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Adaptations of insects to hypoxia: Morphological, metabolic and behavioural responses

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Abstract

Insects rely on oxygen for oxidative metabolism, which is crucial for ATP production and overall metabolic functions. Hypoxia, defined as inadequate oxygen supply, poses significant challenges to insect survival, reproductive success, and flight performance. This review categorizes hypoxia into environmental and functional types and examines its physiological and ecological impacts on insects inhabiting hypoxic environments, such as aquatic systems, flood-prone soils, and high altitudes. Critical adaptations include alterations in tracheal morphology, metabolic shifts from aerobic to anaerobic pathways, and specific behavioural responses aimed at enhancing oxygen uptake. Notably, signalling pathways like Nitric Oxide Synthase (NOS) and Hypoxia-Inducible Factor (HIF) are activated under low-oxygen conditions, promoting necessary physiological changes. The review highlights how hypoxia leads to decreased survivability, reduced fecundity, extended developmental times, and altered flight activity. Moreover, it discusses the variation in sensitivity to hypoxia among terrestrial and aquatic insects. Understanding these adaptations provides insight into the resilience of insects in fluctuating oxygen environments and underscores the evolutionary significance of hypoxia tolerance in ecological contexts.

Keywords: Hypoxia, oxygen metabolism, oxidative metabolism, ATP production

Introduction

All living organisms require oxygen for oxidative metabolism, which produces ATP essential for various metabolic activities. Insects utilize oxygen both as a substrate and a signalling molecule (Hochachka and Somero, 2002; Corbucci *et al.*, 2005) [22, 5]. However, oxygen availability can fluctuate based on habitat and environmental conditions, impacting insect survivability, reproductive capacity, and flight metabolism (O'Farrell, 2001; Feala *et al.*, 2007) [31, 12]. To adapt to low-oxygen environments, insects may shift to anaerobic pathways and undergo morphological and behavioural changes (Piacentini and Karliner, 1999; Wingrove and O'Farrell, 1999; Lavista-Llanos *et al.*, 2002; Pan and Hardie, 2002) [33, 41, 29, 32].

What is Hypoxia?

Hypoxia occurs when a body or tissue is deprived of adequate oxygen supply. The partial pressure of oxygen (PO₂) represents the pressure exerted by oxygen in a gas mixture. At mean sea level, atmospheric gases include 78% nitrogen and 20.9% oxygen, resulting in a normoxic PO₂ of approximately 21 kPa s (Almaas *et al.*, 2004 & Reed and Palsson, 2004) [1, 34]. When PO₂ drops below this level, hypoxia occurs. In terrestrial environments, hypoxia is defined as oxygen concentrations below 20.94%. Aquatic insects can remain non-hypoxic in water with 7 ml/L of dissolved oxygen, while terrestrial insects submerged in oxygen-saturated water may quickly experience hypoxia.

Types of Hypoxia

Hypoxia is categorized into environmental and functional types. Environmental hypoxia refers to a PO₂ lower than normoxia, while functional hypoxia occurs when oxygen demand surpasses supply, leading to decreased internal PO₂ and impaired biological functions.

Effects of Functional Hypoxia

In functional hypoxia, insufficient oxygen supply impairs oxidative metabolism and ATP production (Harrison and Greenlee, 2018; Harrison *et al.*, 2015) [15, 19].

Factors such as tracheal morphology and ventilation influence oxygen delivery. Hypoxic conditions trigger signalling pathways that promote anaerobic ATP production, increase spiracular opening frequency, and alter

tracheal structure (Harrison *et al.*, 2013) ^[18]. Severe hypoxia can lead to protein unfolding, tissue inflammation, and reduced growth in insects (Hsia *et al.*, 2013; Burggren *et al.*, 2017) ^[23, 2] (Fig.1).

