Life above the surface: Using the aerial environment

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Abstract

Water and air differ dramatically in their physical properties, posing numerous physiological challenges for animals that move between aquatic and terrestrial habitats. Nevertheless, more than 200 fish species have evolved an amphibious lifestyle. In this article, the remarkable diversity in amphibious lifestyles among fishes is highlighted, along with a discussion on the potential evolutionary drivers for amphibiousness. Moreover, the article delves into the critical physiological challenges associated with terrestrial exposure, as well as the innovative ways in which amphibious fishes have overcome these challenges to survive and thrive out of water.

Key points

- The ability to tolerate life on land has evolved independently many times among fishes.
- Like aquatic air-breathing fishes, amphibious fishes are also capable of aerial O_2 uptake, but these two groups differ in their physiology, ecology, and evolutionary origins.
- Amphibious fishes can differ dramatically in their amphibious habits, from the duration of terrestrial excursions to the reason(s) for leaving water.
- Amphibious fishes have evolved numerous innovative strategies for tolerating the physiological challenges associated with life on land.

Glossary

Amphibious fishes Fish species that spend time on land as a routine part of their natural history.
Emerse The act of moving from an aquatic to terrestrial habitat. In contrast, the term **immerse** describes the act of moving from a terrestrial to an aquatic habitat.
Aquatic air-breathing fishes Fish species that spend the entirety of their life in water but periodically surface to obtain O_2 from air.
Air-breathing organ (ABO) An anatomical structure found in fishes that facilitates aerial O_2 uptake. ABOs may include lungs, labyrinth organs, the buccopharyngeal cavity, the swim bladder, or the alimentary canal. In amphibious fishes, the skin and gills may also serve as ABOs.
Continuum hypothesis The ability to breathe air evolved first in otherwise fully-aquatic fishes, enabling the subsequent evolution of strategies to cope with the challenges associated with life on land.
Emersion first hypothesis The inhabitation of periodically dry habitats (or other emersion behavior) evolved first and imposed strong selective pressure for the acquisition of air breathing.
Cutaneous respiration A form of respiration in which gas exchange occurs across the skin's surface.
Angiogenesis The physiological process through which new blood vessels form from pre-existing vessels.
Bohr effect Effect of pH on the oxygen affinity of hemoglobin.
Root effect Property of hemoglobin in some fishes such that, in the presence of acid, it is impossible for hemoglobin molecules to be completely saturated with O_2, even at extremely high O_2 partial pressures.
Ionocyte Mitochondria-rich cells in the gills and/or skin that are specialized for either ion secretion (marine fishes) or uptake (freshwater fishes).
Aestivation A state of dormancy characterized by inactivity and a lowered metabolic rate. Aestivation is entered in response to limited food and water availability, which are often accompanied by hot summer temperatures.
Volatilization The conversion of a liquid chemical into a vapor, which escapes into the atmosphere.
Skeletal muscle plasticity The ability of an organism with a given genotype to express alternative muscle phenotypes in response to various environmental, mechanical, and/or physiological stimuli.

Introduction

The colonization of land by fishes represents one of the most extreme habitat transitions in evolutionary history. Water and air differ dramatically in their physical properties (e.g., density, viscosity), which poses critical challenges for processes such as respiration, osmoregulation, ionoregulation, nitrogen excretion, feeding, and locomotion (**Dejours, 1988**; **Sayer, 2005**). These challenges suggest that traversing the air-water interface should be difficult for fishes and consequently rare; yet, numerous species have evolved the ability to survive out of water (**Damsgaard et al., 2020**). Perhaps the most notable land invasion occurred during the Devonian epoch (~400 million years ago), in which early lobe-finned fishes emerged onto land before giving rise to land-dwelling tetrapods. However, the ability to leave water has evolved an additional 87 times among fishes in the last 65 million years (**Fig. 1**; **Damsgaard et al., 2020**) resulting in well over 200 extant species of **amphibious fishes** that spend time on land as a routine part of their natural history (**Wright and Turko, 2016**).

