



Graphical reviews

How insects transition from water to air: Respiratory insights from dragonflies

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ABSTRACT

The transition of animal life from water onto land is associated with well-documented changes in respiratory physiology and blood chemistry, including a dramatic increase in blood $p\text{CO}_2$ and bicarbonate, and changes in ventilatory control. However, these changes have primarily been documented among ancestrally aquatic animal lineages that have evolved to breathe air. In contrast, the physiological consequences of air-breathing animals secondarily adopting aquatic gas exchange are not well explored. Insects are arguably the most successful air-breathing animals, but they have also re-evolved the ability to breathe water multiple times. The juvenile life stages of many insect lineages possess tracheal gills for aquatic gas exchange, but all shift back to breathing air in their adult form. This makes these amphibiotic insects an instructive contrast to most other animal groups, being not only an ancestrally air-breathing group of animals that have re-adapted to life in water, but also a group that undergoes an ontogenetic shift from water back to air across their life cycle. This graphical review summarizes the current knowledge on how blood acid-base balance and ventilatory control change in the dragonfly during its water-to-air transition, and highlights some of the remaining gaps to be filled.

Animals moving from water to air must overcome the physiological challenges associated with breathing two very different respiratory media. Amphibians are perhaps the best-known animal group to straddle the air/water divide, with studies examining the respiratory changes associated with their metamorphosis from gilled larvae to air-breathing adults dating back well over a century. Indeed, some of the first measurements to reveal that frogs exchange respiratory gases using both pulmonary and cutaneous pathways were undertaken by August Krogh as part of his doctoral dissertation (Krogh, 1904). Since then, a clear picture has been built up to reveal how the transition from water to air, over both evolutionary and developmental time scales, drives animals to undergo similar changes in blood acid-base balance and ventilatory control (Fig. 1).

As the shift from water to air is associated with a drastic increase in oxygen (O_2) availability, air-breathing animals can reduce their ventilation substantially due to the increased O_2 content (Rahn, 1966). This enables much lower rates of ventilation, and leads to an increase in blood carbon dioxide partial pressure ($p\text{CO}_2$) compared with water-breathers (Ultsch, 1996). To maintain a constant blood pH in the face of elevated $p\text{CO}_2$, air-breathers increase their strong-ion difference, causing an associated increase in bicarbonate concentration [HCO_3^-].

Finally, as a consequence of the elevated $p\text{CO}_2$ and [HCO_3^-], air-breathers control their ventilation to regulate $p\text{CO}_2/\text{pH}$ in addition to $p\text{O}_2$, while the ventilatory drive of water-breathers responds primarily or exclusively to $p\text{O}_2$ (Bayley et al., 2019). These differences in physiology between water- and air-breathing animals appear to be consistent, leading Dejours (1989) to suggest that all animals could be separated along physiological lines into either water- or air-breathing groups according to the shared physiological traits that arise from a shared respiratory medium. However, while these changes have been observed in aquatic animals that either evolved into obligate air-breathers, or those that transition from water to air during development, there has been comparatively little work done to understand what occurs when an air-breathing animal transitions to breathing water. Given that animals re-invading the aquatic environment must experience the same physico-chemical differences between air and water, one would predict the same differences in physiology to arise, albeit in the opposite direction, with water-breathing and its associated physiological changes being the derived condition. The insects present an ideal group to examine this air-to-water transition, as water-breathing has evolved among the juvenile life stages of insects from at least 10 orders, with 5 insect orders being almost exclusively amphibiotic (Balian et al., 2008). While the acid-base