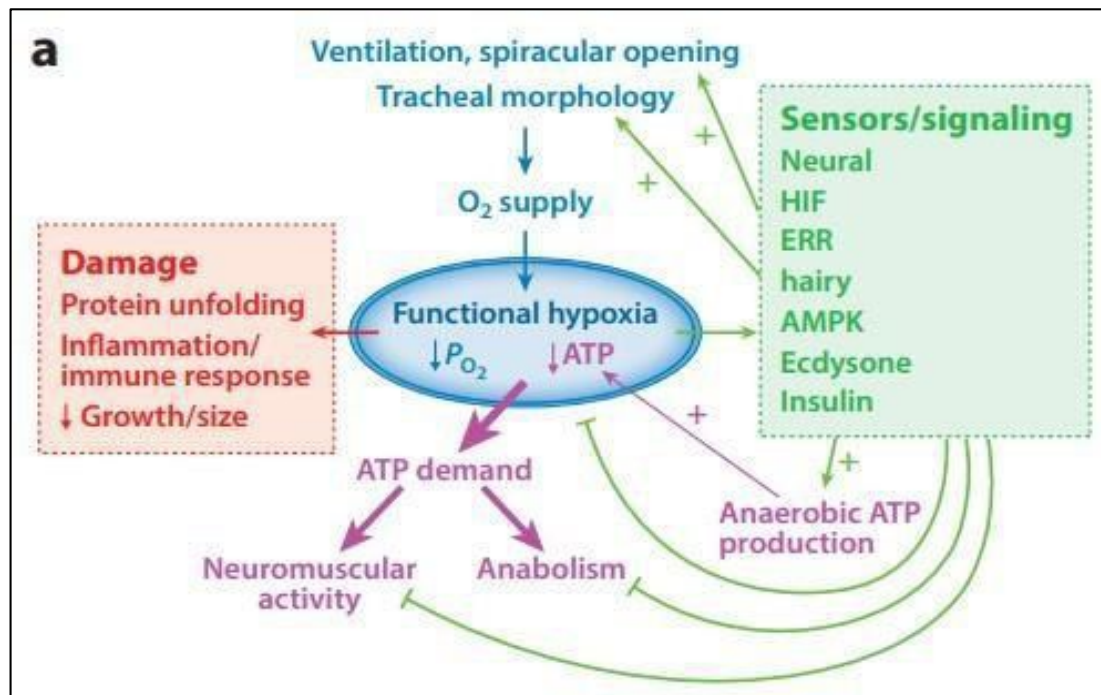


Fig 1: Series of events that occur during hypoxia in an insect

Critical Partial Pressure of Oxygen and Insect Performance

The critical partial pressure of oxygen (PO_2) is the threshold at which an insect's normal functioning occurs. When oxygen levels fall below this critical point, the performance of the insect declines, although it does not necessarily cease entirely due to a shift from aerobic to anaerobic metabolic pathways (Verberk and Bilton, 2013) ^[38, 39]. Above this critical level, insect performance is optimal; however,

further increases in oxygen can be detrimental, leading to the production of reactive oxygen species, which can damage cellular structures (Harrison and Lighton, 1998) ^[16]. Decreasing O_2 levels in a neuroendocrine-mediated manner, suppressing behaviour and growth even at PO_2 levels that are still above those leading to ATP limitation. This state is termed mild functional hypoxia, as it correlates with a decline in functions relevant to fitness (Harrison *et al.*, 2018) (Fig. 2).