Extant amphibious fishes can differ dramatically in their amphibious habits, from the duration of their terrestrial excursions to the reason(s) that they leave water. Some species live in intertidal environments where receding tides force them out of water for short, but predictable periods of time (e.g., **Ebeling et al., 1970**). Others live in tropical regions where seasonal droughts can dry aquatic environments for several weeks or months (e.g., **Delaney et al., 1974**). Still, others actively leave water for brief periods to escape unfavorable aquatic conditions (e.g., hypoxia; **Regan et al., 2011**) or to exploit terrestrial resources (e.g., terrestrial food sources; **Bob-Manuel, 2011**). In general, amphibious fishes that leave water (**emerse**) for prolonged periods tend to remain inactive or dormant on land (e.g., *Neochanna* spp.), possibly as a strategy to conserve limited “on board” energy stores (**Rossi and Wright, 2020**). Conversely, those that make brief terrestrial excursions tend to remain highly active when out of water and exploit terrestrial resources more extensively (e.g., *Periophthalmus* spp.; **Wright and Turko, 2016**; **Turko et al., 2021**). This article highlights the remarkable diversity in amphibious lifestyles among fishes and discusses the potential evolutionary drivers for amphibiousness. Moreover, the article explores the critical physiological challenges associated with terrestrial exposure and the innovative ways amphibious fishes have overcome these challenges to survive and thrive out of water.

Air-breathing versus amphibious fishes

The ability to breathe air has evolved numerous times in fishes (**Damsgaard et al., 2020**). However, there are distinct physiological and evolutionary differences between **aquatic air-breathing fishes** and amphibious fishes (**Turko et al., 2021**). Aquatic air-breathing fishes are species that spend the entirety of their life in water, but periodically surface and gulp air to obtain O_2. Some species are considered facultative air-breathers because aerial O_2 uptake is merely used to supplement water-breathing when aquatic O_2 availability is insufficient to meet metabolic requirements (e.g., under hypoxic conditions). In contrast, obligate air-breathers require regular surface breathing to obtain enough O_2 to meet their metabolic requirements, even in well-oxygenated water. Obligate air-breathing tends to occur in species where the gill surface area has been reduced to the point where fish would drown if denied access to the surface. A notable example is the Amazonian pirarucu (*Arapaima gigas*) that undergoes a transition from water-breathing to obligate air-breathing during development. When these fish are small (10 g), the gills of the Amazonian pirarucu are similar in appearance to closely related water-breathing fishes. However, as these fish grow (100–1000 g), the surface area of the gills becomes reduced, resulting in a shift to obligate air-breathing (**Pelster et al., 2020**). Both facultative and obligate air-breathers require specialized respiratory adaptations to facilitate aerial O_2 uptake

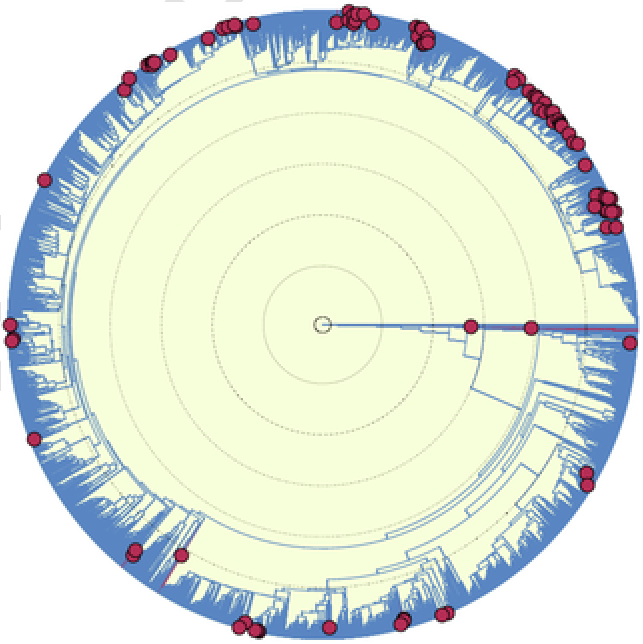

Fig. 1 The evolution of the amphibious lifestyle traced across the osteichtyan fish tree of life. Red circles indicate branches where the amphibious lifestyle evolved. The time from the center to periphery is 615 million years with dashed circles for each 100-million-year period. Retrieved from **Damsgaard et al. (2020)**.

while in water, such as extra-branchial **air-breathing organs (ABOs)**. Indeed, some species have evolved organs specifically designed for air-breathing (e.g., lungs, labyrinth organs); whereas, other fish species have evolved the capacity to air-breathe using existing structures (e.g., swim bladder, buccopharyngeal cavity, alimentary canal). The retention of the gills in all aquatic air-breathers allows these fishes to continue to excrete CO_2 into water, as well carry out other important functions of the gills, such as ion regulation and ammonia excretion. Thus, aside from respiratory adaptations that enable aerial O_2 uptake, the physiology of aquatic air-breathing fishes is fundamentally similar to that of fully aquatic species.

