



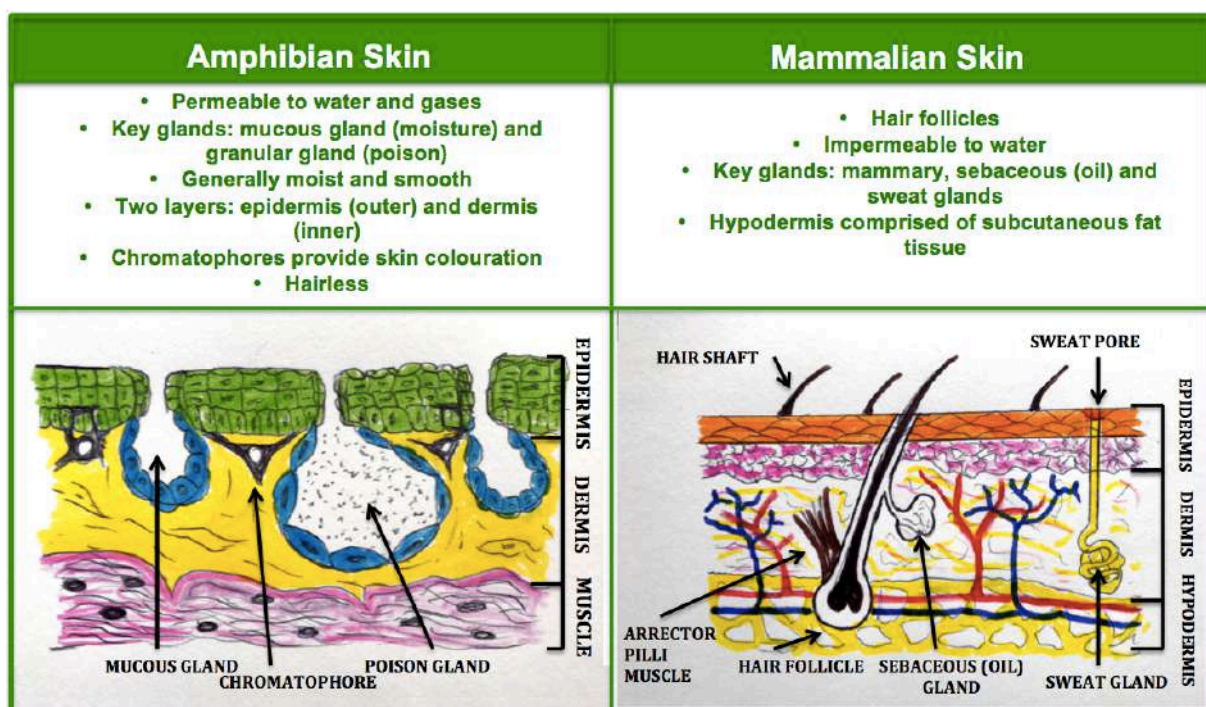
Amphibians are currently undergoing rapid population declines, with at least 42% of species now threatened or extinct worldwide. The amphibians make up three taxonomic orders: frogs and toads (anurans), newts and salamanders (caudata) and the caecilians (gymnophiona). All three orders are regarded as environmental indicator species, as populations are highly sensitive to changes in their environment. Therefore, fluctuations in amphibian populations can be used to monitor ecosystem quality, making them more than just fascinating creatures. Human driven impacts, such as deforestation, the release of environmental pollutants and the introduction of invasive species, are already devastating global numbers. However, the primary culprit for the amphibian population crisis is a fungal pathogen, which inflicts a fatal disease: chytridiomycosis. The fungus infects the amphibian host via their skin. Hence, understanding the unique ways in which amphibian skin allows them to interact with their physical environment can inform about how species respond to these human driven environmental changes, this deadly fungus and ultimately focus conservation efforts.

Amphibian skin

Amphibians have highly specialised skin in order to balance their internal and external environments. It is comprised of two layers: the dermis (inner) and epidermis (outer), the latter of which is shed every few days. Typically their skin is smooth and moist, however toads are an exception to this as they have rough, warty skin due to the aggregation of glands on the surface. Amphibian skin is permeable to water and gases and is fundamental for maintaining a constant internal environment via homeostasis. Contrastingly mammals and reptiles (squamates) typically have a waterproof non-moist epidermal layer; in addition, mammals have multiple hair follicles projecting from the inner dermis, whereas the reptilians have scaly skin.