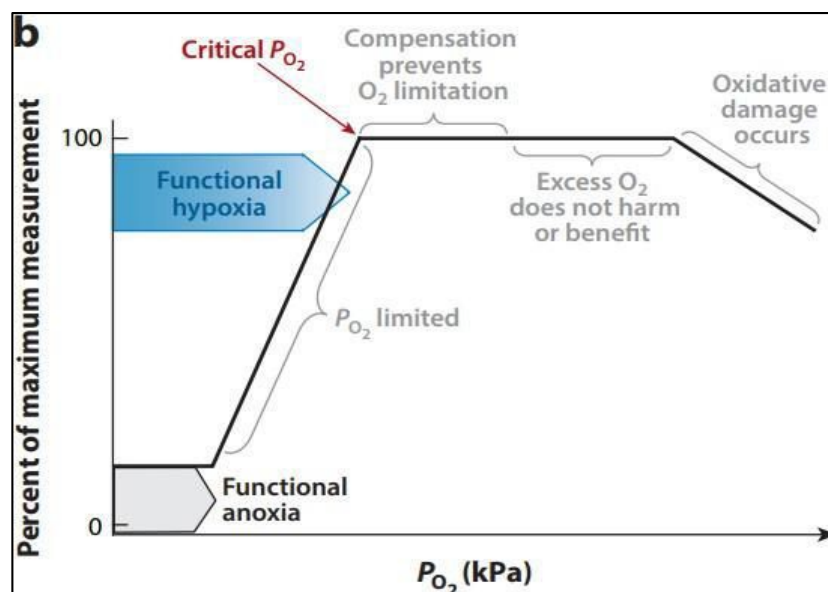


Fig 2: A hypothetical performance curve for O_2 effects on an organism Hypotoxic habitats of insects

When the metabolic rate becomes oxygen-limited and ATP is generated anaerobically, this indicates that the ATP demand exceeds what can be supplied through oxidative

catabolism, resulting in severe functional hypoxia. In many cases, insects respond to

Hypoxic Habitats of Insects

Insects inhabit various hypoxic environments, including aquatic systems, flood-prone soils, ice encasement, high altitudes and unique microhabitats such as dung and carrion.

Aquatic Habitat

Aquatic insects, which comprise only 3-5% of all insect species, are sensitive to hypoxia, and entire species assemblages are defined by the oxygenation of their habitats (Dejours, 1975; Verberk and Bilton, 2011) [7, 38, 39]. They can experience severe hypoxia; chronic exposure to sub-lethal conditions can lead to reduced growth, as seen in the mayfly *Heagenia limbata*. The equilibrium of PO₂ in aquatic

environments is slow due to the limited diffusion of oxygen. Aquatic insects can endure hypoxic conditions through various adaptations. For instance, an experiment by (Sesterhenn *et al.*, 2013) [35] on the respiration behaviour of *Ischnura posita* naiads demonstrated that ventilation rates significantly increase in low dissolved oxygen levels (Vander, 2007; Walshe, 1950) [36, 40]. At 50% dissolved oxygen, naiads had an average of two ventilations per minute, which rose to five and eight at 25% and 10% levels, respectively, indicating a compensatory response to acquire more oxygen in hypoxic situations (Wong *et al.*, 2014) [42] (Fig. 3).