Amphibious fishes are often viewed as a specialized group of air-breathing fishes, but it has been emphasized that these two groups should not necessarily be considered together (**Turko et al., 2021**). First, not all amphibious fishes are capable of breathing air. For example, the California grunion (*Leuresthes tenuis*) leaves water at very high tides between February and September to spawn on the beach. Females first dig their tails into the sand to deposit eggs; males then wrap themselves around the head of the female, depositing milt (sperm) on the eggs. This fascinating reproductive behavior occurs within a matter of minutes, during which time the grunions rely solely on anaerobic metabolism to meet energy demands (**Martin et al., 2004**). Second, amphibious fishes spend time in terrestrial habitats and therefore face a unique set of challenges compared with all other fishes (see section below on *Physiological challenges and innovative solutions*). Notably, the gill lamellae of many amphibious fishes collapse and coalesce without the buoyant support of water, thereby reducing the surface area available for gas exchange (**Lam et al., 2006**). Like aquatic air-breathers, most amphibious fishes rely on ABOs to facilitate aerial O_2 uptake, but additional strategies are required to deal with CO_2 and nitrogenous waste accumulation, and to osmo- and ionoreguate without water flow over the gills (for reviews, see **Sayer, 2005**; **Wright and Turko 2016**; **Damsgaard et al., 2020**). Furthermore, fishes out of water must contend with the constant threat of desiccation, as well as an increase in apparent gravity owing to the lower density and viscosity of air compared to water (**Dejours, 1988**; **Sayer, 2005**). These physical disparities between air and water can affect locomotion (**Standen et al. 2014**) and impair the ability of fishes to consume food out of water (**Heiss et al., 2018**). Additionally, amphibious fishes may face sensory challenges (e.g., vision) as they traverse the air-water interface, particularly when sensory systems are predominately suited for an aquatic environment. Thus, while aquatic air-breathing and amphibious fishes share the ability to obtain O_2 from air, they differ dramatically in other aspects of their physiology, ecology, and in the environmental challenges that they face.

The evolution of amphibiousness

Aquatic air-breathing is widely considered to be a critical first step in the evolution of amphibiousness in fishes. This **continuum hypothesis** suggests that ability to air-breathe allowed fishes to first meet one of the most acute challenges associated with invading land (i.e., maintaining aerobic metabolism), enabling the subsequent evolution of strategies to cope other challenges that fishes face in terrestrial environments (**Fig. 2**A). While this sequence of events is probable for the invasion of land by tetrapods, it may not necessarily reflect broader patterns of terrestrial evolution in fishes (for a review, see **Turko et al., 2021**). It has been demonstrated that, among the 87 independent origins of amphibiousness, only 7 (~8%) evolved from lineages in which aquatic air-breathing had previously evolved. Ancestral state reconstruction suggests that air-breathing evolved in tandem with amphibiousness for the remaining ~92% of amphibious origins (**Turko et al., 2021**). Consistent with this finding, numerous amphibious fishes are incapable of aquatic air-breathing and only breathe air when emersed. For example, the mangrove rivulus (*Kryptolebias marmoratus*) uses **cutaneous respiration** for aerial gas exchange and must therefore leave water for its primary ABO (i.e., the skin) to engage in aerial respiration (**Blanchard et al., 2019**). Thus, the evolution of aquatic air-breathing is unlikely to precede amphibiousness in this species, as well as in other skin-breathers. Furthermore, severe aquatic hypoxia is widely considered to be the primary driver for the evolution of both aquatic air-breathing and amphibious lifestyles in fishes (e.g., **Graham and Lee, 2004**). However, why would fishes leave water and confront the challenges associated with life on land when they could circumvent aquatic hypoxia by remaining in water and breathing air? Indeed, only a small proportion (16%) of amphibious lineages contain species that emerse in response to aquatic hypoxia (**Turko et al., 2021**). Thus, the factors underpinning the evolution of aquatic air-breathing in fishes may differ from those that lead amphibiousness, lending evidence against the continuum hypothesis.