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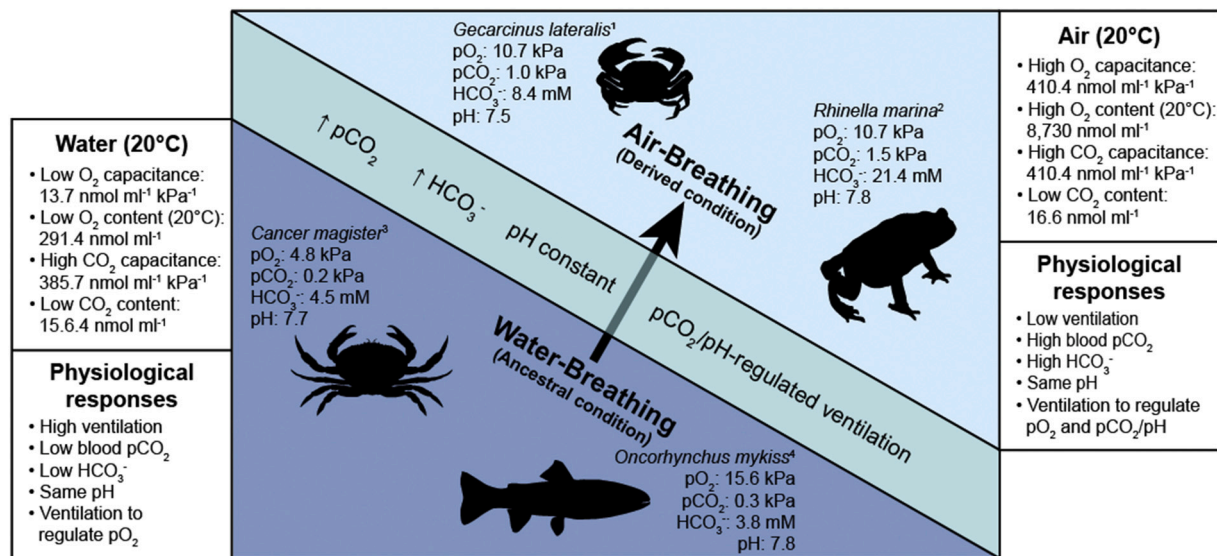


Fig. 1. The water-to-air respiratory transition. Oxygen (O_2) is relatively insoluble in water, with air-equilibrated water holding 30 times less O_2 compared with an equivalent volume of air at 20 °C. However, air and water have an equally high capacitance for CO_2 , allowing both media to contain approximately the same quantity of CO_2 at the same CO_2 partial pressure (pCO_2). Thus, the low O_2 solubility forces water-breathing animals to ventilate large amounts of water in order to meet their aerobic requirements, while the combination of increased ventilation and high CO_2 solubility results in these animals maintaining a low blood pCO_2 . In contrast, the higher O_2 content of air reduces the air-breather's ventilatory requirements. With air-breathers hypo-ventilating relative to their water breathing ancestors, blood pCO_2 naturally increases. Thus, the increase in blood pCO_2 during the transition from water- to air-breathing is a universal consequence of the different physico-chemical properties of the two media (Rahn, 1966), and while air-breathing animals have adapted to a state of higher blood pCO_2 and HCO_3^- compared with the water-breathers, both lineages maintain the same blood pH (Dejours, 1989). Gas content calculated for air-equilibrated distilled water at 1 atm. Arterial blood gas and pH values are reported here, measured between 17 and 25 °C. 1. Taylor and Davies (1981) 2. Toews and Heisler (1982) 3. McMahon et al. (1978) 4. Eddy et al. (1977).

and respiratory physiology of secondarily aquatic insects has been considered previously (Cooper, 1994), measurements of the respiratory changes that occur in insects as they transition from water-to-air have been sorely lacking. In this review we summarize the recently published data on the respiratory and acid-base physiology of the amphibiotic dragonflies (Odonata, Anisoptera) as they shift from breathing water using tidally-ventilated rectal gills to breathing air using spiracles, and explore how dragonfly respiratory physiology compares with other aquatic and terrestrial animal groups.