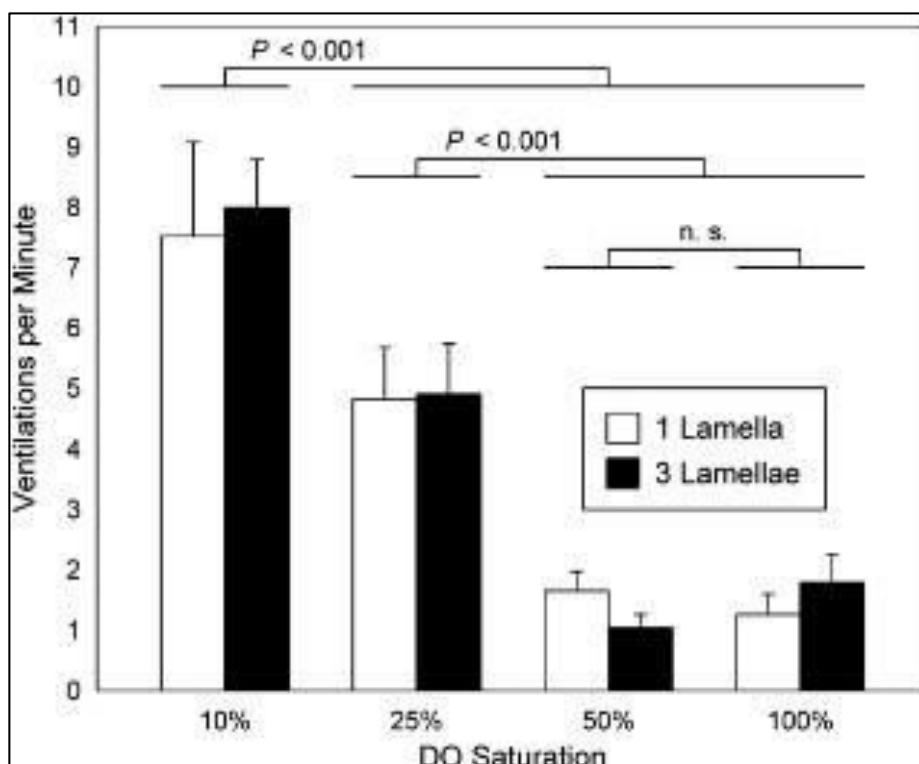


Fig 3: Ventilations of damselfly naiads to different oxygen levels Ground burrows and flooding

Ground Burrows and Flooding

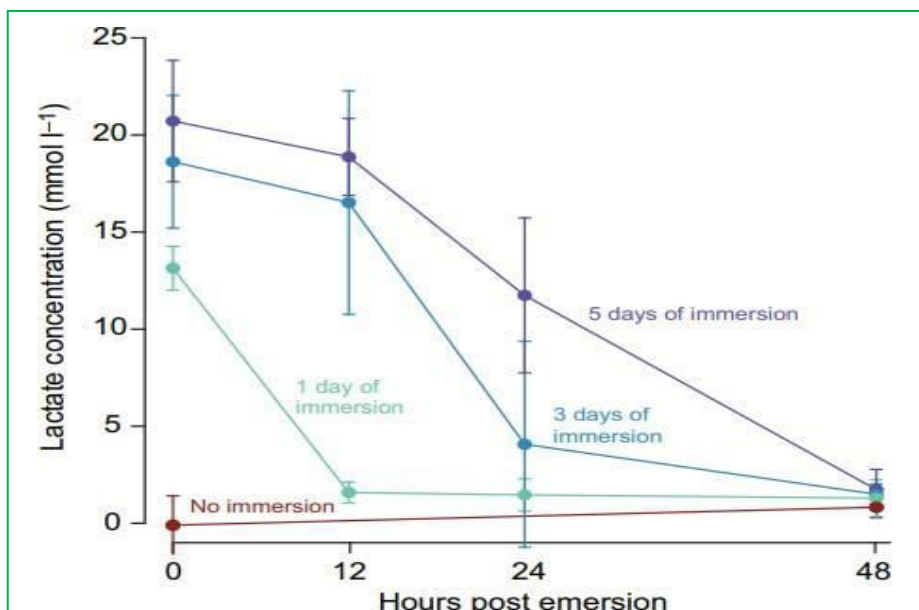


Fig 4: Lactate concentrations in *Manduca sexta* pupa after immersion in water

Soil-dwelling insects also face severe hypoxia during flooding. While upper soil layers may have atmospheric oxygen levels, deeper layers often experience intense hypoxia (Hoback and Stanley, 2001; Cavallaro and Hoback, 2014) [21, 4]. Flooding conditions can vary significantly; some insects have adapted by modifying their metabolic pathways. For example, a study by Woods and Lane (2016) [43] on *Manduca sexta* pupae showed that under anoxic conditions, lactate concentrations increased initially but decreased over time, indicating a metabolic shift from aerobic to anaerobic processes (Woods and Lane, 2016) [43] (Fig. 4).

High Altitudes

Insects have adapted to high altitudes, reaching elevations of up to 6,800 meters, where the atmospheric pressure and oxygen levels are significantly lower, creating chronic hypoxia (Dillon *et al.*, 2006; Dillon and Frazier, 2006;) [9, 10].

These insects often exhibit reduced body size and wing morphology, adaptations that lower their oxygen requirements. Some species can survive anoxia, such as adult ground beetles, which showed nearly 100% survival for 127 days in anoxic conditions at 0 °C (Zhang *et al.*, 2013; Verberk and Atkinson, 2013) [46, 38, 39].