An alternative hypothesis to explain the evolution of amphibiousness is the **emersion-first hypothesis**. If the inhabitation of periodically dry habitats and/or other emersion behavior (e.g., exploitation of terrestrial resources) evolved first, it would have imposed strong selective pressure for the acquisition of air breathing (**Fig. 2**B). In support of the emersion-first hypothesis, the majority amphibious lineages (67%) contain species that live in habitats that dry periodically (e.g., ephemeral pools, intertidal zones), which may explain why air breathing appears to have evolved in tandem with amphibiousness in the majority of amphibious lineages (**Turko et al., 2021**). Unlike the presence of aquatic hypoxia, being forced out of water due to habitat drying is uniquely tolerable by only amphibious species and may therefore be a critical driver for the evolution of amphibiousness. Overall, there are likely multiple driving forces behind the amphibious lifestyle, and while the continuum hypothesis appears to be the default hypothesis, it does not convincingly explain all origins of amphibiousness in fishes.

Diversity in amphibious lifestyles

Given the multiple independent origins of amphibiousness among fishes, it is not surprising that there is remarkable diversity in amphibious lifestyles. Fishes differ dramatically in their amphibious habits, from the duration of their terrestrial excursions to the reason(s) that

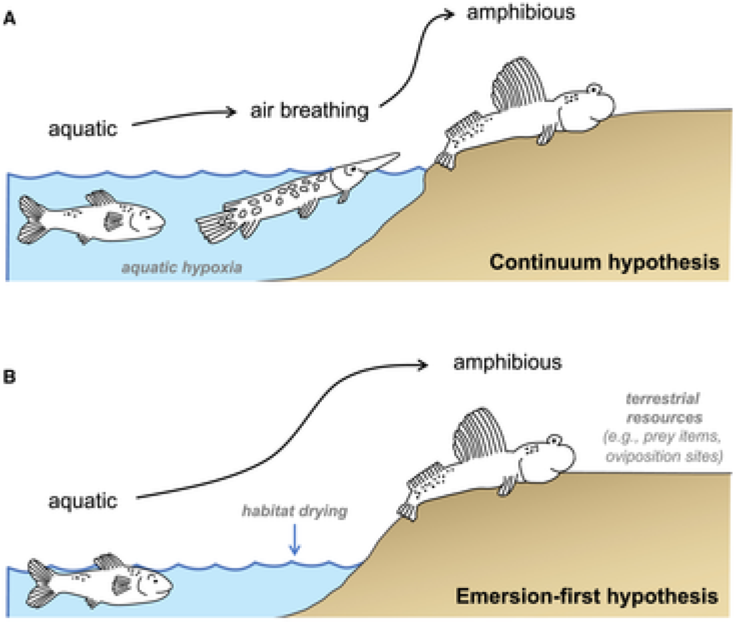

Fig. 2 (A) Schematic representation of the "continuum hypothesis," in which the evolution of air breathing is thought to precede the evolution of amphibiousness. According to the "continuum hypothesis," severe aquatic hypoxia is likely the primary driver for the evolution of air breathing and amphibiousness in fishes. (B) Schematic representation of the "emersion-first hypothesis," in which the inhabitation of periodically dry habitats and/or other emersion behavior (e.g., to exploit terrestrial resources) evolved first, imposing strong selection pressure for the acquisition of air breathing. Initial figure design by P.A. Wright with fish schematics by I. Smith.

they leave water. For example, intertidal clingfish (*Sicyases sanguineus*) are forced out of water as tides recede, leaving them stranded on land for short, but predictable periods of time (**Ebeling et al., 1970**). The marbled lungfish (*Protopterus aethiopicus*) is similarly forced out of water when its shallow lake or river evaporates during seasonal droughts but may remain out of water for many months until heavy rains return (**Delaney et al., 1974**). Fishes may also leave water when aquatic conditions fall beyond tolerable limits (e.g., extreme hypoxia; **Regan et al., 2011**), returning only when more favorable aquatic conditions return. For instance, the mangrove molly (*Poecillia orri*) occupies mangrove pools rich in toxic hydrogen sulfide (H_2S) but emerges when H_2S concentrations exceed 0.5 mM (**Rossi et al., 2019a**). Retreating tides, habitat drying, and harsh aquatic conditions are considered ecological factors that "push" fish out water and may be important drivers for the evolution of amphibiousness in many species (**Turko et al., 2021**).

