Developmental plasticity of the cardiovascular system in oviparous vertebrates: effects of chronic hypoxia and interactive stressors in the context of climate change

Running title: Hypoxia and the oviparous embryonic heart

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Summary statement – We discuss the phenotypic consequences of developmental hypoxia on the cardiovascular system of oviparous vertebrates. We focus on species-specific responses, critical windows, high-altitude adaptations and interactive effects of other stressors.

Glossary

Adrenergic - a substance, receptor or transporter that involves adrenaline (epinephrine) or noradrenaline (norepinephrine)

Baroreflex – a mechanism that regulates blood pressure by altering autonomic nervous output

Bradycardia – a reduction in heart rate

β-adrenergic sensitivity – sensitivity of β-adrenergic pathways to stimulation by agonists

Cardiac hypertrophy – an increase in the mass or size of the heart

Cardiac output - the product of heart rate (HR) and stroke volume (SV), measured in liters per minute

Cholinergic – a substance, receptor or synapse that involves acetylcholine, or butyrylcholine

Chronic developmental hypoxia (CDH) - defined here as periods of hypoxia during development that last for days, weeks or months

Convective cardiovascular function – the movement of solutes and O_2 through the flow of blood

Critical O_2 tension - the O_2 concentration where animals transition from oxy-regulation (i.e. maintaining a stable rate of oxygen consumption as environmental oxygen concentration declines) to oxy-conformation (i.e. when oxygen consumption declines linearly with environmental oxygen concentration)

Critical Window: Periods of heightened plasticity during development where environmental stress can affect morphology and physiology

Diastolic or diastole – referring to the stage of the cardiac cycle when the heart is relaxed

Eutrophication – a process where excessive plant and algal growth occurs, mainly due to increased availability of nutrients

Hypercapnia – excess carbon dioxide

Hypobaric - having less than normal atmospheric pressure

Isobaric hypoxia – reduced O_2 with normal atmospheric pressure

Oxidative phosphorylation – a process in the mitochondria which generates ATP by the reduction of O_2

Sea-level equivalent oxygen concentration – the amount of oxygen available at high altitude that is equivalent to the oxygen concentration at sea level

Secretory granules – organelles that contain specific proteins and other macromolecules that are destined for secretion into the extracellular space

Systolic or systole - referring to the stage of the cardiac cycle when the heart is contracted

Tachycardia – an increase in heart rate

Abstract

Animals at early life stages are generally more sensitive to environmental stress than adults. This is especially true of oviparous vertebrates that develop in variable environments with little or no parental care. These organisms regularly experience environmental fluctuations as part of their natural development, but climate change is increasing the frequency and intensity of these events. The developmental plasticity of oviparous vertebrates will therefore play a critical role in determining their future fitness and survival. In this Review, we discuss and compare the phenotypic consequences of chronic developmental hypoxia on the cardiovascular system of oviparous vertebrates. In particular, we focus on species-specific responses, critical windows, thresholds for responses and the interactive effects of other stressors, such as temperature and hypercapnia. Although important progress has been made, our Review identifies knowledge gaps that need to be addressed if we are to fully understand the impact of climate change on the developmental plasticity of the oviparous vertebrate cardiovascular system.

Introduction

Oviparous (egg-laying) vertebrates typically develop in fluctuating environments with little or no parental care. This reproductive strategy has some advantages over viviparity (Shine, 2015), but it exposes the embryo to environmental stress at a critical stage of life when defence mechanisms may not be fully developed. The consequences can be severe, because environmental fluctuations during development can permanently alter organismal structure, function and behaviour, and these traits can even be inherited by subsequent generations (Sultan, 2017). Therefore, the developmental plasticity of oviparous vertebrates plays a critical role in determining their future fitness and survival. This is especially true in an era of climate change, where rising concentrations of CO_2 in the atmosphere are driving global warming and increasing the frequency and intensity of environmental hypoxia and hypercapnia (Pörtner et al., 2014). Such rapid changes in the severity, frequency and spatial scale of these stressors will significantly challenge the developmental plasticity of oviparous species. Thus, it is important to gain an understanding of both the short- and long-term consequences of environmental stress on the embryonic physiology of these vulnerable animals.

Oviparous vertebrates commonly experience hypoxia during embryonic development (Box 1). Importantly, studies across a wide range of species have shown that chronic developmental hypoxia (CDH; see Glossary) has persistent effects on the cardiovascular system of oviparous vertebrates (Fig. 1). It appears that some cardiovascular responses to CDH are well-conserved among mammals, birds, reptiles and fish (Galli et al., 2023); Tables S1, S2 and S3). However, there are many interspecific differences, and the outcome of CDH appears to be dependent on multiple factors, including the magnitude and duration of hypoxia, as well as developmental stage. Furthermore, the hypoxic response can be altered by the interactive effects of other environmental stressors, such as temperature and hypercapnia (Box 2). These interactions are becoming increasingly important in the context of climate change.

The overall aim of this Review is to discuss and compare the phenotypic consequences of CDH on the cardiovascular system of oviparous birds, fish and reptiles. We define CDH here as periods of hypoxia that last for days, weeks or months. When we refer to oxygen levels, we present values as % O_2 saturation (21% O_2 = 100% air saturation). We start the Review with

an overview of the effects of CDH on the embryonic cardiovascular system of each vertebrate class, as well as the persistent effects on juvenile and adult life stages. Unless specified, the data we present on juveniles and adults are taken from studies that exposed embryos to CDH for a defined period during development, and then returned them to normoxia and investigated the cardiovascular phenotype in later life. These kinds of studies reveal traits that arise from persistent developmental plasticity, rather than plasticity due to acclimation (Earhart et al., 2022). Where possible, we attempt to identify species-specific responses, the threshold for response and critical windows. Although data is extremely limited, we also review the interactive effects of CDH, hypercapnia and temperature on cardiovascular outcomes. Surprisingly, we were unable to find sufficient literature to warrant a review of the effects of CDH on the cardiovascular system of amphibians (only one relevant paper: (Fritsche and Burggren, 1996).

Effects of developmental hypoxia on the avian cardiovascular system

Much of what we know about the effects of CDH on the avian cardiovascular system comes from studies on domestic chickens (Table S1). These investigations were largely devised to improve farming practices or to study the clinical implications of CDH without the confounding influence of maternal responses (Itani et al., 2018). However, the levels of hypoxia used in these studies (13–17% O_2 saturation) are within the natural range of some avian nests (Box 1), which makes them ecologically relevant.

Effects of CDH on embryonic somatic growth and heart mass

The most common consequence of CDH is embryonic growth restriction. In chickens, isobaric or hypobaric hypoxia (see Glossary) at 13–15% O_2 (≡2500–3500m) consistently leads to a reduction in embryonic body mass (Table S1A), and the critical window occurs at 30–60% of incubation (Dzialowski et al., 2002; Ruijtenbeek et al., 2000). In addition, embryos from various chicken strains (broilers, red junglefowl, white Leghorn) exposed to isobaric or hypobaric hypoxia have an increased brain-to-body weight ratio (Giussani et al., 2007; Salinas et al., 2010; Skeffington et al., 2020) and/or an increased heart-to-body weight ratio (Table S1C). Asymmetric growth restriction is usually a consequence of the 'brain-sparing' response (Giussani, 2016), which involves a systemic vasoconstriction that shunts blood to hypoxia-sensitive organs, such as the brain and heart. Although protective in the short term, it can

ultimately lead to systemic hypertension and cardiac remodelling (Giussani, 2016). Indeed, some studies have shown that isobaric or hypobaric hypoxia leads to an increase in chick embryonic heart mass, aortic wall thickness and ventricular wall thickness (Table S1B; (Salinas et al., 2010; Villamor et al., 2004). However, other studies have found a decrease in heart mass, or no effect (Table S1B), and there appears to be no clear correlation between the outcome and the length or duration of the hypoxic exposure.