Research by (Ding *et al.*, 2018) [11] on the migratory locust, *Locusta migratoria*, revealed metabolic adaptations for high-altitude survival. Locusts from the Tibetan plateau maintained stable levels of trehalose and glucose under hypoxic conditions, unlike lowland locusts, which exhibited elevated PTP1b enzyme levels that inhibited insulin signalling, resulting in decreased glucose and trehalose levels. This metabolic regulation helps prevent tissue damage during hypoxia (Jacobsen, 2008) [24].

Mechanisms of Hypoxia

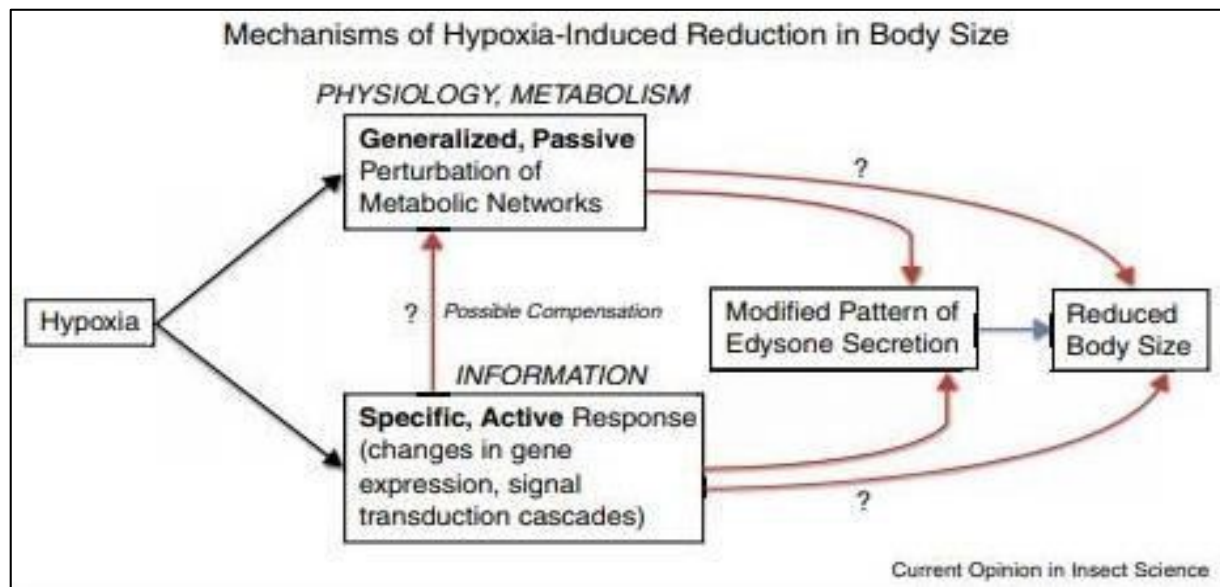


Fig 5: Mechanism of hypoxia induced reduction in body size

Hypoxia affects organisms through two principal mechanisms (Fig. 5). Firstly, it can impair biophysical processes and biomolecular interactions, similar to the effect of temperature on enzymatic reactions (Zitova, 2010) [49]. Low oxygen levels can limit ATP production, slowing down all cellular processes, including growth and development (Kacser, 1981; Callier, 2014) [25, 3]. This biochemical perspective highlights the importance of oxygen-dependent enzymes and metabolic pathways, with passive metabolic perturbations negatively impacting growth and size.