In addition to factors that "push" fish out of water, there are ecological factors that "pull" amphibious fishes into terrestrial habitats. In some cases, fishes leave water to exploit terrestrial food sources (e.g., *Periopthalmus koelreuteri*; **Bob-Manuel, 2011**) or to disperse to new aquatic environments via overland movement (e.g., *Clarias batrachus*; **Bressman et al., 2020**). Furthermore, several fishes are known to exhibit reproductive behaviors on land (for reviews, see **Martin et al., 2004**; **Ishimatsu et al., 2018**). For example, the midshipman (*Porichthys notatus*) is usually found in deep water but moves into the rocky intertidal zone to spawn. Spawning takes place underwater at high tide once males have attracted a female to their spawning site. Males then remain in the intertidal zone—even when air-exposed at low tide—to guard the developing embryos and larvae for many months (**Houpt et al., 2020**). Although terrestrial oviposition sites are most commonly used by intertidal fishes, there are a few freshwater species that also spawn and deposit eggs out of water. An extreme example is the splashing tetra (*Copella arnoldi*), in which spawning pairs first leap out of water onto an overhanging leaf. Eggs are deposited onto the leaf by the female and immediately fertilized by the male before the pair returns to water. Males then care for their incubating eggs by going to the surface and periodically splashing them to prevent desiccation (**Krekorian, 1976**). Finally, there have even been reports of fishes voluntarily leaving water for no apparent reason whatsoever (e.g., **Szelistowski, 1990**). Some have speculated that these voluntary emersions may be a form of exploratory behavior driven by hunger (;**Turko et al., 2011**) or possibly a way in which fish prime their physiology for future bouts of air exposure (**Rossi et al., 2020**).

While the diversity in amphibiousness across species is impressive, amphibious habits can also vary at the population- or individual-level. Notably, **Turko et al. (2018)** demonstrated that the mangrove rivulus (*Kryptolebias marmoratus*) from one wild population voluntarily spent up to 90% of their time on land; whereas, a second population in close proximity spend only one-third as much time on land. Fish can also exhibit differences in emersion behavior within their lifetime. In some species where the adult life stage is amphibious, the larval form is unable to tolerate periods of air exposure. For example, climbing perch (*Anabas testudineus*) develop their ABO (i.e., labyrinth organ) during the juvenile life stage and only make terrestrial excursions once its development is complete (**Morioka et al., 2009**). Moreover, the mangrove rivulus shows age-related deterioration in a number of physiological traits important for tolerating life on land (e.g., terrestrial locomotion), suggesting that fish may alter their emersion behavior as they senesce (**Rossi et al., 2019b**). Taken together, amphibious species show tremendous interspecific diversity in the frequency and duration of their terrestrial excursions, but there may also be significant population- and individual-level variation in amphibious habits.

Classifying amphibiousness

Amphibious fishes have long interested biologists because they can provide important clues about the adaptations required for transitioning between two dramatically different environments—water and land. However, it is not easy to generalize these physiological adaptations because of the diverse natural histories within this group of fishes. Whereas some fishes can survive out of water for weeks or months, others can only tolerate a few minutes or hours in air. Still, some species exploit the terrestrial environment extensively and voluntarily, while others emerse only when absolutely necessary. Thus, a staging scheme has been proposed that scores amphibiousness in fishes and is accommodating of the diverse natural histories within this group (**Turko et al., 2021**).

The scale categorizes fishes into four levels of amphibiousness: mostly aquatic (Level 1), mildly amphibious (Level 2), moderately amphibious (Level 3), and highly amphibious (Level 4). Levels are assigned by plotting a species' tolerance for air exposure (e.g., minutes, weeks) against their “land use score,” which reflects how extensively fish exploit terrestrial landscapes (**Fig. 3**). To determine land use scores, a value of 1 or 2 is assigned to each of the reasons amphibious fishes are found out of water. Factors that “push” fish out of water (i.e., habitat drying, unfavorable aquatic conditions) are given a value of 1, whereas factors that “pull” fish out of water (i.e., terrestrial food, reproduction, dispersal/exploration) are given a value of 2. Ecological pulls are valued more than pushes because they often require adaptions beyond those required to simply tolerate air exposure, such as specialized jaws for eating out of water (e.g., *Periophthalmus koelreuteri*) or modified appendages for effective movement overland (e.g., *Beaufortia kweichowensis*). A species' land use score is the sum of all the pushes and pulls that result in its terrestrial exposure. For example, the climbing perch (*Anabas testudineus*) has a land use score of 3 because it is forced out of water during periods of drought (1 point), but also leaves water to disperse to new aquatic habitats (2 points). Overall, species that can survive on land for several weeks or months are considered more amphibious than those that can