Recent work on dragonflies (families Libellulidae and Aeshnidae) has found that these insects occupy an unusual position in relation to other animal groups that move from water to air, as the water-breathing dragonfly nymph possesses a hemolymph [HCO_3^-] that is not only elevated relative to other water-breathers, but is also higher than the few measurements available for other air-breathing insects (Fig. 2A). Even more curious is that the average in vivo hemolymph pCO_2 recorded in aquatic aeshnid nymphs (0.9 kPa) (Lee et al., 2018) exceeds the theoretical maximum expired pCO_2 predicted for any water-breathing animal (0.7 kPa), assuming that the animal had a respiratory quotient (RQ) of 1, was inspiring water with a pO_2 of 20 kPa, a pCO_2 of 0, and all the O_2 was extracted from the inspired water (Rahn, 1966). However, this expired maximum does not account for the hemolymph-to-water pCO_2 gradient across the gill necessary to achieve it, nor the degree of hemolymph perfusion around the gill. It also neglects the elevated gill water pCO_2 due the mixing of aerated and deoxygenated water in the branchial chamber. If the expired gill water has a pCO_2 of 0.7 kPa, hemolymph pCO_2 must be somewhat higher, equivalent to the gradient between the mixed hemolymph and end-tidal gill water pCO_2 . Thus, the dragonfly nymph's unusual tidally-ventilated rectal gill likely explains why the internal hemolymph pCO_2 is higher than Rahn's theoretical expired maximum pCO_2 . Dragonfly hemolymph pH is also more alkaline

relative to those few measurements available for other insect groups (Fig. 2B). Interestingly, the only published insect pH values that overlap substantially with those recorded from dragonfly nymphs are those from various dipteran larvae, all of which are either aquatic water-breathers (i.e., chironomid larvae), or aquatic air-breathers (i.e., mosquito larvae). Unfortunately, there is insufficient data to determine whether this similarity is due to the shared aquatic habits of these insects or some other cause.

Although water-breathing dragonfly nymphs have a hemolymph [HCO_3^-] that is high relative to other water-breathers, they still possess much lower HCO_3^- levels compared with the air-breathing adults (Lee et al., 2018; Lee and Matthews, 2019) (Fig. 2A), reflecting the different demands placed on ventilation in water and air (Bayley et al., 2019). These differences are also apparent in the ventilatory responses of dragonfly nymphs and adults exposed to ambient hypoxia or hypercapnia. While the air-breathing adult increases abdominal ventilation amplitude when exposed to hypoxia and hypercapnia, water-breathing nymphs hyperventilate only in response to hypoxia (Fig. 3A) (Ubhi and Matthews, 2018). This demonstrates that aquatic dragonfly nymphs are like most other water-breathing animals: they regulate their gas exchange to control internal pO_2 , and are insensitive to pCO_2 . Thus, while pCO_2 induces a strong ventilatory drive in typical air-breathing insects (e.g. Buck, 1962), even a pCO_2 of 10 kPa is insufficient to elicit hyperventilation in the aquatic dragonfly nymph (Fig. 3B). Other water-breathing insect larvae, including dobsonflies (Megaloptera) and damselflies (Odonata), show this same blunted or absent response to hypercapnia (Ubhi and Matthews, 2018), suggesting that water-breathing insects have secondarily adopted the same ventilation control used by most water-breathers in general (Bayley et al., 2019).

The challenges facing amphibiotic animals are likely to be greatest during the actual transition between water and air – a process that may