Effects of CDH on embryonic O_2-carrying capacity and cardiac function

CDH triggers a range of responses to improve O_2-carrying capacity and delivery in vertebrates (Galli et al., 2023). Embryonic capillary density and chorioallantoic membrane (CAM) vascularity is increased in the CDH-exposed Canada goose and domestic chicken, respectively ((Snyder et al., 1984); Table S1E). Hematocrit is also increased in chicken embryos exposed to CDH (13–15%, Table S1E), and the critical window lies between day 6 and 12 of development (Dzialowski et al., 2002). However, at least in the case of sea-level chickens, the increase in embryonic O_2-carrying capacity is not enough to offset the negative effects of hypoxia, and cardiac function is compromised. In one study, hypoxia reduced chicken embryonic ventricular peak systolic pressure, dorsal aortic peak systolic pressure (see Glossary), stroke volume and cardiac output (see Glossary), while diastolic function (see Glossary) was preserved (Sharma et al., 2006). In other studies, hypoxic chick embryos had signs of cardiomyopathy, including left ventricular dilatation, reduced ventricular wall thickness, increased apoptosis (Tintu et al., 2009), a reduced left ventricular ejection fraction, aortic thickening, reduced contractility, reduced cardiac output and diastolic dysfunction (Itani et al., 2016; Itani et al., 2020; Jonker et al., 2015; Rouwet et al., 2002). These problems were associated with a significant increase in cardiac oxidative stress and a reduction in cardiac antioxidant capacity (Itani et al., 2016; Itani et al., 2020). Heart rate is generally reduced by acute hypoxia in chickens (Akiyama et al., 1999; Altimiras and Phu, 2000; Crossley et al., 2003; Mortola et al., 2010; Sharma et al., 2006; Tazawa, 1981), but it eventually returns to control values with longer hypoxic periods; Table S1D). This is despite a significant increase in adrenal concentrations of adrenaline and noradrenaline, which is associated with a greater sensitivity of cardiac β-receptors and enhanced sympathetic innervation in the peripheral vasculature (Table S1H).

Effects of CDH on embryonic avian cardiomyocytes

The effects of CDH on chicken embryonic cardiac structure and function are associated with multiple cellular abnormalities. Ventricular protein content and protein/DNA ratios are reduced in hypoxic embryonic chickens, which is associated with a reduction in heart mass (Asson-Batres et al., 1989). In another study, CDH initially caused cardiac myocyte hyperplasia in chicken embryos, but this eventually led to hypertrophy (see Glossary) with more myofibrils, larger Golgi complexes, less glycogen and fewer, larger secretory granules (see Glossary; (Maksimov and Korostyshevskaia, 2012). This response was also accompanied by an increase in cardiac collagen (Table S1G), and a decrease in myosin heavy chain and titin proteins (Tintu et al., 2009). There is also reduced expression of genes involved in cardiac calcium handling, as well as a shift from compliant to stiff isoforms of titin and increased vascular endothelial growth factor (VEGF) expression (Jonker et al., 2015; Tintu et al., 2009). CDH also increases mitochondrial-derived oxidative stress in the hearts of chicken embryos and reduces mitochondrial efficiency and capacity (Table S1F).

Long-term effects of avian CDH on the cardiovascular system

Numerous studies have shown that developmental hypoxia has long-term effects on the avian cardiovascular system. Firstly, the growth restriction and increased heart-to-body weight ratio associated with CDH often continues into adulthood in chickens (Table S1A; (Lindgren and Altimiras, 2013). Changes in absolute heart mass are often absent after hatching or later in life, indicative of a degree of cardiac plasticity (Table S1B). However, the cardiomyopathy in hypoxic embryonic chickens observed by Tintu et al. (2009) persists into adulthood, with severe left ventricular dilatation, decreased left ventricular ejection fraction, fibrosis and diastolic dysfunction. Lindgren and Altimiras (2013) showed that adult chickens exposed to CDH have signs of systolic, but not diastolic, dysfunction and increased expression of B1 adrenoreceptors without any change in collagen content. Lastly, Skeffington et al. (2020) found a range of cardiovascular abnormalities in adult chickens exposed to CDH, including hypertension, increased cardiac work, enhanced baroreflex gain (see Glossary), left ventricular wall thickening and increased contractility. Overall, adult chickens exposed to CDH share many of the pathological cardiovascular signatures observed in adult mammals from hypoxic pregnancies (Table S3; (Itani et al., 2018), and the phenotype is reminiscent of both compensated and decompensated heart failure. This makes chickens an excellent model

for studying the programming of cardiovascular disease by CDH in the absence of confounding maternal factors.

Effects of developmental hypoxia on the cardiovascular system of reptiles

Many embryonic reptiles experience CDH as part of their natural development, but climate change is increasing the frequency and intensity of these events (Box 1 and 2). It is particularly interesting to study cardiovascular programming in reptiles, because cardiac design differs substantially between the reptilian classes (Burggren et al., 2020). Most turtles (testudines), snakes and lizards (squamates) have a single undivided ventricle with no pressure separation between the pulmonary and systemic circulations. However, monitor lizards and pythons have a functionally divided ventricle, and crocodilians (alligators, crocodiles, caimans and gharial) have a fully divided ventricle, allowing for high systemic arterial pressures and an elevated metabolic rate. These differences place variable metabolic demands on the reptilian cardiovascular system, which could be expected to lead to species-specific responses to CDH.

Effects of CDH on reptilian embryonic somatic growth and heart mass

As in chickens, there is no effect of CDH on reptilian embryonic body mass if the O_2 concentration is at or above 17% (Table S1A). However, isobaric and hypobaric hypoxia at O_2 concentrations of 10–15% consistently reduces embryonic body mass and/or body length in American alligators, snapping turtles, Florida red-bellied turtles, common wall lizards, viperine snakes and leopard geckos; but total incubation time is unchanged (Table S1A). The critical window for growth restriction is between 70 and 90% incubation in American alligators (Tate et al., 2016), whereas embryonic mass in common snapping turtles is dependent on the total hypoxic exposure time (Tate et al., 2015). Hypoxia also causes an increase in the total amount of yolk present at the end of development in American alligators, Florida red-bellied turtles, common wall lizards and viperine snakes; indicating a reduced conversion of yolk to tissue (Crossley et al., 2017; Crossley and Altimiras, 2005; Kam, 1993; Owerkowicz et al., 2009).

CDH is also associated with an increase in heart-to-body weight ratio in American alligators, snapping turtles and Florida red-bellied turtles (Table S1C). The critical window for the response in American alligators is at 20–40% of development (Tate et al., 2016), and 50–70%

in common snapping turtles (Tate et al., 2015). The asymmetric growth restriction suggests that reptiles exhibit the brain-sparing response, which is supported by a recent study that found a modest increase in brain blood flow in embryonic turtles exposed to CDH (Sartori et al., 2019). An increase in absolute heart mass is also evident in hypoxic embryonic snapping turtles, lizard geckos and common wall lizards (Table S1B). However, in most studies, absolute heart mass does not change with hypoxia, suggesting that heart growth is preserved at the expense of somatic growth. Nevertheless, Crossley's laboratory thoroughly investigated the critical windows for this response and showed that cardiac enlargement occurs before somatic growth restriction (Tate et al., 2015; Tate et al., 2016). This finding suggests that cardiac enlargement in reptiles is a direct response to CDH, rather than a consequence of reduced somatic growth.

Effects of CDH on reptilian embryonic O_2 carrying capacity and heart function

In contrast to birds, CDH leads to long-term changes in reptilian heart rate, but the responses are species-specific. CDH causes bradycardia in American alligator embryos (70–90% development, 10% O_2) and common wall lizards chronically exposed to high-altitude hypoxia [15–17% O_2 sea-level equivalent (SLE, see Glossary); Table S1D, but it causes a significant tachycardia (see Glossary) in embryonic snapping turtles (10% O_2) and scincid lizards (Table S1D. The underlying reason for these species-specific differences is unknown, and it is also unclear why reptiles modulate heart rate during CDH, whereas mammals and birds do not (Table S1D Table S2C).