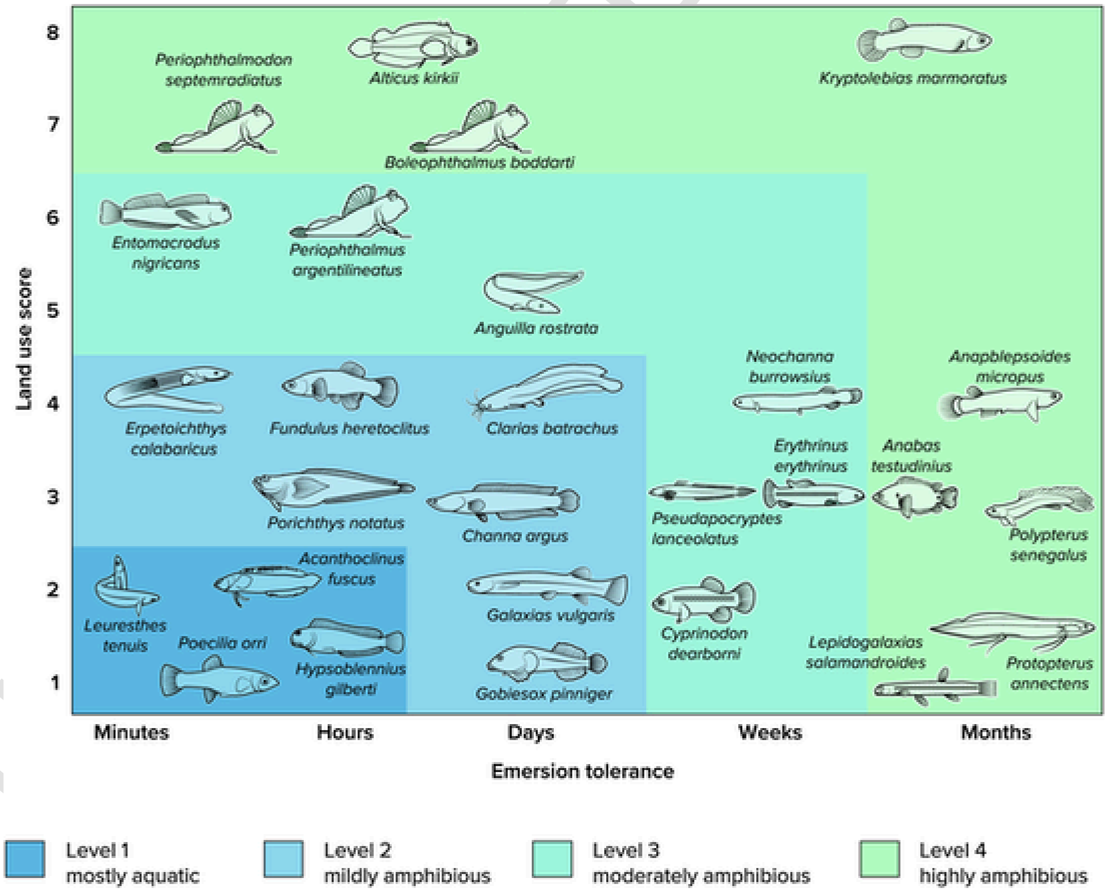

Fig. 3 A scoring system that assigns fishes varying degrees of amphibiousness (Level 1–4). The degree of amphibiousness is determined according to emersion tolerance and use of terrestrial habitats. Retrieved from **Turko et al. (2021)**.

only tolerate a few minutes of air exposure. Likewise, species are considered more amphibious if they use the terrestrial environment for multiple reasons, as this more closely resembles the lifestyle of terrestrial vertebrates. Understanding which species are “more amphibious” than others may provide important insights into the adaptations required for the colonization of land by aquatic taxa.

Terrestrial challenges and innovative solutions

O_2 uptake

When on land, amphibious species encounter high environmental O_2 because the concentration of O_2 is ∼30 times higher in air compared to water (**Dejours, 1988**). However, accessing aerial O_2 can be challenging. Without the buoyant support of water, the gill lamellae of many amphibious fishes collapse and coalesce when they are on land, thereby reducing the surface area available for respiration (**Lam et al., 2006**). Some species have modified gills, which partially alleviates this problem (for a review, see **Sayer, 2005**). For example, the bearded mudskipper (*Scartelaos histophorus*) possesses stout gills with widely spaced lamellae to reduce the degree of collapse and coalescence during air exposure (**Tamura et al., 1976**). Other amphibious species rely on extra-branchial ABOs to facilitate aerial O_2 uptake and, much like in aquatic air-breathers, these ABOs may include the lungs, swim bladder, buccopharyngeal cavity, or alimentary canal. For many amphibious species, the skin is also an important exchange site not only for respiratory gasses, but also for water, ions, and nitrogenous waste (for a review, see **Wright, 2021**).