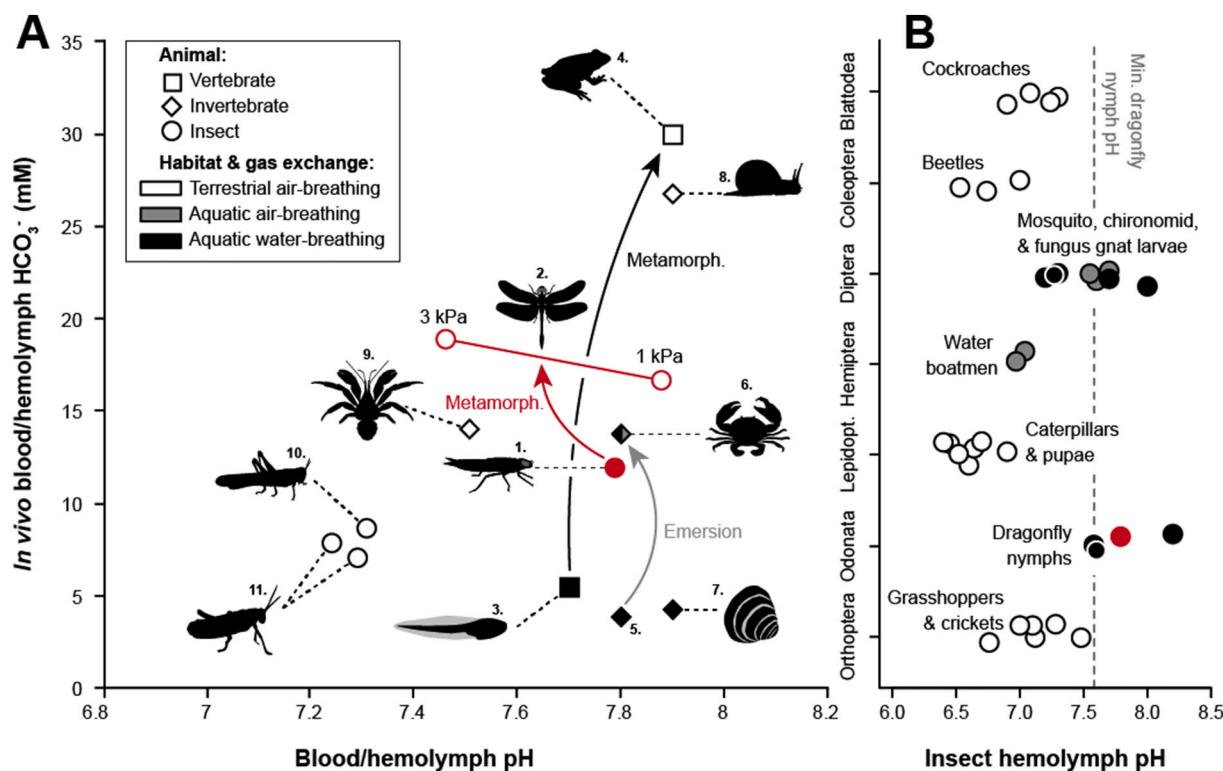


Fig. 2. Changes in acid-base balance across the water-to-air transition in dragonflies and other animal groups (A), and insect hemolymph pH in relation to Order and respiratory medium (B). A) Hemolymph $[\text{HCO}_3^-]$ increases when dragonflies transition from aquatic to aerial gas exchange (1 to 2 (Lee and Matthews, 2019)). However, the magnitude of this increase (39–58%) is small compared with those experienced by a metamorphosing amphibian (445%: 3 to 4) or a crustacean moving onto land (258%: 5 to 6). This arises from the elevated $[\text{HCO}_3^-]$ and pCO_2 of the water-breathing nymph relative to other water-breathers (e.g., tadpole 3, crab 5, and clam 7), and the relatively low levels in the adult dragonfly. Although in vivo pCO_2 and $[\text{HCO}_3^-]$ have not been measured in the adult dragonfly (2), using their blood buffer line and assuming a pCO_2 range similar to that seen in other insects, shows they too possess a $[\text{HCO}_3^-]$ well below air-breathing amphibia (4) and mollusks (8), but similar to air-breathing crustaceans (6 and 9). Experimental temperatures ranged from 15 to 25 °C, except for the coconut crab (27–30 °C). B) In relation to other insects, dragonfly nymphs have a higher hemolymph pH and $[\text{HCO}_3^-]$ than those measured from other air-breathing insect orders, but partly overlap with both water- and air-breathing dipteran larvae from aquatic habitats (i.e., mosquitoes). Refs for data in A: 1. Lee et al. (2018), 2. Lee and Matthews (2019), 3–4. Erasmus et al. (1970), 5–6. Truchot (1975), 7. Byrne et al. (1991), 8. Barnhart (1986), 9. Cameron and Mecklenburg (1973), 10. Harrison et al. (1990), 11. Harrison et al. (1995). Data in B. from Matthews (2017) and refs therein. Experimental temperatures were reported for only 14 of 36 values, and ranged from 20 to 37 °C.