As in birds, chronic levels of hypoxia in embryonic reptiles trigger adaptive cardiovascular responses that improve O_2-carrying capacity and delivery. American alligators and Florida red-bellied turtles increase haematocrit during chronic hypoxia exposure (Kam, 1993; Warburton et al., 1995), but haemoglobin isoform expression and affinity is unchanged (Bautista et al., 2021; Grigg et al., 1993). CDH also increases angiogenesis in the CAM in American alligators (Corona and Warburton, 2000), which lowers the resistance of the chorioallantoic circulation by adding parallel vascular beds. This response ultimately reduces systemic blood pressure (Crossley and Altimiras, 2005; Eme et al., 2011b; Eme et al., 2013). The critical window for hypotension is at 20–70% of development in snapping turtles and 50–70% in American alligators (Tate et al., 2015; Tate et al., 2016). However, despite arterial

hypotension, blood flow to the American alligator CAM increases during hypoxia, which presumably serves to enhance gas exchange (Eme et al., 2011a; Sartori et al., 2019). Given that total blood flow remains constant, the increase in CAM blood flow may be driven by increased intraembryonic vascular resistance, which could also explain the observed cardiac enlargement in snapping turtles and lizard geckos (Eme et al., 2021; Parker and Dimkovikj, 2019).

Effects of CDH on the embryonic reptilian acute hypoxia tolerance

In addition to baseline changes in cardiovascular function, CDH alters the embryonic cardiovascular response to acute hypoxia in reptiles. American alligator and snapping turtle embryos exposed to CDH have an attenuated response to an acute hypoxic challenge, with blunted heart rate and blood pressure responses (Crossley and Altimiras, 2005; Eme et al., 2011b). In agreement with these findings, critical O_2 tension (P_{Crit}; see Glossary) is lower in snapping turtles and American alligator embryos exposed to CDH, compared to their normoxic counterparts (Crossley et al., 2017; Kam, 1993). However, the enhanced hypoxia tolerance does not appear to be associated with mitochondrial remodelling (Galli et al., 2016). Collectively, these results suggest that embryos exposed to CDH are less responsive to acute hypoxic stress and may tolerate lower levels of hypoxia.

Long-term effects of CDH on the reptilian cardiovascular system

Most of our understanding of the long-term effects of CDH have come from studies on American alligators and common snapping turtles. It is interesting to compare and contrast these two reptiles because crocodilians are archosaurs and more closely related to birds than testudines and squamates (Brusatte et al., 2010). Given that crocodilians also have a fully divided heart and higher metabolic rates, one may expect American alligators to respond to CDH more similarly to birds than to snapping turtles.

Juvenile American alligators and snapping turtles exposed to CDH most commonly experience catch-up growth, but some studies have reported persistent growth restriction (Table S1A), as well as an increased heart-to-body weight ratio (Crossley et al., 2022; Galli et al., 2016; Galli et al., 2021; Joyce et al., 2018; Ruhr et al., 2021; Smith et al., 2023; Smith et al., 2019). Despite cardiac enlargement, most resting cardiovascular parameters in juvenile American alligators

and snapping turtles are similar between individuals from normoxic or hypoxic incubations. In particular, the systemic and pulmonary hypertension, as well as systolic and diastolic ventricular dysfunction that is often present in mammals and birds exposed to CDH appears to be absent in American alligators and turtles (Table S3). However, there are some reptilian cardiovascular parameters that are permanently affected by CDH. Left ventricular stroke volume is increased and pulmonary blood flow is decreased in juvenile American alligators exposed to CDH (Joyce et al., 2018; Smith et al., 2019). Likewise, heart rate is reduced, and total cardiac output is increased in juvenile common snapping turtles exposed to CDH (Wearing et al., 2017; Wearing et al., 2016).

More differences in the long-term cardiovascular phenotype are revealed when reptiles are placed under physiological stress. Compared to normoxic controls, juvenile American alligators from hypoxic incubations that are swimming or stimulated with β-adrenergic agonists (see Glossary) have a faster rate of ventricular relaxation, greater left ventricle stroke volume, increased carotid blood flow and lower pulmonary blood flow (Joyce et al., 2018; Smith et al., 2019). Furthermore, the blunted cardiovascular response to acute hypoxia that is observed at the embryonic level is also present in juvenile alligators, suggesting a long-term improvement in hypoxia tolerance (Crossley et al., 2022; Crossley et al., 2023; Smith et al., 2019). This is also the case for juvenile turtles exposed to CDH, as they are able to maintain cardiac output two-fold higher than controls during two hours of anoxia (Ruhr et al., 2021). The improved anoxia tolerance is also apparent at the cellular level, and is associated with increased myofilament calcium sensitivity, a superior ability to suppress cardiac myocyte reactive oxygen species (ROS) production during anoxia and lower basal cardiac ROS production (Galli et al., 2021; Ruhr et al., 2019). These adaptations could be useful for turtles in juvenile and adult life stages, as they often experience long bouts of anoxia and reoxygenation following breath-hold dives and overwintering under ice-covered lakes (Jackson, 2002). Exposure to CDH also affects the response to digestion in snapping turtles. Compared to controls, peak postprandial metabolic rates are higher in juvenile turtles exposed to CDH (suggesting an increased metabolic cost of digestion) and this is supported by higher systemic blood flows (Wearing et al., 2017).

The cellular and molecular mechanisms driving cardiovascular programming in reptiles may involve mitochondrial remodelling, as CDH appears to improve mitochondrial efficiency in American alligators and snapping turtles, and this is driven by a lower proton leak (Galli et al., 2016; Galli et al., 2021). Furthermore, CDH induces substantial changes in the cardiac proteome of American alligators prior to hatching, and these changes are largely maintained into juvenile life, with animals from hypoxic incubations showing a shift in protein synthesis (transcription and translation), cellular organization, metabolic adjustments and protein degradation (Alderman et al., 2019). Proteins involved in metabolism are particularly enriched in juvenile alligator hearts from hypoxic incubations, including those with roles in fatty acid oxidation, the citric acid cycle and oxidative phosphorylation. Also worth noting is an increased protein expression of the antioxidant superoxide dismutase, which – in addition to the improved ability to recycle proteins – may help to manage ROS production (Alderman et al., 2019). Finally, we have recently shown that cardiac programming by CDH in snapping turtles is supported by differential expression and DNA methylation of genes associated with sarcomere function, ion-channels, cardiomyocyte survival and heart rate (Ruhr et al., 2021).

In summary, it is clear that CDH programmes the cardiovascular physiology of American alligators and snapping turtles, but in contrast to birds and mammals, the phenotype is not overtly dysfunctional; in fact, in many cases, it appears to be beneficial. The fact that these two species lack many of the pathological signatures associated with CDH (Table S3) suggests the long-term outcome of CDH may be more dependent on body temperature and metabolic rate, rather than phylogeny. It is possible that the higher metabolic costs associated with endothermy place an additional metabolic burden on juvenile and adult birds and mammals exposed to CDH, leading to pathological outcomes.

Effects of hypoxia on the cardiovascular system of fishes

Among the vertebrate classes, fish are prone to experiencing the most severe levels of hypoxia during development, particularly in climate change scenarios (Box 1). Previous work has shown that CDH alters a wide range of phenotypic traits in teleosts, including metabolic rate (Del Rio et al., 2021), swimming performance (Johnston et al., 2013), sex ratios (Robertson et al., 2014), the balance of sex hormones (Shang and Wu, 2004) and brain development (Mikloska et al., 2022). Nevertheless, surprisingly little is known about the

effects of CDH on the teleost cardiovascular system. Comparisons to the other oviparous classes is also difficult because the levels of hypoxia used in fish studies are considerably more severe than those used in studies of reptiles and birds.

Effects of CDH on growth and cardiac mass in fishes

Similar to the other vertebrate classes, fish embryos or larvae exposed to CDH have reduced body mass (Table S1A), which renders the individuals less competitive and more vulnerable to predation (Mason, 1969). The growth restriction is driven by the activation of hypoxia inducible factor (HIF), which ultimately supresses the insulin-like growth factor (IGF) pathway (Kajimura et al., 2004; Sun et al., 2011). Fish embryos exposed to CDH also have slower developmental rates, delayed hatching and delayed heart morphogenesis (Bagatto, 2005; Ciuhandu et al., 2005; Del Rio et al., 2021; Kajimura et al., 2005; Miller et al., 2011; Miller et al., 2008). These effects are particularly prevalent when fish are exposed to hypoxia in the later embryonic stages, presumably due to the increasing O_2 demands of the developing organism and the O_2-diffusion limitations across the egg membrane (Rombough, 1988).