Amphibious fishes may use a variety of respiratory organs to meet metabolic O_2 requirements when out of water. For example, **Urbina et al. (2014)** found that approximately 60% of O_2 uptake occurred across the skin in air-exposed inangas (*Galaxias maculatus*), with the remainder occurring across the gills. Interestingly, some species can reversibly alter features of their ABOs to enhance their contribution toward aerial O_2 uptake. A notable example is the mangrove rivulus that frequently “gulps” air immediately following emersion, presumably because a significant proportion of O_2 uptake occurs within the buccopharyngeal cavity. After only a few days in air, gulping behavior is reduced by more than 50% and fish exhibit significant **angiogenesis** in the skin, suggesting a greater reliance on the skin for O_2 uptake (**Turko et al., 2014**; **Blanchard et al., 2019**). Indeed, increased skin capillarity has been linked with improved aerobic performance out of water in this species (**Rossi et al., 2020**).

CO_2 accumulation

Metabolically-produced CO_2 readily diffuses out across a fish's gills into the water. When on land, however, many amphibious fishes retain CO_2 because the absence of water flow over the gills impairs its excretion. Indeed, the partial pressure of CO_2 (PCO_2) in the blood more than doubles in the gold wolf fish (*Hoplerythrinus unitaeniatus*) upon emersion (**Randall et al., 1978**). Elevated blood PCO_2 causes a reduction in blood pH (respiratory acidosis), which can reduce both the affinity of hemoglobin for O_2 (**Bohr effect**) and O_2 carrying capacity of the blood (**Root effect**) (for a review, see **Brauner et al., 2019**). Both of these changes can negatively affect the transport of O_2 throughout the body. To mitigate respiratory acidosis and it consequences on blood O_2 transport, some amphibious fishes may accumulate bicarbonate (HCO_3-) in the plasma to enhance the buffering capacity of the blood (e.g., *Protopterus aethiopicus*; **DeLaney et al., 1977**), increase the affinity of hemoglobin for O_2 (e.g., *Kryptolebias marmoratus*; **Tunnah et al., 2021**), and/or alter ventilation patterns to restore acid-base balance (e.g., *Protopterus annectens*; **Gilmour et al., 2007**). In other cases, amphibious fishes may avoid respiratory acidosis altogether by effectively eliminating CO_2 into air. **Martin (1993)** showed that many intertidal fishes can exchange O_2 and CO_2 in air at similar rates to their respiration in water using the skin as the primary site of gas exchange.

Water and ion balance

In an aquatic setting, the gills are the primary site of ion regulation in fishes. The gills contain **ionocytes**, which are mitochondria-rich cells specialized for either ion secretion (marine fishes) or uptake (freshwater fishes) (**Evans et al., 2005**). When emersed, gill ion exchange is diminished or eliminated in amphibious fishes, as water ceases to flow across the gills. Thus, for many amphibious species, the skin may function as an important site for ion exchange out of water (for a review, see **Wright, 2021**). Indeed, ionocytes have now been reported in the skin of a few amphibious fishes (e.g., **Martin et al., 2019**).

When emersed, freshwater fishes may encounter low ion concentrations in the substrate, resulting in the passive loss of Na^+ and Cl^-, but gain of water across the skin. Cutaneous ion uptake and renal water excretion is therefore necessary to maintain water and ion balance. In contrast, marine fishes may encounter high ion concentrations in the substrate, leading to the passive gain of Na^+ and Cl^-, but loss of water across the skin. To maintain balance, ion secretion and water uptake across the cutaneous surface is necessary, as drinking to obtain water is not an option (for a review, see **Wright, 2021**). For the few amphibious fish species that can feed on land (see section below on **Feeding**), ions and water may also be obtained across the gut upon feeding. Behavioral mechanisms may also be employed to reduce cutaneous water loss, such as seeking humid terrestrial habitats, periodically rolling in shallow pools to keep the skin wet, and/or spending time in burrows to avoid elevated air temperatures (e.g., **Daxboeck and Heming, 1982**).