Amphibian skin is highly distinguishable due to the two primary glands found within it; the mucous and granular glands, which are not found in reptiles, as they lack skin glands. Amphibian mucous glands ensure that their outer epidermis remains moist, facilitating respiration through the skin. On the other

hand, their granular glands play a part in skin immune defence against infecting bacteria and fungi. In some frog species these granular glands have specialised into a form of chemical defence against predation. For example, the poison dart frogs have adapted their granular glands to produce toxic secretions lethal enough to kill multiple humans. Contrastingly, mammals lack granular glands. Mammals have their own array of skin glands such as mammary glands and the sebaceous glands, which release oily secretions within the hair follicle. These are all types of saccular glands, with the amphibian granular and mucous glands defined as simple saccular structures comprised of a singular bulb. Whereas mammalian mammary glands are termed compound saccular, as they are made up of multiple simple saccular glands, which form a larger lobule. Amphibians would not survive long in the absence of their primary skin glands, as they would quickly dry out. Further to this, poisonous species that are typically active during the day rely on their granular glands for toxicity and use aposematic signals to deter predators.



Respiration: the role of the skin

Amphibian offspring start off life by breathing through paired gills formed of feathery filaments; these provide a large surface area for aqueous gas exchange. In order to maintain a fresh supply of dissolved oxygen to the gills, tadpoles pump and draw water over these filaments, where gases diffuse into and out of the small blood vessels near the gill surface. The passive uptake of oxygen occurs simultaneously with the diffusion of carbon dioxide out of the blood. Amphibians unlike mammals undergo a process called metamorphosis; when juveniles transition to adulthood their body shape changes radically. The tadpoles first lose their tails, which are designed for an aquatic environment and develop limbs; further to this their gills are absorbed and covered with skin. Following metamorphosis they now rely entirely upon two structures for gas exchange: newly formed lungs and their skin. The permeable skin of amphibians is tailored towards cutaneous (skin) gas exchange as it has a rich supply of capillaries near the surface and a moist layer maintained by the mucous glands.

However, amphibians that live in arid environments have fewer mucous glands on their backs and legs to minimise water loss via evaporation. Generally it is observed that the larger the surface area to volume ratio of the amphibian, the greater the efficiency of cutaneous respiration. One freshwater species, the Lake Titicaca frog, has adapted to have multiple folds in its skin in order to increase its surface area and maximise its ability to exchange gases through the skin. This species of frog also sways about to disturb the surrounding water to ensure a constant supply of dissolved oxygen.

Terms Explained

Chytridiomycosis: a fatal amphibian disease caused by the chytrid fungus, *Batrachochytrium dendrobatidis*.

Osmosis: net movement of water from a region of high water potential to a region of low water potential.

Hyperosmotic: region of higher solute concentration.

Zoospores: motile spores with a single flagellum, these are asexually produced in sporangium.

Aposematism: animal pattern/colouration acts as warning signal of their toxicity.



Lake Titicaca
frog

Maintaining Water Balance

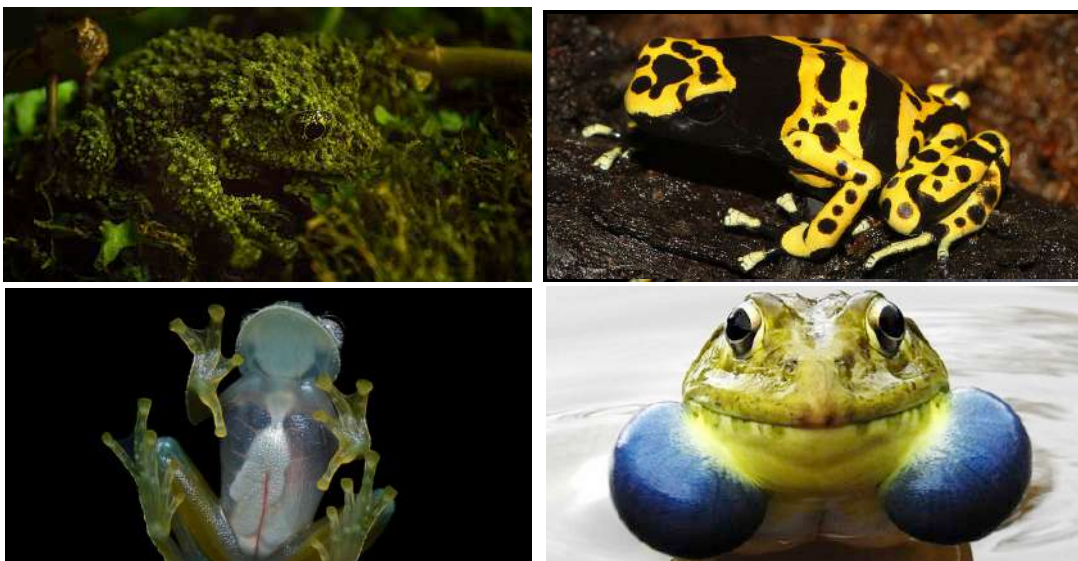
Amphibians circumvent the need to drink water by absorbing it through their skin. Therefore, they need to maintain a balance between evaporative water loss and contact mediated uptake. However, when submerged in water they don't want to take on too much water, so will expel large volumes of very dilute urine. It is also very important that an amphibian retains their body salts, as ensuring they have a higher internal concentration of salts relative to their environment facilitates water uptake; hence their urine is dilute to conserve salts. Amphibians live in both aquatic and terrestrial habitats, but predominantly terrestrial species must prevent themselves from drying out. Each individual species has its own adaptations to avoid excessive water loss, including the development of unique skin structures and changes in behavioural and physiological traits.