Although acute hypoxia exposure slows growth and delays development during embryogenesis, upon reoxygenation, hypoxia-exposed embryos often (but not always) return to the same size as control animals (Table S1A). Zebrafish embryos exposed to ~1–2% O_2 from 24 to 36 hours post-fertilisation (hpf) are shorter than control animals, but the embryos catch up if they are returned to normoxia (Kamei et al., 2018). The catch-up growth in zebrafish embryos is mediated in part by the IGF pathway (Kamei et al., 2011). Specifically, IGF pathway activity, stimulated by insulin receptor substrate 1 (IRS1)-mediated IGF signalling, helps maintain neural crest cell populations during hypoxia (Kamei et al., 2018). Reductions in neural crest cell numbers – either through ablation or by a combination of hypoxia and reduced IRS1-stimulated IGF signalling – prevents catch-up growth upon reoxygenation in zebrafish (Kamei et al., 2018).

To our knowledge, the effects of CDH on cardiac mass in embryonic/larval fishes have not been directly studied, but there have been measurements of ventricular volume. In zebrafish, hypoxia (3% O_2) leads to a reduction in ventricular end diastolic and systolic volume at 96 hpf, but an increase at 5 days (Table S1B). This suggests that hypoxia initially causes a reduction

in heart size in embryonic zebrafish, but cardiac enlargement occurs once they reach the larval stages. Interestingly, *in vivo* imaging of zebrafish larvae has shown that brain blood flow is unchanged by hypoxia (Schwerte et al., 2003), despite an overall redistribution of blood to the red layer of muscle to enhance O_2 uptake at seven days post fertilisation (dpf). This suggests that although blood flow distribution is changed, the brain-sparing effect is absent (El-Fiky and Wieser, 1988). Although these studies have only been performed on one species, it is possible that the brain-sparing effect is unnecessary in fish. Instead, blood is redistributed towards the muscle to enhance O_2 uptake to the body.

Effect of CDH on O_2 carrying capacity and cardiac function in fishes

Like other vertebrates (Galli et al., 2023), embryonic and larval fishes exposed to hypoxia trigger mechanisms to enhance O_2 extraction. Stage-matched comparisons reveal a greater expression of the higher-O_2 affinity embryonic haemoglobin in fish incubated in hypoxia (6% O_2) compared to those in normoxia (Bianchini and Wright, 2013). Similarly, erythropoiesis is stimulated from 7dpf in hypoxic zebrafish larvae (Schwerte et al., 2003), and intersegmental blood vessel vascularisation is increased from 6dpf (Yaqoob and Schwerte, 2010). O_2 extraction may also be enhanced through the activation of O_2-sensitive transcription factors, such as HIF. Lake whitefish and zebrafish embryos and larvae show hypoxia-induced, stage-specific changes in the expression of HIF1a and its associated downstream targets, which are known to stimulate haematopoiesis (Wang and Semenza, 1996) and angiogenesis (Iyer et al., 1998), and have been shown to enhance hypoxia tolerance in early life in some studies (Mandic et al., 2020; Robertson et al., 2014; Whitehouse and Manzon, 2019), but not others (Levesque et al., 2019). Finally, behavioural adaptations may also lead to increased O_2 extraction. For example, hypoxia (3% O_2) has been shown to induce pectoral fin motions in zebrafish (from 2dpf) to aid O_2 uptake (Jonz and Nurse, 2005), and acute hypoxia exposure causes suppression of O_2 uptake while simultaneously increasing tail beat frequency – potentially in an attempt to reoxygenate the egg case – in little skate embryos (Di Santo et al., 2016).

In addition to increasing O_2 extraction, embryonic and larval fishes can also increase O_2 transport to the tissues through alterations in cardiovascular dynamics. During early embryogenesis under normal conditions, fishes rely on diffusion for the supply of O_2 to their

427 respiring tissues (Burggren, 2004; Grillitsch et al., 2005). This has been demonstrated in
428 developing zebrafish where, prior to ~14dpf, reducing the blood's O_2-carrying capacity elicits
429 no changes in either cardiac output or anaerobic metabolism, implying that under standard
430 conditions, there is no essential role for convective O_2 (Jacob et al., 2002). However, this is
431 not the case under hypoxic conditions. Zebrafish incubated in hypoxia (~10% O_2) display
432 greater heart rate and cardiac output than those in normoxia from 4dpf onwards, which is
433 likely to increase convective O_2 transport and act to complement the O_2 obtained through
434 diffusion to meet the organism's total O_2 demand (Grillitsch et al., 2005; Jacob et al., 2002).
435 Interestingly, this implies that the afferent nervous system can sense and respond to hypoxia
436 by increasing heart rate from 4 dpf, around 10 days before convective O_2 transport is required
437 under normoxic conditions. These studies suggest that CDH hastens the shift from diffusion
438 to convection-based O_2 provision in zebrafish embryos (Jacob et al., 2002), but further work
439 is required on this topic. Similar to embryonic reptiles, there is evidence that these
440 cardiovascular adjustments may improve hypoxia tolerance in the short-term, as P_{Crit} is lower
441 in hypoxic zebrafish (Robertson et al., 2014) and Atlantic salmon (Wood et al., 2019b)
442 compared to that of normoxic counterparts.

443 CDH also causes long-term changes in heart rate in embryonic zebrafish (Table S1D), but the
444 magnitude and direction are variable. In general, tachycardia is the dominant response for
445 embryonic zebrafish exposed to relatively mild or moderate levels of hypoxia (8–10% O_2) at
446 temperatures of 28–31^{o}C. However, severe hypoxia (2–4% O_2) causes bradycardia (Table
447 S1D), which is mediated by a release of vagal tone or increase in catecholamines (Steele et
448 al., 2011; Steele et al., 2009). Nevertheless, cardiac output remains constant in chronically
449 hypoxic larval or embryonic zebrafish due to an elevated stroke volume, and in some cases it
450 is even increased (Cypher et al., 2018; Jacob et al., 2002; Moore et al., 2006; Yaqoob and
451 Schwerte, 2010). Larval zebrafish subjected to hypoxia (4% O_2) also have significantly
452 increased gene expression of β1, β2a and β2b adrenergic receptors (Ars) at 4dpf relative to
453 normoxic fish (Steele et al., 2009), and CDH increases cardiac responsiveness to agonists of
454 adrenergic signalling and delays the onset of cholinergic control (see Glossary) in the rainbow
455 trout (Miller et al., 2011). However, sympathetic sensitisation in zebrafish is likely to be
456 dependent on the duration of hypoxia exposure and developmental stage, as the expression

of β1AR does not change in whole zebrafish embryos (2dpf) exposed to only 12h or 24h of hypoxia (5% O_2; (Ton et al., 2002; Ton et al., 2003).

The long-term effects of CDH on fish growth and the cardiovascular system

Despite the ecological importance, the long-term effects of CDH are poorly studied in fishes, and the results are highly variable. Trout larvae exposed to CDH exhibit catch-up growth with a significantly greater increase in weight (278% versus 188%) and length (64% versus 27%), eventually leading to significantly larger fry body weights and lengths compared to controls (Johnston et al., 2013). In contrast, juvenile Chinook salmon and European seabass exposed to CDH during embryogenesis are significantly smaller than controls (Del Rio et al., 2019), and growth restriction in hypoxic zebrafish embryos also persists into adulthood (Table S1A). However, no effect of CDH has been found on body weight in adult Atlantic salmon (Wood et al., 2017). Collectively, these studies show that the long-term effect of CDH on body mass is extremely variable in teleosts, and it depends on multiple factors, including species and body temperature.

To our knowledge, nothing is known about the long-term effects of CDH on juvenile and adult teleost cardiac structure or function. However, there is evidence of differential cardiac gene expression in rainbow trout exposed to CDH, including that of the common house-keeping genes 18s ribosomal RNA and acidic ribosomal phosphoprotein, and protein expression of cardiac troponin I (Johnston et al., 2013). Furthermore, previous work has shown that zebrafish cardiac morphology can be altered by other environmental stressors during development, including temperature and CO_2 (see below), as well as crude oil and polycyclic aromatic hydrocarbons (for a review see (Takeshita et al., 2021). Therefore, there is ample evidence that the fish heart is capable of developmental plasticity, but there is a distinct lack of studies on CDH.