Alternatively, hypoxia can trigger specific signalling pathways that elicit adaptive responses. Notable among these are the Nitric Oxide Synthase (NOS) and Hypoxia-Inducible Factor (HIF) pathways, which regulate growth and development by promoting tracheal proliferation. This informational view emphasizes how external stimuli are transduced into cellular responses via kinase signalling cascades and gene regulatory networks, allowing for coordinated adaptations to low oxygen

Induction of NO and HIF Signalling Pathways

Under hypoxic conditions, both the NO/cyclic GMP and HIF pathways become activated (Fig.7) (Zagorska, 2004; Dekanty *et al.*, 2005) [45, 8]. Local hypoxia leads to increase

NO production in tissues near spiracles and imaginal disks, which then diffuses and activates guanylyl cyclase (Callier, 2014; Frei, 2004) [3, 13]. This cascade includes the activation of protein kinase G (PKG), influencing behaviours such as escape responses and tracheal growth. Overexpression of NOS enhances hypoxic responses, while knocking out PKG or inhibiting NOS reduces these effects (Harrison *et al.*, 2006; Davies, 2000) [14, 6].

Effects of Hypoxia on Insects in several ways:

Decrease in Adult Survivability: Low oxygen levels can lead to central nervous system damage and increased mortality (Dekanty *et al.*, 2005) [8]. An experiment with *Tribolium castaneum* revealed nearly 90% mortality at 2% oxygen after 10 days.

Decrease in Fecundity: Exposure to low oxygen negatively impacts reproductive capacity. In *Callosobruchus maculatus*, total egg production was significantly reduced after 72 hours at 2% oxygen.

Increase in Developmental Time: Larvae exposed to low oxygen experienced prolonged developmental periods (Callier, 2014) [3]. In *Tribolium castaneum*, developmental

time increased from 38 days under normoxia to 50-55 days at reduced oxygen levels.

Decrease in Adult Mass: Lower oxygen levels resulted in reduced adult mass. An experiment on *Blattella germanica* showed that females weighed an average of 55 mg at 12 % oxygen compared to 84 mg at normoxia.

Altered Flight Activity: Flight metabolism in insects like *Libellula saturata* is affected by hypoxia, with significantly fewer flight bouts recorded at lower oxygen concentrations (Zitova, 2010) [49].

Tracheal Compression and Insect Adaptations to Hypoxia

Tracheal systems in insects are significantly affected by low oxygen levels. An experiment on *Manduca sexta* revealed that caterpillars exposed to varying oxygen levels (21, 2, 1 and 0 kPa) exhibited different tracheal compression frequencies. Using X-ray imaging, it was observed that those in 1 kPa had a higher compression frequency compared to those at 21 kPa, indicating their tracheae compress to enhance oxygen uptake.

Insects have evolved various adaptations to match with hypoxia, including switching from aerobic to anaerobic metabolic pathways. During glycolysis, glucose is broken down into intermediates, ultimately producing pyruvate (Sesterhenn *et al.*, 2013) [35]. Depending on oxygen availability, pyruvate may enter the TCA cycle under aerobic conditions or shift to lactate production in hypoxic environments. This metabolic flexibility, along with reduced basal metabolic rates and enlarged tracheal systems, enables insects to survive in diverse hypoxic habitats (Verberk and Bilton, 2013) [38, 39].

Behavioural Reactions to Hypoxia in Insects

Insects exhibit distinct behavioural changes in response to hypoxia, aiming to increase local oxygen availability (Ding *et al.*, 2018) [11]. Upon exposure to low oxygen levels, immediate reactions include increased spiracular openings, enhanced ventilatory movements, and heightened activity levels (Klok *et al.*, 2009) [27]. For instance, the American locust *Schistocerca americana* demonstrates this by increasing its ventilatory frequency and tracheal conductance, while also entering a state of quiescence during prolonged hypoxic conditions (Harrison *et al.*, 2009; Zhang *et al.*, 2013) [27, 46].

Behavioural responses vary significantly between terrestrial and aquatic insects (Lack *et al.*, 2016) [28]. Aquatic insects, such as damselfly nymphs, react to hypoxia by intensifying their ventilatory movements, spreading their gills, and performing vigorous body shakes to ascend towards the surface. This behaviour helps expose their respiratory structures to air, thereby increasing oxygen uptake. In contrast, terrestrial insects tend to reduce their aerobic metabolic rate and rely more on anaerobic metabolism as hypoxic conditions persist. Notably, aquatic insects are generally more sensitive to hypoxia and less tolerant of severe hypoxic or anoxic environments than their terrestrial counterparts.