Nitrogen excretion

Ammonia is a toxic end product of metabolism that fishes typically excrete rapidly and efficiently across the gills into water. The absence of water flow over the gills can therefore be lethal for fishes owing to the accumulation of ammonia. Consequently, amphibious fishes have adopted a number of strategies for dealing with ammonia production. Some species convert ammonia into relatively non-toxic compounds (e.g., urea, glutamine) that can be stored in the body until water becomes available (e.g., *Protopterus dolloi*); whereas, others supress protein and amino acid catabolism to minimize ammonia production (e.g., *Boleophthalmus boddaerti*) (for a review, see **Chew and Ip, 2014**). In some cases, fishes may even continue to excrete ammonia while in air using extra-branchial surfaces. For example, **Livingston et al. (2018)** found that several amphibious killifishes have ammonia-transporting Rhesus proteins in the skin, which facilitate the movement of ammonia from the blood to mucous film on the skin from which its **volatilization** into air can occur.

Feeding

Most fishes capitalize on the high density and viscosity of water to help capture, transport, and swallow food (for a review, see **Heiss et al., 2018**). Thus, amphibious fishes on land must either find new ways to consume food or refrain from eating altogether. Among the most unique adaptations for feeding out of water is the “hydrodynamic tongue” of the Atlantic mudskipper (*Periophthalmus barbarus*). When these mudskippers begin to envelop a terrestrial prey item, they use water stored in their mouth cavity as a protruding and retracting “tongue,” thereby allowing the manipulation and swallowing of prey (**Michel et al., 2015**). While several amphibious species are also capable of capturing terrestrial prey items (e.g., *Channallabes apus*), many must return to water in order to swallow their food.

Many other amphibious fishes refrain from eating when on land. While the inability to feed may not be problematic for short-term emersers, long-term emersers must find ways to conserve their endogenous energy stores to prevent starvation. Some species can tolerate months (and in rare cases, years) on land without feeding by entering a state of dormancy (**aestivation**), in which their metabolic rate is significantly reduced. For example, lungfish can reduce O_2 consumption by more than 90% during aestivation, with concomitant declines in ventilation, heart rate, and blood pressure (**Fishman et al., 1992**). Interestingly, amphibious species may seek terrestrial microhabitats that accentuate metabolic depression during dormancy (e.g., hypoxic burrows or tunnels) to further slow the depletion of endogenous energy stores (**Rossi and Wright, 2020**). Thus, both physiological and behavioral strategies may work in concert to help fishes tolerate long periods on land in the absence of food.

Locomotion

Many amphibious fishes that remain active on land rely on terrestrial locomotion to accomplish important daily tasks (e.g., predator avoidance, prey capture). However, locomotor movement on land is far more difficult than movement in water. Fish are effectively weightless in aquatic environments, owing to the buoyant support of water, but must contend with increased effective body weight on land. To facilitate effective overland movement, many amphibious fishes have evolved specialized locomotor structures. Notable examples include the gray bichir (*Polypterus senegalas*) that “walks” on land using modified pectoral fins that appear to work like limbs (**Standen et al., 2014**) and the climbing perch (*Anabas testudineus*) that uses modified spikey gill covers to grip the substratum during forward movement on land (**Davenport and Matin, 1990**). Amphibious species that lack apparent specialized locomotor structures produce directed movements on land using only the axial skeleton and associated musculature. For instance, amphibious eels use serpentine movements to move overland (Gillis, 2000) , and many killifishes use jumping behaviors to traverse terrestrial landscapes (**Minicozzi et al., 2019**). Perhaps the best-studied terrestrial jumper is the amphibious mangrove rivulus, which can travel several hundred body lengths on land via repeated jumps before reaching exhaustion (**Turko et al., 2022**). Interestingly, the skeletal musculature of the mangrove rivulus is highly responsive to air exposure, producing a more aerobic phenotype on land that results in improved jumping performance (**Brunt et al., 2016**; **Rossi et al., 2018**). It is unclear whether **skeletal muscle plasticity** can similarly improve the locomotor performance of other amphibious fishes out of water, but this remains an interesting avenue for future work.

Conclusion

Amphibious fishes have long interested biologists because they transition between two of the most physically divergent environments that can be experienced by any animal. For decades, biologists have carefully documented the amphibious habits of fishes in their environment and investigated their physiology under laboratory settings. Research findings are beginning to shed light on the remarkable physiological and behavioral adaptations that enable highly amphibious lifestyles in fishes, yet much remains to be uncovered. Given that the colonization of land by extant amphibious fishes has clear parallels with the origin of all land vertebrates, including humans, insights into the factors facilitating successful land invasions in extant fishes may offer valuable perspectives into the evolutionary path to terrestrial life.

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