Although few studies have explicitly investigated hypoxic programming in the fish heart, several studies address aspects of whole-organism performance and fitness that potentially link to cardiac performance. Hypoxic-incubated (10% O_2) rainbow trout show a consistently lower maximum relative swimming speed than normoxic controls across three developmental stages, which is thought to be caused by a delay in cardiac maturation (Johnston et al., 2013). Zebrafish and Atlantic salmon larvae exposed to CDH also show an

improved whole-animal hypoxia tolerance. However, this phenotype does not persist into the juvenile and adult life stages (Del Rio et al., 2021; Robertson et al., 2014; Vanderplancke et al., 2015; Wood et al., 2019a; Wood et al., 2017). In fact, European seabass larvae raised in hypoxia (8% O_2) show a reduced hypoxia tolerance as juveniles, which is associated with an increased prevalence of opercular abnormalities (Cadiz et al., 2017). Similarly, 15 month-old Atlantic salmon exposed to CDH (10% O_2) are marginally less hypoxia tolerant than normoxia-incubated animals, although their aerobic scope is similar (Wood et al., 2017), and there is no effect of CDH (10% O_2) on hypoxia tolerance in juvenile Chinook salmon (Del Rio et al., 2021). Nevertheless, the physiological response to hypoxia can be affected by CDH in some fish. For example, when seabass are exposed to hypoxia as juveniles, fish that experienced hypoxia during embryogenesis show different changes in haemoglobin sub-type expression, but no differences in overall haemoglobin concentration (Cadiz et al., 2017).

Climate change and the interactive effects of CDH with other stressors

Oviparous vertebrates rarely experience CDH in isolation because other developmental stressors often occur simultaneously (Box 2). Indeed, under natural conditions, CDH often occurs alongside fluctuations in CO_2, temperature, pH and salinity. Given that climate change is increasing the magnitude and frequency of these events, it is becoming increasingly important to study these interactive effects.

Interactive effects of CDH and temperature

Although maternal nest choice and behaviour may partly shield terrestrial embryos from thermal stress, recent models suggest that global warming will increase the incubation temperatures of avian and reptilian eggs (Du et al., 2023; DuRant et al., 2019). Extensive research has shown that thermal stress can dramatically alter the morphology and physiology of reptilian and avian embryos, including changes in growth, body mass, cardiac mass, heart rate, mitochondrial density and respiration (Ben-Ezra and Burness, 2017; Du et al., 2023; Du and Shine, 2015; Du et al., 2010; Singh et al., 2020). Although data is scarce, some studies have investigated the interactive effects of temperature and hypoxia in avian and reptilian embryos. For example, Lourens et al. (2007) undertook a study in chickens where incubation temperature was increased from 37.8°C to 38.9°C at either 17% or 21% O_2. Temperature and hypoxia had independent effects on hatch time, body weight, yolk-free body weight and

517 relative heart weight; however, there were no interactions between O_2 and temperature
518 (Lourens et al., 2007). Another study in chickens found that mild levels of hypoxia (17% O_2)
519 did not produce any effects on embryonic body mass or heart mass, even when temperature
520 was increased from 37.8 or 38.9^{o}C (Table S1A). By contrast, the negative effects of hypobaric
521 hypoxia (2877m, 15% O_2 SLE) during embryonic development on body mass, swimming speed
522 and heart rate in adult viperine snakes at 28^{o}C disappear when temperature is reduced to
523 24^{o}C (Souchet et al., 2020a; Souchet et al., 2020b). Interestingly, increasing the temperature
524 to 32^{o}C produces a completely different phenotype, with a reduced heart rate, smaller body
525 mass and faster swimming speed. The surprising improvement in swimming performance in
526 adult snakes at high altitude at the warmest temperature persisted after relocation to low
527 elevation (Souchet et al., 2020a). The authors suggest that constraints on development may
528 be offset by the preservation of performance traits (perhaps through cardiorespiratory
529 plasticity). Collectively, these studies suggest that the vertical colonisation potential of
530 reptiles and birds (see below) will be affected by the interaction between temperature and
531 O_2 availability.
532
533 Interestingly, a recent meta-analysis found that aquatic embryonic ectotherms are more
534 than three times as plastic as terrestrial ectotherms when exposed to thermal stress during
535 development (Pottier et al., 2022). Indeed, a large body of literature has shown that an
536 increase in developmental temperature affects embryonic and larval fish growth rate, sex
537 ratio, body size, metabolism, heart rate, cardiac morphology, hypoxia tolerance and
538 swimming performance (Dimitriadi et al., 2018; Eme et al., 2015; Melendez and Mueller,
539 2021; Mueller et al., 2011; Pelster, 1999; Vagner et al., 2019; Zambonino-Infante et al.,
540 2013). Some of these studies found effects that lasted into adulthood, including increased
541 ventricular roundness in juvenile and adult male zebrafish exposed to elevated
542 temperatures during embryogenesis (Dimitriadi et al., 2018; Dimitriadi et al., 2021).
543 However, the short and long-term effects of developmental temperature are highly variable
544 in fish, and interestingly, the same meta-analysis found that persistent effects on thermal
545 tolerance limits in adulthood were surprisingly weak (Pottier et al., 2022). Whether the
546 same is true when elevated temperature occurs in combination with hypoxia is largely
547 unknown, because surprisingly little is known about this interaction. One study on Chinook
548 salmon investigated developmental outcomes in fish that were reared from fertilization to

549 the fry stage at two temperatures (10°C and 14°C) and two O_2 levels (100% or 50% air
550 saturation). Although temperature and O_2 saturation had independent effects on growth
551 and acute hypoxia tolerance, there was no interaction between the two stressors (Del Rio et
552 al., 2019). This was also the case in European sea bass exposed to different temperature
553 and hypoxia combinations (40% or 100% air saturation x 15 °C and 20 °C) from the flexion
554 stage until the end of larval development (Cadiz et al., 2018). However, there were
555 significant interactions on hatching success and thermal tolerance in Chinook salmon, with
556 higher temperature generally potentiating the effects of hypoxia (Del Rio et al., 2019).
557 Lastly, CDH causes an increase in cardiac output and heart rate in zebrafish embryos at 25–
558 31^{o}C, but the magnitude of the response is lowest at 31^{o}C, presumably because the fish had
559 neared their maximal cardiovascular capacity (Jacob et al., 2002). Clearly, more studies are
560 warranted and necessary to understand the physiological implications of temperature and
561 hypoxia interactions during development.
562
563 *Climate-driven elevational range shifts and high-altitude acclimatization*
564 Global warming is driving some reptilian and avian species to shift their geographical
565 distributions towards higher-elevation habitats with lower O_2 availability (Neate-Clegg and
566 Tingley, 2023; Rubenstein et al., 2023). Developmental plasticity will therefore play a
567 pivotal role in successful colonization of high-altitude environments. One approach to
568 predicting the effects of climate-driven elevational range shifts is the so-called 'transplant'
569 experiment, whereby gravid females or embryos from one elevation are transported and
570 maintained at another. In this regard, recent work on the viperine snake has been
571 particularly insightful, because this species has repeatedly migrated across elevational
572 gradients to colonise high-altitude environments, in association with historical warming and
573 cooling cycles (Gómez and Lunt, 2007). Transplanting viperine snake embryos at 28^{o}C from
574 436m (20% O_2 SLE) to 2877m (15% O_2 SLE) increases heart rate, reduces body mass and
575 decreases swimming ability (Souchet et al., 2020b). Importantly, post-hatching reciprocal
576 transplant of snakes back to 436m does not fully recover swimming performance, and the
577 response is significantly temperature sensitive (see temperature section, above). Similar
578 results were found in common wall lizards, where transplantation of embryos from sea-level
579 to 2877m (15–16% O_2 SLE) leads to suppressed embryonic metabolism, cardiac hypertrophy
580 and larger eggs that produce hatchlings with relatively low mass (Cordero et al., 2017). In

contrast, transplantation of lowland Mongolia racerunner lizards to 2036m (16–17 O_2 SLE) had no effect on embryonic development (hatching time and success) or hatchling phenotypes (body size and locomotor performance), which suggests this species can buffer the impact of hypobaric hypoxia (LI et al., 2020).