Nocturnal amphibians are active at night and therefore avoid exposure to the sun and the higher temperatures of the day, reducing evaporative water loss. Alternatively amphibians can seek shelter under leaf litter or by digging down into the damp soil below the surface. Species like the Couch's and European spadefoot toad have specialised spurs on their feet, which allow them to find moisture below the ground. However, fire salamanders prefer to crawl under rocks and hide within vegetation, living sedentary lifestyles. Whereas, frogs that remain visible to the sun will minimise their exposed skin surface by hunching and tucking all their limbs beneath their body.

The skin of every species functions in relation to their environment, for example tree frogs, which are exposed to the sun in the canopy, have a greater risk of drying out. Therefore, tree frogs typically have a reduced permeability to water on their backs and will concentrate their permeability to their unexposed underbelly and legs. The Budgett's frog has evolved to burrow into mud, where they actively shed excess skin to form a protective outer layer in which they can trap moisture. Not only have they specialised to cocoon themselves in their dead skin, but they are also capable of reabsorbing water they have stored in their bladders. Physiologically amphibians can maintain a high concentration of internal solutes by producing highly concentrated urea, ensuring the net movement of water into the body via osmosis. African grey tree frogs take this one step further by excreting their toxic urea as uric acid and thus avoid water loss by not diluting urine. A further example of host physiological change is seen in the waxy tree frogs, which have specialised lipid glands. These frogs then rub their secretions all over their skin, creating a protective layer against water loss.

Box | Funky frogs

The world is full of unique and frankly bizarre creatures and amphibians provide their fair share. Their skin not only provides vital life function, but also has other roles including camouflage and display. The Vietnamese mossy frogs seen below are a great example of how amphibian skin has adapted to blend in to the habitat and help avoid predation by camouflage. Whereas the yellow-banded poison dart frog displays its toxicity via bright colouration patterns, a form of aposematism. Another bizarre and incredible group of frogs are the glass frogs whose transparent skin leaves their internal organs visible and in some species you can even see their hearts beating. Another fascinating role of skin can be observed in the vocal sacs of Indian bullfrogs, used for vocal communication in many anurans. This species uses their bright blue vocal sacs to call out to other nearby frogs. The diversity of different amphibians and the range in the patterns, textures and colours of their skin is vast, making them a truly astonishing group of organisms to study.



Top left: **Vietnamese mossy frog** (*Thelederma corticale*)

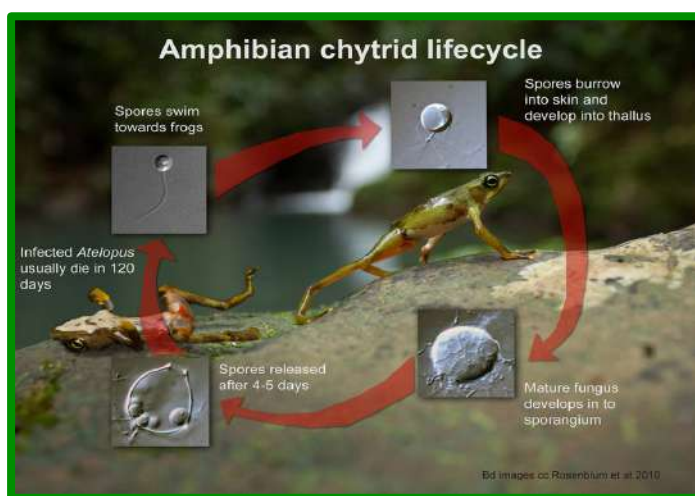
Top right: **Yellow-banded poison dart frog** (*Dendrobates leucomelas*)

Bottom left: **Glass frog** (*Hyalinobatrachium yaku*)

Bottom right: **Indian bullfrog** (*Hoplobatrachus tigerinus*)