last from seconds (e.g., emerging mayflies) to days or weeks involving an extended period of bimodal gas exchange (e.g., tadpole to frog). The dragonflies examined here fall in the latter category. In the days preceding metamorphosis, the final instar nymphs develop functional thoracic spiracles while retaining their rectal gill to become bimodal breathers (Fig. 3A) (de Pennart and Matthews, 2020). Thus, they display a period of acclimation where gas exchange is partitioned between both water and air, allowing for a gradual rise in internal pCO_2 and $[\text{HCO}_3^-]$ before the insect's final metamorphosis and loss of its rectal gill (Figs. 3A, 4). Amphibians undergo an equivalent developmental shift as the tadpole simultaneously develops lungs while absorbing their gills, resulting in a progressive increase in pCO_2 and $[\text{HCO}_3^-]$ and a period of bimodal breathing (Erasmus et al., 1970; Just et al., 1973) (Fig. 4). These changes in acid-base chemistry experienced during the frog's ontogeny is reminiscent of those that must have occurred during the ancestral vertebrate's invasion of land hundreds of millions of years ago. Dragonflies, on the other hand, represent an equally ancient shift from air back to water, but still display the same general pattern of physiological changes across ontogeny as the amphibian.

The biggest difference between dragonflies and other animal groups is the magnitude of the change in internal CO_2 levels. This is likely due to basic differences in the respiratory physiology of insects compared with other animal groups: Air-breathing insects possess an open, air-filled

tracheal system that is capable of maintaining some of the highest rates mass-specific rates of O_2 uptake in the animal kingdom, and equally enables them to maintain a comparatively low internal pCO_2 . This, combined with the elevated pCO_2 of the aquatic nymph relative to other water-breathers, attributable to gas exchange across a tidally-ventilated rectal gill, makes the overall increase in hemolymph pCO_2 as they move between water and air comparatively small. What remains to be seen is how other amphibiotic insects compare to dragonflies. The rectal gill is unique to the Odonata suborder Anisoptera, with other aquatic insects possessing external tracheal gills which may be functionally more like those of aquatic crustaceans and vertebrates. Thus, whether other amphibiotic insects moving from water to air conform to the patterns described here for the dragonflies, or must contend with a greater changes in pCO_2 , remains to be seen.

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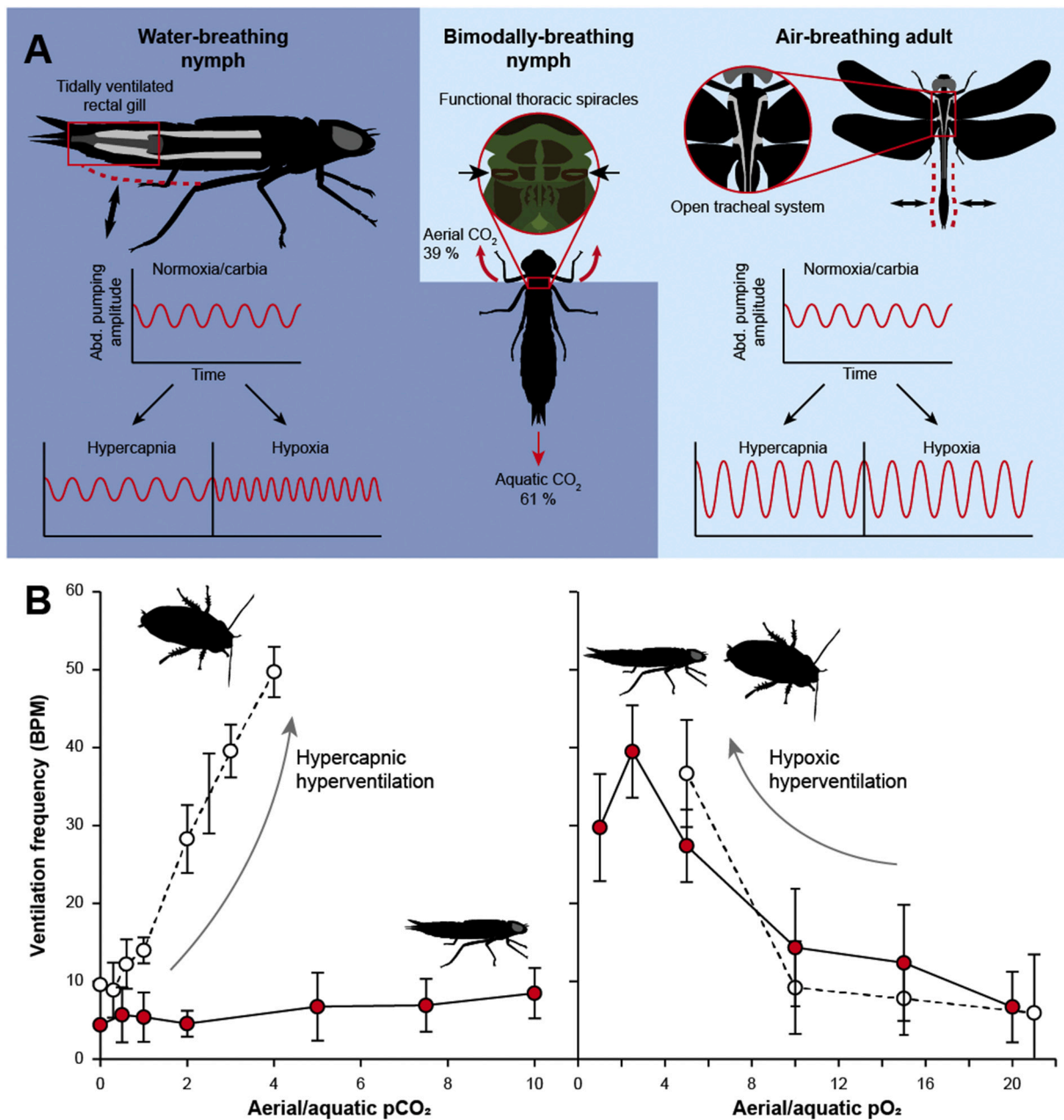