Another approach to predicting the effects of climate-driven elevational range shifts is to compare embryonic outcomes in native highland versus native lowland individuals from the same species. These types of studies reveal genetic adaptations that arise over successive generations. Perhaps unsurprisingly, numerous studies have clearly shown that embryonic highland oviparous species are less sensitive to hypoxia than their lowland counterparts. For example, there is no effect of 12% O_2 exposure on embryonic body weight in geese raised at high altitude (1600m, Table S1A), and hatchling masses of high-altitude coots (4100m) are similar or slightly greater than those at sea-level (Carey et al., 1989). Native high-altitude ptarmigan and coot embryos (4200m; (León-Velarde and Monge-C, 2004) and bar-headed goose embryos (Snyder et al., 1984) have a greater O_2-carrying capacity than their sea-level counterparts, with increased hematocrit, haemoglobin, capillary density and blood O_2 affinity. Adult fishes from high-altitude habitats in China also possess adaptations related to haemoglobin, as well as expansions of gene families associated with energy metabolism, ion transport and the response to hypoxia (Kang et al., 2017; Lei et al., 2021; Tong et al., 2017). Lastly, cardiac citrate synthase activity in white-tailed ptarmigan (4200m) is higher than that of its sea-level counterparts, suggesting increased mitochondrial density and oxidative capacity (Carey and Martin, 1997). These studies demonstrate that prolonged high-altitude residence in oviparous vertebrates confers some protection against hypobaric hypoxia (similar to humans; (Giussani et al., 2001), and this is associated with adaptations in both O_2 carrying capacity and utilisation. Nevertheless, living at high altitude for six successive generations does not completely protect chicken embryos from the effects of hypobaric hypoxia. Growth restriction in chickens is improved by high-altitude residence, but there is still a significant reduction in embryonic body mass with hypobaric hypoxia, as well as cardiac hypertrophy, ventricular wall thickening, aortic medial thickening and an increase in adrenal catecholamines (Giussani et al., 2007; Salinas et al., 2010; Salinas et al., 2011). The effects can be prevented if high-altitude hens are given O_2 supplementation, which confirms that hypoxia rather than hypobaria is driving the cardiovascular response.

Furthermore, the effects persist into adulthood when chickens are maintained at high altitude for a further 6 months, and there is also evidence of pulmonary hypertension, right-sided heart dysfunction and hypotension (Herrera et al., 2013; Salinas et al., 2014). Interestingly, American alligators exposed to CDH and maintained in hypoxia into juvenile life also have signs of pulmonary hypertension, including a decreased ratio of the right ventricle to left ventricle (Owerkowicz et al., 2009). Collectively, these studies suggest some of the problems associated with CDH in chickens cannot be prevented by residence at high altitude (at least across six generations) and post-hatch exposure to hypoxia may cause further damage, including pulmonary hypertension. Whether later generations would eventually evolve better protection awaits investigation.

Potential interactive effects of CDH and CO_2 concentration

Despite the fact that oviparous vertebrates often experience hypoxia and hypercapnia simultaneously (both naturally and in climate change scenarios; Box 2), we are unaware of any studies that have investigated the combined effects of CDH and chronic hypercapnia. There are however, several studies that have shown interactive effects of acute hypoxia and hypercapnia (< 1 day) on chick embryonic O_2-carrying capacity and acid–base balance (Andrewartha et al., 2011; Andrewartha et al., 2014; Burggren et al., 2023; Burggren et al., 2012; Mueller et al., 2017). Furthermore, there is ample evidence that embryonic growth and cardiovascular outcomes can be affected by chronic hypercapnia alone, even at physiological levels. For example, exposure of embryonic chickens and ducks to CO_2 concentrations that they would normally encounter in the nest (1%) or higher (4%) increases body mass, compared to atmospheric levels (0.004%), and this effect persists into adulthood (De Smit et al., 2006; El-Hanoun et al., 2019; Everaert et al., 2007; Fares et al., 2012; Verhoelst et al., 2011). A similar observation has been made in common snapping turtles exposed to 3.5% CO_2 (Wearing et al., 2014), and American alligator embryos exposed to 3.5% and 7% CO_2 have increased relative heart mass and reduced arterial blood pressure (Eme and Crossley, 2015). Lastly, embryonic chickens and ducks exposed to 1% CO_2 have increased embryonic hemoglobin, packed cell volume (proportion of blood made up of cells) and red blood cell count (El-Hanoun et al., 2019; Fares et al., 2012). Collectively, these studies suggest that hypercapnia during development could offset hypoxic growth

642 restriction in birds and reptiles, and it could potentiate some of the cardiovascular
643 responses to hypoxia.

644 A large body of evidence suggests that juvenile and adult fish possess sufficient acid–base
645 and osmoregulatory capabilities to tolerate very high CO_2 levels (> 2000 µatm; Murray et al.,
646 2016). However, a recent metanalysis confirmed that fish embryos and larvae are
647 significantly more sensitive to hypercapnia than their adult counterparts (Cattano et al.,
648 2018). Indeed, embryonic or larval fish have significantly higher levels of mortality and
649 reduced growth at PCO_2 levels consistent with climate change projections (~1000 atm). The
650 increased sensitivity is likely due to ontogenic differences in respiration modes (dermal
651 versus gills) and insufficient acid–base regulation prior to gill formation (Ishimatsu et al.,
652 2008). There is also evidence that chronic hypercapnia affects cardiac function in some
653 larval fish species. Chronic exposure to PCO_2 at ~1100–1300 µatm causes tachycardia in
654 Pacific herring, garfish and zebrafish larvae (Alter and Peck, 2021; Miller, 2013; Villalobos et
655 al., 2020). However, numerous other studies have found no effect of hypercapnia on
656 growth, heart rate, haemoglobin and mitochondrial function, and some have even found
657 increased growth (Esbaugh, 2018; Leo et al., 2018; Mu et al., 2015; Scheuffele, 2017; Sun et
658 al., 2019). Therefore, although there is certainly a case to study the interaction between
659 hypercapnia and hypoxia in fish embryos and larvae, the effects may be relatively modest
660 compared to those of temperature.

661 **Conclusions and perspectives**

662 Oviparous ectotherms produce viable young when eggs are exposed to CDH, but there are
663 numerous effects on the cardiovascular system at multiple levels of biological organisation,
664 both during development and in postnatal life (Figure 1). Despite vastly different cardiac
665 designs and body temperatures, the embryonic cardiovascular responses are generally well
666 conserved among vertebrates, and include asymmetric growth restriction, relative cardiac
667 enlargement, alterations in heart rate, enhanced sympathetic activity and an increase in O_2-
668 carrying capacity. In the long term, these phenotypic changes programme cardiovascular
669 abnormalities in chickens that are very similar to those of mammals, leading to reduced
670 cardiac performance and pathological cardiovascular signatures. The impact of CDH in
671 American alligators and snapping turtles is less severe in juvenile life and may even be
672 beneficial under circumstances of increased physiological stress. This suggests that the

673 increased metabolic demand associated with endothermy places an additional burden on the
674 avian and mammalian heart.
675
676 Unsurprisingly, the embryonic and postnatal response to CDH depends on the severity of
677 hypoxia. In birds and reptiles, most responses are only evident at O_2 concentrations at or
678 below 15% saturation. These levels of O_2 are commonly experienced by many embryonic
679 reptilian species, which suggests that CDH is a significant driver of individual variation. In
680 contrast, most lowland embryonic avians are unlikely to experience O_2 concentrations below
681 20% O_2, which makes CDH less ecologically relevant. However, megapode species develop at
682 O_2 concentrations below 15%, so it would be interesting to see whether these species are
683 uniquely adapted to hypoxia. The situation in fishes is far more complex, and there doesn't
684 seem to be any obvious O_2 threshold for a cardiovascular response, even within the same
685 species. This is probably because the levels of CDH are much more severe in the fish studies
686 (45–95% reduction in O_2) versus the avian and reptilian studies (20–50% reduction in O_2),
687 probably leading to higher levels of variation, and making comparisons between these groups
688 complicated.

