and conservation. The rainforest café already takes advantage of this using the red eyed tree frog as their mascot with great success. The use of colorful and interesting amphibians as symbols of ecotourism, such as the brilliant sapphire tree frog *Dendrobates tinctorius "azureus"*, that has a vibrant color not seen on the skin of any other animal, more people will be attracted to the ecotourism destinations, and more ecotourism habitats can be preserved and created. The increased number of ecotourism locations will create more protected areas, so the image of amphibians generate greater environmental protection and therefore stability. By reserving the habitat where colorful and charismatic (Fig 3-6) amphibians are found as an ecotourism destination where tourists can be taken, conservation of the amphibians living in that area will be improved. The habitat will become a valuable resource for the local people in its natural state, and it will be protected and preserved. Setting aside habitats as amphibian-based ecotourism destinations, will not only protect amphibians but also other native species in the area as well. This is the principle of surrogate species, and the enhanced biodiversity will in turn benefit the amphibians who will have a diverse array of invertebrates to eat, a variety of plants to live on and in, and a variety of predators to regulate their populations, and prevent overpopulation and the accompanying over exploitation and depletion of food resources.

Amphibians are colorful and charismatic animals with interesting behavioral patterns, life history strategies, and natural histories, making them an object of significant public interest. These characteristic enable amphibians to increase the number of ecotourism visitors to certain area, where amphibians are found, especially areas with very colorful species (Fig 3-6). Amphibians make an ideal focal organism in ecotourism, especially in developing countries where high amphibian richness and endemism is threatened by an areas anthropocentric unsustainable land-use practices such as farming, logging, mining or oil drilling high (Hausmann et al. 2016). Australia and South America have some extremely colorful frogs; these colors arise due to either aposematism or mimicry (Fig 3-6). For example, the South American Blue poison dart frog *Dendrobates tinctorius "azureus"* uses its brilliant sapphire blue color to warn predators about the potent pumiliotoxins that interfere in muscle contractions by affecting calcium channels causing extreme pain, muscular paralysis, hyperactivity, or death (Daly et al. 2003).

Figure 3: *Dendrobates tinctorius* of the subspecies *azureu*s from South America Photo:https://commons.wikimedia.org/wiki/File:Dendrobates_azureus_(Dendrobates_tinctorius)_Edit.jpg

Figure 4: *Ameerega macero*, or Manu Poison dart frog from Brazil and Peru. Photo: Paul

Figure 5: *Dendrobates pumilio*, or strawberry poison frog native to Central America. Photo: https://allanimalia.com/256/poison-dart-

Figure 6: Notaden bennettii, Crucifix frog from Australia. Photo: Dr. Paul Anthony Stewart, Sydney University

Educational Value of Amphibians:

Amphibians are excellent model organisms for both research and teaching. Amphibians have distinct physical characteristics, life histories, and evolutionary histories. Being tetrapod vertebrates, amphibians share many similarities with other vertebrates including humans, thus are effective model organisms to teach vertebrate anatomy as well as physiology. Studies have also shown that students learn information better and are more motivated to learn about the natural and life sciences when living organisms such as amphibians are used to educate them (Wünschmann et al. 2017). For instance, when elementary students in Landau, Germany went to an amphibian and reptile zoo, the normally present gender gap seen in the motivation to learn, self-confidence, and knowledge was lost (Wünschmann et al. 2017).

By bringing amphibians into the classroom, a greater appreciation of and understanding of nature can be generated at a younger age. If public schools routinely used charismatic and interesting amphibian species to teach children about ecology, evolution, anatomy, physiology, and the general public's literacy on these topics would increase. This in turn would enhance conservation efforts, as there would be more public support for legislation aimed at setting aside protected wildlife areas and at limiting the use of chemicals damaging to amphibians and the environment. A greater public understanding of science would also allow the general public to better see through false arguments aimed at derailing and destroying legislation aimed at protecting the environment, such as has been accomplished by the factions of society attempting to convince Americans that climate change is not real. This improved public scientific literacy would also increase the desire for the general public to get involved with conservation world as citizen scientists who are familiar with the natural world, and want to engage with it to learn more about it and protect it.

Recent Trends in Public Interest in Conservation

In the recent decades, the global conservation community— including conservation biologists and ecologists, natural resource managers, and conservation authorities of governments— have underscored the importance of and the need for amphibian conservation at the local, regional, as well as global scale (Gadgil 1992, Mittermeier et al. 1992, Rabb and Sullivan 1995). Consequently, even the pubic, particularly environmental enthusiasts and citizen scientists have expressed interests in and needs for amphibian conservation (Gascon 2007, Dickinson et al. 2012). Interestingly, the public's enthusiasm for learning about amphibians and their conservation has grown substantially (Burne and Griffin 2005, Schmeller et al. 2009). To satisfy the public demands for education and awareness as well as to implement scientifically-informed conservation actions, development of a strong knowledge-base on amphibian biology is imperative. In the global context of biodiversity loss, amphibians have received increasing attention. The year 2008 was declared as the year of the frog, with the intention of increasing the conservation efforts of amphibians and to promote awareness about the importance of amphibian conservation among public (Pavajeau et al. 2008).