Harrison *et al.* (2015) [19] studied the behavioural reactions of the Madagascar hissing cockroach *Gromphadorhina portentosa* to varying oxygen levels in a controlled environment. They employed a Peltier temperature cabinet

to regulate oxygen partial pressures and recorded the insects' responses. After a 30-minute acclimatization period at normoxic conditions (21 kPa), the oxygen level was gradually reduced to lower set points (21, 15, 10 and 5 kPa). Following equilibration, the abdominal pumping frequency was measured. The results indicated that, regardless of temperature, a significant increase in abdominal pump frequency occurred when oxygen levels dropped to 5 kPa (Fig. 6). This finding suggests that insects ramp up ventilatory efforts to enhance oxygen supply to their tissues under hypoxic conditions.

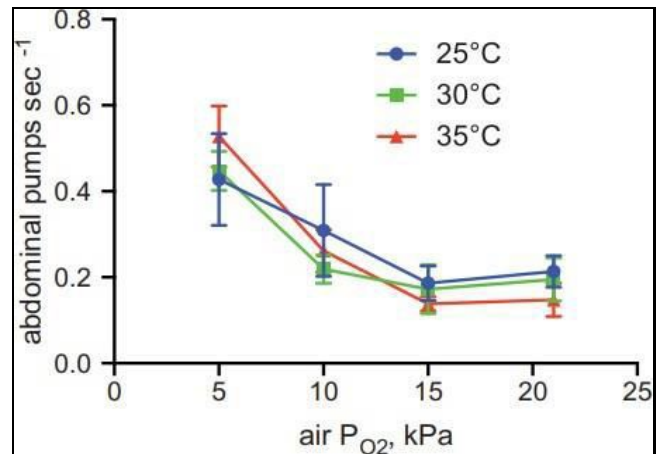


Fig 6: Frequency of abdominal pumping at different oxygen levels

Morphological and Metabolic Changes in Insects Under Hypoxia

Insects exhibit significant morphological and metabolic adaptations to cope with low oxygen levels (Feala *et al.*, 2007) [12]. One key morphological change is the increase in tracheal volume, which facilitates greater oxygen uptake. (Vandenbrooks *et al.*, 2012) [37] studied adult cockroaches reared from the egg stage under varying oxygen levels (12, 16, 21, 24, 27, 31 and 40 kPa). Using X-ray imaging, they measured tracheal volumes, finding that at 21 kPa, the volume was 0.008 mm³/g. In contrast, cockroaches reared at 16 and 12 kPa showed tracheal volumes of 0.016 and 0.018 mm³/g, respectively (Fig. 7). According to Fick's law of diffusion, increased cross-sectional volume enhances gas diffusion rates, indicating that reduced oxygen levels prompt insects to enlarge their tracheal systems to boost oxygen supply to tissues.