Fig. 3. Change in gas exchange strategy and ventilatory control during dragonfly ontogeny. A) During their larval stages, dragonflies are aquatic and breathe water using a tidally-ventilated rectal gill. Regulation of ventilation frequency is determined by oxygen (O₂), as the nymphs increase their breathing frequency when exposed to progressive hypoxia, but not in the presence of even severe hypercapnia. This insensitivity to carbon dioxide (CO₂) is seen in the ventilatory control of other water-breathing animals (Ubhi and Matthews, 2018). As the nymphs approach metamorphosis, they no longer rely solely on their rectal gill and begin to breathe air through functional spiracles, becoming bimodal breathers (de Pennart and Matthews, 2020). Although the ventilatory control in this bimodal stage is unknown, it likely represents a transitional state between water- and air-breathing. The adults breathe air using an open tracheal system, and their ventilatory control changes to respond to both O₂ and CO₂, similar to other air-breathing animals (Ubhi and Matthews, 2018). B) Exposing either a water-breathing dragonfly nymph (*Anax junius*; red circles) or an air-breathing cockroach (*Nauphoeta cinerea*; white circles) to hypoxia and hypercapnia reveals a clear difference – while the cockroach responds to both hypoxia and hypercapnia by increasing abdominal ventilation (Matthews and White, 2011), the aquatic dragonfly nymph hyperventilates in response to hypoxia only (Ubhi and Matthews, 2018). This blunted or absent hypercapnic response is typical of water-breathing animals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