It is essential that logistic support and public enthusiasm for research and conservation in amphibians continue so that unprecedented extinctions and population declines can be prevented. While charismatic megafauna such as large carnivores, avifauna, and marine mammals attract much of the conservation dollars and public support, ensuring continued interests in amphibian conservation, research, and education can be very challenging. Sustaining and enhancing public interests will be essential to securing financial, logistic and intellectual resources for research and conservation of amphibians.

Suggested Future Directions and Conclusion

My suggestion for amphibian conservation is to take advantage of the uniqueness and interesting physical, developmental, and evolutionary characteristics of amphibians to engage public school children in the classroom at an early age. I also suggest that parallel outreach programs are performed with adults at aquariums, zoos, wildlife centers, on television and the radio. These outreach programs should clearly articulate the direct benefits the conservation of amphibians will give to people in their daily lives, and also the benefits these animals confer to the environment. The full repercussions of amphibian extinction should be highlighted including the reduced ecosystem productivity, impaired ecosystem stability and loss of native biodiversity, and the increased numbers of disease vectors and ectoparasites. The irreplaceable lost potential for new medicine discovery, and new regenerative treatment development should also be highlighted. The funding saved by cutting down application of synthetic pesticides, and the profits gained by the creation of new medicines and the use of amphibians as a renewable food source, will not only benefit society, but can be partially directed to amphibian conservation. A healthy dose of worst case scenarios resulting from the loss of amphibians should be described to galvanize people into quick and passionate action to save amphibians. One such scenario is that when bacteria develop resistance to all currently available antibiotics, and then amphibians are driven to extinction we will not have these new medicines to treat infections. In this scenario even minor cuts and operations like the removal of wisdom teeth could result in untreatable bacterial infections that can be lethal.

Once sufficient public interest is generated, public fundraising events should be held in the name of amphibian conservation at all the locations outreach programs are held. The public support of amphibian conservation and funding raised should be used to purchase areas where endangered amphibians live, and to pass legislation that stops the activities that increase the risk of amphibian extinction, such as releasing pollution into the environment, the release of greenhouse gasses that increase global warming and climate change.

The federal government as well as state and local governments should give tax breaks, grants, subsidies, interest free loans, and other incentives to companies, towns and cities that take steps to educate their citizens and employees about the value of amphibians and begin using local amphibians in ways that benefit both the amphibians and the humans of the area. One example of a program like this could be the protection of several ponds and the areas around them to allow the amphibians in those ponds to act bio control agents and consume mosquito eggs, larvae, and adults. Frog farms that use native species could be created, like an American Bullfrog farm outside Boston that donates some of the profits raised to amphibian conservation. There are currently no frog farms in Massachusetts, so government subsidies should be provided to encourage the opening of new companies. Another good example is a biotech company in Boston that agrees to donate a quarter of the profits from the sale of its new antibiotic derived from frog skin polypeptides to amphibian conservation. Another example is if a farm was given tax breaks and subsidies for using local amphibians to control the crop eating pests instead of pesticides. The general public and the scientific community should also get involved and express support and solidarity with the companies, farms, and communities that attempt new strategies to conserve and protect amphibians and the environment.

If people are informed about how much they have to gain in terms of a better standard of living with better medicine, fewer diseases, and more stable and renewable food supplies from the conservation of amphibians, I believe many people will support conservation efforts and become involved in the process. The critical part will be effectively getting the truth about all the value amphibians have out to the public. I believe the measures I have suggested will be an effective way to educate people about the services amphibians can provide to both humans and the environment. It will take solidarity from the scientific community, and tireless work on behalf of conservationists everywhere, but thanks to the multitude of services amphibians provide, I believe people will realize amphibians are so valuable and important to humanity and the environment, they have to be saved. According to the IUCN Red List approximately 25% of all the assessed species of Amphibians are listed data deficient and their conservation status remains unassessed as a result. Data deficiency is a major challenge particularly when prevalent when assessing the conservation status of tropical amphibians. Because of this I would advise that data deficient tropical species receive priority funding and attention and are researched first, to find out their risk of extinction. This is an essential course of action because data deficient species could be critically endangered, and if the scientific community does not know that, no steps can be taken to protect them, and they could be lost forever. With climate change expanding the range of tropical regions, tropical invertebrate pathogen vectors are migrating to greater ranges, and the tropical amphibians that are specifically adapted to effectively prey on them will be needed to control them.

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