689 More work needs to be done to characterise the phenotypic responses and thresholds for
690 CDH in the presence of other stressors, such as hypercapnia and temperature in all oviparous
691 vertebrate groups. Interestingly, hypercapnia alone appears to have both synergistic and
692 antagonistic responses to hypoxia in oviparous vertebrates, which means that the
693 combination of these two stressors is expected to produce entirely different phenotypes. This
694 is relevant to normal development because reptiles and birds experience hypoxia and
695 hypercapnia simultaneously, and most studies use non-physiological levels of CO_2 when
696 investigating hypoxia. It is also important in the context of climate change because the
697 prevalence and intensity of hypercapnia is increasing, particularly in aquatic environments.
698 Unsurprisingly, warming temperatures exacerbate the effects of developmental hypoxia in
699 some oviparous species, which is concerning considering global warming and the increased
700 prevalence and intensity of heat waves. The timing of extreme weather events is also crucial,
701 because most species possess critical windows in development where the cardiovascular
702 system is especially sensitive to stress. Furthermore, we expect species with faster
703 developmental rates and shorter gestations to be disproportionately affected by heat waves

and extreme weather events, compared to shorter-gestation species, because a greater proportion of their development will be affected. Obviously, the challenge is to study the integrative effects of CDH, hypercapnia and warming on embryonic and adult phenotypic outcomes. In this regard, it is also critically important to gather accurate data about the effects of climate change on nest gas tensions and temperatures.

Future work should also focus on transplantation studies to determine the effects of high-altitude acclimation on reptilian and avian developmental outcomes. Studies like these are important because the phenotypic response to high-altitude hypoxia in lowland species will ultimately determine the colonization potential of these animals as the planet continues to warm. From the limited data available, it is clear that reptiles and birds respond to hypobaric hypoxia in a similar fashion to isobaric hypoxia, and some of the traits cannot be reversed by returning the animals to sea level. Long-term residence at high altitude affords protection in most avian and reptilian species, but domestic chickens raised at high altitude for six generations still undergo some level of growth restriction and cardiac remodelling in response to CDH. Importantly, the phenotype worsens with continued exposure to hypoxia post-hatch. Clearly, more multigenerational studies are necessary to understand the impact of cardiovascular plasticity on the vertical colonisation potential of oviparous birds and reptiles.

Lastly, there are some questions in this field that are almost completely unstudied. For example, our understanding of the effects of CDH and other stressors on the amphibian cardiovascular system is severely lacking. This is surprising, as this class of vertebrates is one of the most likely to experience fluctuations in developmental O_2, CO_2 and temperature (Box 2). There is also very little known about sex-dependent differences in the response to CDH among oviparous vertebrates. It is well established in the mammalian literature that cardiometabolic responses to developmental stressors are strongly sex-dependent, with females often being protected against detrimental long-term health outcomes compared to males (Giussani, 2021; Sandovici et al., 2022). Sex-dependent differences have been observed in some avian studies, but these effects are largely unstudied in ectothermic vertebrates. Similarly, the transgenerational effects of CDH and the underlying epigenetic mechanisms are very poorly studied in oviparous vertebrates. In this regard, several studies have shown that parental exposure to hypoxia can improve hypoxia tolerance in zebrafish offspring (Burggren,

2014; Ragsdale et al., 2022). These kinds of phenomena are particularly important to study, because transgenerational plasticity will play a crucial role in determining a species' ability to cope with a rapidly changing environment (Donelson et al., 2018).

Box 1: Incidence and prevalence of chronic developmental hypoxia in oviparous vertebrates

Although most avians develop at atmospheric levels of O_2 (~21% saturation), megapode birds bury their eggs in mounds where O_2 concentration can range from 13 to 17% (Seymour and Ackerman, 1980). Certain reptiles also exhibit this behaviour (mainly crocodilians and chelonians), with some nest O_2 concentrations as low as 10% (Seymour and Ackerman, 1980). Hypoxia develops in these nests because of gas diffusion limitations, embryonic metabolism, the decomposition of matter and the activity of microorganisms (Seymour and Ackerman, 1980). Subterranean nests are also prone to flooding, which can cause unpredictable temporal changes in O_2 (Doody and Refsnider, 2023). Many birds and reptiles also experience hypobaric hypoxia as a consequence of living at high altitude (1500 to 6500m), where effective O_2 concentrations can range between 10 and 19% (sea-level equivalent; León-Velarde and Monge-C, 2004). However, the most severe levels of hypoxia are observed in aquatic environments, because O_2 concentration and diffusion rates are lower in water than in air, and they change diurnally and seasonally (Wu, 2009). For example, fish that develop in intertidal environments can transition from hyperoxia (four times air saturation) to severe hypoxia (5% O_2 saturation) and even anoxia (zero O_2) within 24 hours (Richards, 2011). Similarly, seasonal increases in temperature can create hypoxic zones in freshwater and marine environments due to evaporation and stratification. This is particularly disruptive for sessile species that have protracted embryonic periods, such as elasmobranchs. Lastly, even in fast-flowing, well-aerated environments, embryos often experience hypoxic conditions due to low water-flow rates within the egg mass (Dhiyebi et al., 2013). These factors make fish embryos particularly vulnerable to chronic developmental hypoxia.

BOX 2: Interactions between chronic developmental hypoxia and other environmental stressors

The phenotypic effects of chronic developmental hypoxia can be modulated by other naturally occurring or anthropogenic environmental stressors, most commonly temperature and CO_2. In avian and reptilian nests, hypercapnia naturally occurs in parallel with hypoxia because embryonic CO_2 production increases as the organism respires. Nest CO_2 concentrations usually rise from ~0.05% to 1.4%, but levels can increase to 4–12% when large amounts of decaying vegetation are present (Seymour and Ackerman, 1980). Similarly, CO_2 fluctuations within aquatic environments can arise from natural phenomena, including variations in photosynthesis and respiration rates, wind speed and direction, ecosystem metabolism, convective mixing and ice phenology (Golub et al., 2023). All these factors are influenced by temperature, which can vary dramatically in terrestrial and aquatic developmental environments, both spatially and temporally (Du et al., 2019). Unfortunately, climate change and other anthropogenic activities are increasing the intensity of these environmental interactions. Extreme weather events, such as heat waves and flooding, are likely to increase the magnitude and duration of hypoxia and hypercapnia in terrestrial nests (Doody and Refsnider, 2023). Within aquatic environments, global warming and extreme heatwaves are increasing water temperatures in rivers (van Vliet et al., 2023), lakes (Woolway et al., 2022) and oceans (Benthuysen et al., 2020). Furthermore, the combination of eutrophication (see Glossary) and warming is increasing the prevalence and intensity of hypoxic zones. Oceanic CO_2 levels are projected to increase from 410 to 1400 μatm by the year 2100, leading to a reduction in seawater pH of up to 0.4 units (Henson et al., 2017). Recent studies have shown that CO_2 is also increasing in freshwater systems (Phillips et al., 2015). This problem is further confounded by anthropogenic eutrophication, which also leads to aquatic hypercapnia due to the decomposition of algal blooms (Cai et al., 2011). It is therefore critically important to study the interactive effects of hypoxia, hypercapnia and temperature on embryonic phenotypic outcomes.

Figure 1: Effects of chronic developmental hypoxia (CDH) on the cardiovascular system (CVS) of oviparous vertebrates. CDH often develops in the nests of oviparous birds, reptiles and fish (see Box 1 for details). CDH can alter embryonic cardiovascular structure and function at multiple levels of biological organisation, and some of these abnormalities persist into adulthood (see Table S1 for full details of species-specific differences. The effects of CDH can be modulated by other environmental stressors that occur during development, including hypercapnia and warming. This figure has been created with Biorender (Agreement number: NG25JUBP7L).