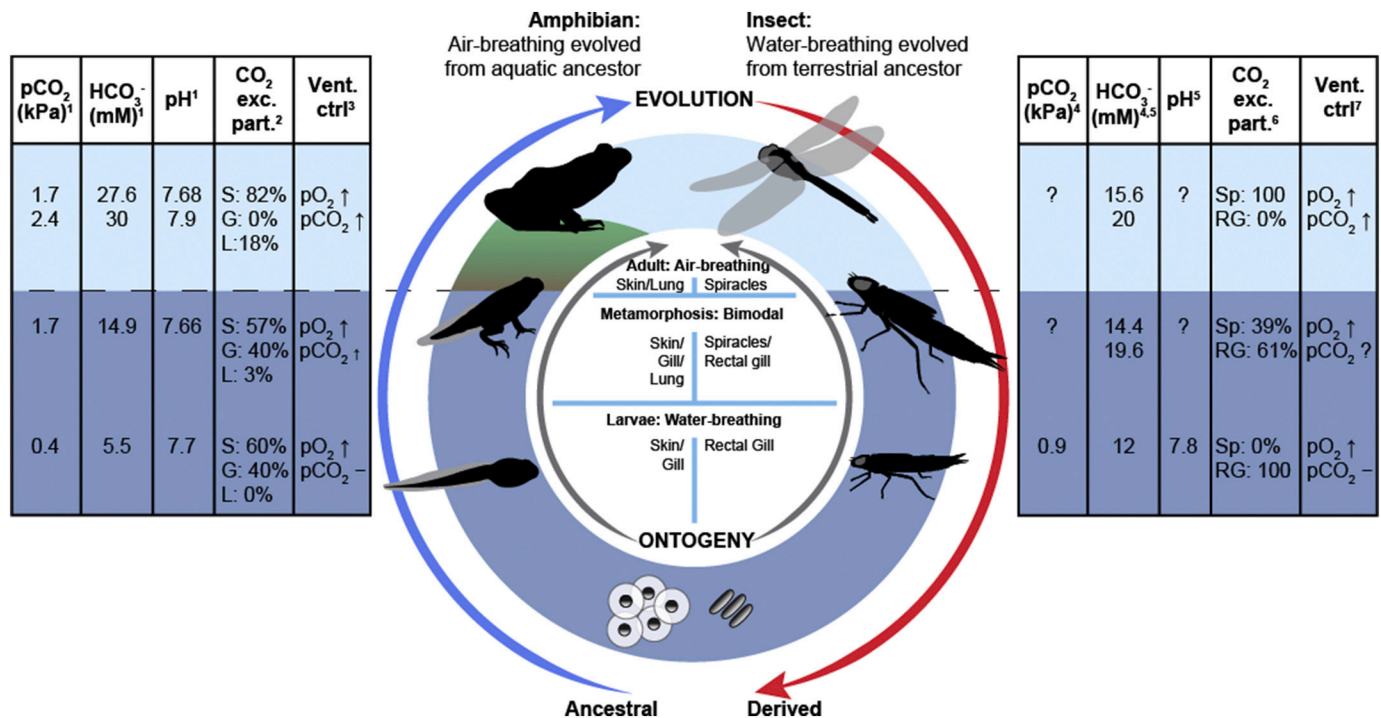


Fig. 4. The evolutionary and ontogenetic transition between water and air. Throughout the frog's ontogeny, tadpoles transition from breathing water using their gills and skin to becoming bimodal breathers as they develop lungs. Associated with this shift in gas exchange medium is a progressive increase in the animals' blood pCO₂ and [HCO₃⁻], along with an increasing ventilatory sensitivity to pCO₂/pH (Torgerson et al., 1997). Upon metamorphosis into adulthood, adult frogs lose their gills and become air-breathers, and must compensate for an even further increase in pCO₂ by adjusting their acid-base balance (Erasmus et al., 1970; Just et al., 1973). Dragonflies also represent a developmental water-to-air transition. Similar to amphibians, these insects breathe water as young nymphs using a rectal gill, breathe both water and air using gill and thoracic spiracles as late final instar nymphs, and eventually metamorphose into air-breathing adults. Although in vivo adult dragonfly pCO₂ and pH remain unknown, blood HCO₃⁻ increases as the insects become more reliant on air-breathing (Lee and Matthews, 2019), reaffirming the typical rise in blood acid-base chemistry as a result of switching from water-breathing to air-breathing. However, while the frog's development repeats the evolutionary water-to-air transition, dragonflies represent an ancestral shift from air to water, requiring the exaptation of their rectum into a gas-exchange organ. The consequence of this appears to be a higher internal pCO₂ in the water-breathing nymph. Refs for data: 1. Erasmus et al. (1970); Just et al. (1973), 2. Burggren and West (1982), 3. Torgerson et al. (1997), 4. Lee et al. (2018), 5. Lee and Matthews (2019), 6. de Pennart and Matthews (2020), 7. Ubhi and Matthews (2018).

Declaration of Competing Interest

We declare no conflict of interest.

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