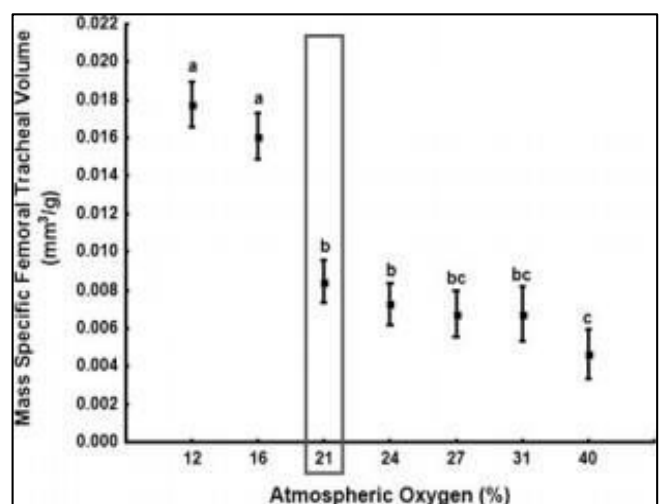


Fig 7: Increased tracheal volume due to hypoxia

Metabolic adjustments are another vital response to hypoxia. Feala *et al.* (2007) [12] investigated the metabolic rates of *Drosophila melanogaster* by comparing “naïve” flies, never exposed to low oxygen, with “adapted” flies raised in hypoxic conditions (Zhou and Haddad, 2013) [47]. Naïve flies were initially kept at 20% oxygen, while adapted flies were maintained at 4%. Upon exposure to 4% and 0.5% oxygen levels for four hours, naïve flies showed no significant change in ATP levels, producing 20 and 22 nmol/min/mg protein, respectively (Zhou *et al.*, 2011; Li *et al.*, 2011) [48, 30]. Conversely, adapted flies exposed to 0.5% oxygen produced 25 nmol/min/mg protein, indicating an increased ATP output in response to hypoxia (Fig. 8).

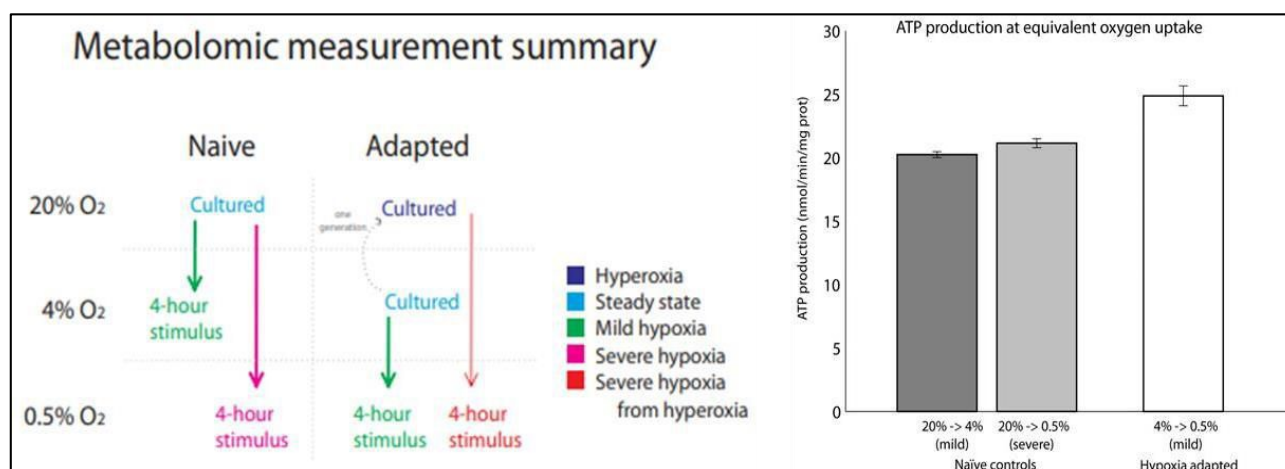


Fig 8: Changes in metabolic rate in *Drosophila melanogaster* due to hypoxia treatment

Conclusion

Insects demonstrate remarkable adaptability to hypoxic conditions through a range of morphological, metabolic, and behavioural responses. These adaptations, including alterations in tracheal structure, metabolic shifts from aerobic to anaerobic pathways, and enhanced ventilatory efforts, enable insects to survive in diverse environments with fluctuating oxygen availability. The activation of signalling pathways like Nitric Oxide Synthase (NOS) and Hypoxia-Inducible Factor (HIF) plays a critical role in facilitating these adaptations, promoting both immediate and long-term physiological changes. However, hypoxia can negatively impact insect fitness, leading to decreased survivability, reduced fecundity, and extended developmental times. Understanding these mechanisms not only sheds light on the ecological resilience of insects in hypoxic habitats but also emphasizes the potential effects of climate change and habitat alteration on their populations. Continued research is essential to explore the full extent of these adaptations and their implications for insect ecology and evolution in a changing world.

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