The global amphibian crisis: Chytridiomycosis

A fungal pathogen known as *Bd* is responsible for the mass declines in amphibian populations and infects its victims via their skin. The fungus is waterborne and therefore particularly lethal to juveniles (tadpoles) that live an aquatic lifestyle, before they metamorphose into adults. The fungus exists as flagellated zoospores, which are attracted towards the protein dense keratin in the outermost layers of amphibian skin. Here the infective zoospores develop into a sporangium. The infective cycle then continues when the sporangium releases more motile zoospores, increasing the level of infection. Once the amphibian is burdened with enough zoospores, the host is killed by the resulting disease chytridiomycosis. The accumulation of zoospores in the skin leads to a process called hyperkeratosis, where the keratin in the outer layers becomes thickened.



Further reading

National geographic:
<https://www.nationalgeographic.com/animals/amphibians/>

The Atlantic, Ed Yong:
<https://www.theatlantic.com/science/archive/2019/03/bd-frogs-apocalypse-disease/585862/>

Amphibians rely on their skin to regulate a constant internal environment, but due to the damage caused by the accumulation of zoospores and the resulting skin thickening, this process is impaired. Amphibians must maintain a hyperosmotic internal environment, where the concentration of solutes is higher inside the body. This facilitates a net movement of water into the organism through their permeable skin. This is achieved by pumping salt into the body and expelling very dilute urine. However, a *Bd* infected species is ineffective at actively pumping salts into their body, creating an osmotic imbalance. When host blood potassium levels fall too low, their muscle function deteriorates, especially in the cardiac muscle, so the ultimate cause of their death is heart failure. In cases where the skin is thickened and damaged, its ability to exchange gases and to absorb water is severely impacted. For example, lungless salamanders that rely solely upon their skin for respiration would no longer be able to provide their body with enough oxygen to survive.

The ways in which amphibians interact with their environment are truly unique and vital to their survival. With increasing human pressure and the emergence of this deadly chytrid fungus, this group of special organisms is on the brink of extinction. The outlook for amphibian populations is currently grave, but by increasing interest and awareness, alongside advances in ongoing research into the dynamics of infection, there could still be hope.

A-LEVIEW REVIEW:

Further reading:

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Yong, E. (2018) The worst disease ever recorded. The Atlantic [Online]. Accessed 28 March 2019. <<https://www.theatlantic.com/science/archive/2019/03/bd-frogs-apocalypse-disease/585862/>>

Image sources:

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Lake Titicaca frog: (Stated source in article Wikimedia commons)

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Vietnamese mossy frog: (Wikimedia commons free for re-use)

Hartmann, T. (2015) In house of reptiles, Oslo. Accessed 9 April 2019. <https://commons.wikimedia.org/wiki/File:Vietnamese_Mossy_Frog.jpg>

Yellow-banded poison dart frog: (Wikimedia commons free for re-use)

Krisp, H. (2011) Yellow-Banded Poison Dart Frog, Yellow-Headed Poison Dart Frog or Bumblebee Poison Frog, *Dendrobates leucomelas*, Family: Dendrobatidae, Germany, Ulm, Zoological Garden. Accessed 9 April 2019. <<https://commons.wikimedia.org/wiki/File:Dendrobates-leucomelas-gelbgebänderter-baumsteiger.jpg>>

Glass frog: (Wikimedia commons free for re-use)

Gallice, G. (2011) Centrolenidae: *Cochranella pulverata*; ventral view. Accessed 9 April 2019. <[https://commons.wikimedia.org/wiki/File:Flickr_-_ggallice_-_Glass_frog_\(4\)_cropped.jpg](https://commons.wikimedia.org/wiki/File:Flickr_-_ggallice_-_Glass_frog_(4)_cropped.jpg)>

Indian bullfrog: (Wikimedia commons free for re-use)

Kasambe, R. (2018) Indian Bullfrog *Hoplobatrachus tigerinus* (Daudin, 1802). Syn. *Rana tigerina* Daudin, 1802. Clicked in BNHS Conservation Education Centre, Goregaon, Mumbai, Maharashtra. Sanjay Gandhi National Park. Male croaking loudly with swollen vocal sacs. The sound-resonating throat pouch of male frogs and toads (amphibians of the order Anura). Vocal sacs are outpocketings of the floor of the mouth, or buccal cavity. Accessed 9 April 2019. <https://commons.wikimedia.org/wiki/File:Indian_Bullfrog_Hoplobatrachus_tigerinus_by_Dr._Raju_Kasambe_DSCN6470_08.jpg>

Amphibian chytrid life cycle: (Free to share)

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