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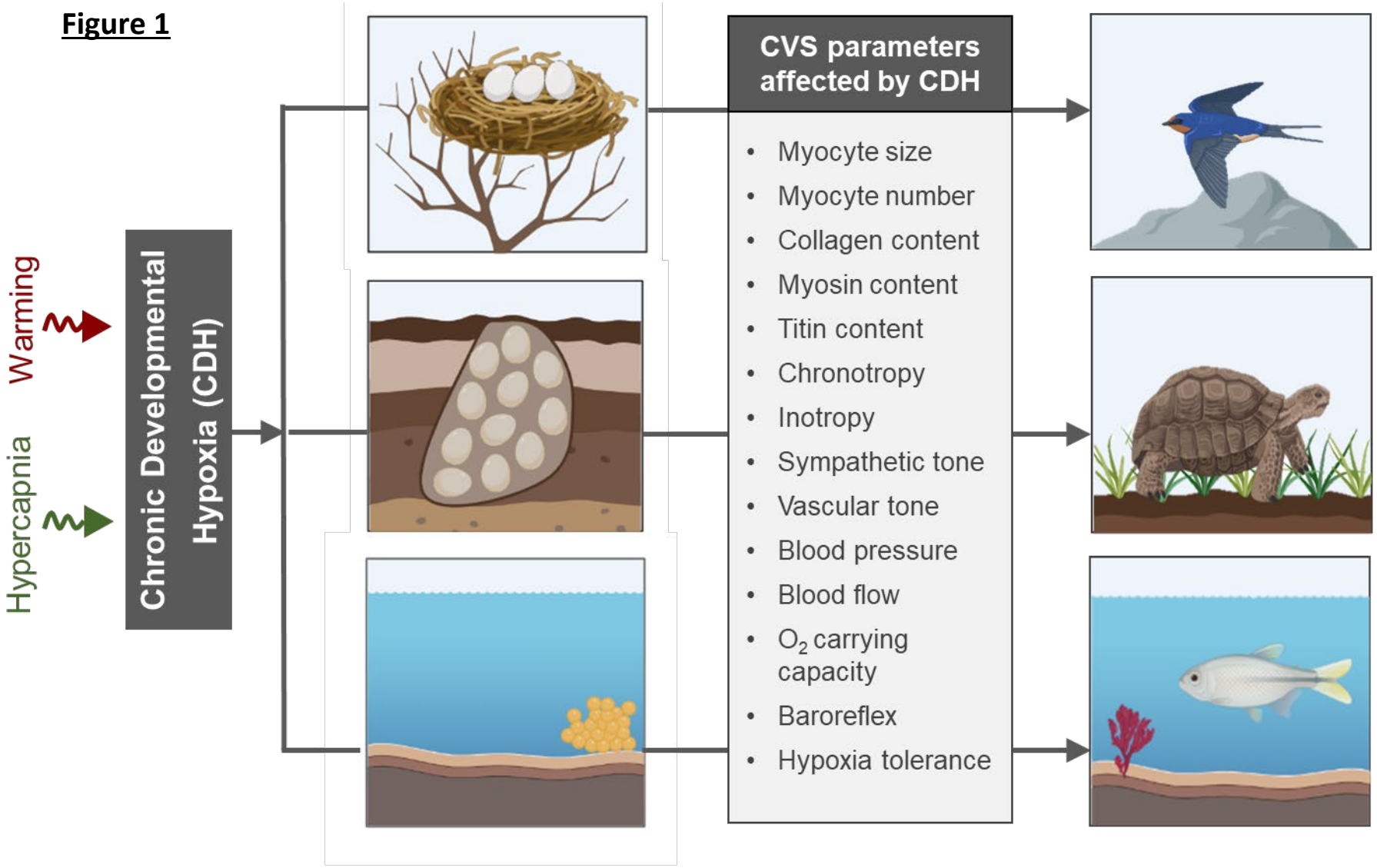
1321

Figure 1



Supplementary Table 1: Effects of chronic developmental hypoxia on body mass and cardiovascular parameters in avians, reptiles and fish

AVIANS	Stage	Body Temp (°C)	$[O_2]$ Control	$[O_2]$ Hypoxia	(A) Body mass		(B) Heart mass		(C) Heart-body weight ratio	(D) Heart rate		(E) O_2 carrying capacity	(F) Mito capacity		(G) Cardiac Fibrosis		(H) Sympathetic activity	
					Emb	Juv or Adult	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Juv or Adult	Emb	Juv or Adult
Gallus gallus domesticus (leghorn): 20-21 days	1-6 doi	37.5	21%O_2	15%O_2	↓ 1 — 2		—1,2	— 1				— 1						
	6-12 doi	37.5	21%O_2	15%O_2	↓ 1		↑ 1	— 1				↑ 1						
	6-19 doi	38	21%O_2	15%O_2	↓ 3-7	— 4	↑ 3 — 5 ↓ 6		↑ 3, 5 — 6				↓ 6				↑ 7	
	7-14	37.5	21%O_2	15%O_2	↓ 8							↑ 8						
	12-18 doi	37.5	21%O_2	15%O_2	↓ 1		— 1	— 1				— 1						
	16-18 doi	37.5	21%O_2	15%O_2	↓ 9		↓ 9											
	0-19 & 1-20 doi	37.5	21%O_2	15%O_2	↓ 10-16		↓ 10,15		— 10 ↑ 11,13	— 14,16		↑ 12,15				↑ 15		
	0-21 doi	37.5	21% O_2	14% O_2	↓ 17,18		↓ 17,18					↑ 17,18						
	1-21 doi	38	21%O_2	HB: 13%O_2	↓ 19												↑ 19	
	0-20 doi	38	21% O_2	HB: 13%O_2	↓ 20							↑ 20						

AVIANS	Stage	Body Temp (°C)	[O_2] Control	[O_2] Hypoxia	(A) Body mass		(B) Heart mass		(C) Heart-body weight ratio	(D) Heart rate		(E) O_2 carrying capacity	(F) Mito capacity		(G) Cardiac Fibrosis		(H) Sympathetic activity	
					Emb	Juv or Adult	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Juv or Adult	Emb	Juv or Adult
Gallus gallus domesticus (bovans brown): 20-21 days	1-21 doi	37.9	21%O_2	14%O_2	↓ 21-23	↓ 23	↑ 21		— 21			↑ 22,23	↓ 22					↑ 23
Gallus gallus domesticus (broiler): 20-21 days	1-21 or 0-19 doi	37.8	21%O_2	14%O_2	↓ 24-26	— 24 ↓ 25	— 25	— 25	↑ 24	— 26						— 25	↑ 24,26	↓ 24 ↑ 25
	1-20 doi	37.8	21%O_2	15%O_2	↓ 10		↓ 10		↓ 10									
	6-19 doi	37.8	21%O_2	15%O_2	↓ 4	↓ 4	↑ 4											
	9-19	37.8	21%O_2	17%O_2	— 27		— 27											
		38.9	21%O_2	17%O_2	— 27		— 27											
Gallus gallus (red junglefowl): 19-21 days	1-20 doi	37.8	21%O_2	15%O_2	↓ 10		↓ 10		↓ 10									
Branta canadensis **(Canada goose) 28 days**	0-28	37	16%O_2	12%O_2	— 28,29							↑ 29						
Anser indicus **(bar-headed goose): 27-30 days**	0-28	37	16%O_2	12%O_2	— 28													

REPTILES	Stage	Body Temp (°C)	[O_2] Control	[O_2] Hypoxia	(A) Body mass		(B) Heart mass		(C) Heart-body weight ratio	(D) Heart rate		(E) O_2 carrying capacity	(F) Mito capacity		(G) Cardiac Fibrosis		(H) Sympathetic activity	
					Emb	Juv or Adult	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Juv or Adult	Emb	Juv or Adult
American alligator (*Alligator mississippiensis*): 63-68 days	0-90%	30	21%O_2	10%O_2	↓ 30-34	— 35-37 ↓ 38,39	— 30,31		↑ 30,31	↓ 30,33,34	— 35	↑ 40	— 38	↑ 38				
	0-80%	30	21%O_2	10%O_2	↓ 30,34		— 30		↑ 30	↓ 34 — 30								
	0-70%	30	21%O_2	10%O_2	— 30 ↓ 33,34		— 30		— 30	— 30 ↓ 33,34							↑ 34	
	0-60%	30	21%O_2	15%O_2	— 30		— 30		— 30	— 30								
	0-90%	30	21%O_2	15%O_2	— 30		— 30		— 30	— 30								
	0-80%	30	21%O_2	15%O_2	— 30		— 30		— 30	— 30								
	0-70%	30	21%O_2	15%O_2	— 30		— 30		— 30	— 30								
	0-60%	30	21%O_2	15%O_2	— 30		— 30		— 30	— 30								
Snapping turtle (Chelydra serpentina): 80-90 days	0-90%	30	21%O_2	10%O_2	↓ 41-43	↓ 44 — 45–47	↑ 43		↑ 41-43,48	— 41 ↑ 42		↑ 49		↑ 47			↑ 42	
	0-70%	30	21%O_2	10%O_2	↓ 41-43		— 43		↑ 41,42,48 — 43	— 41,42				↑ 38			↑ 42	

REPTILES	Stage	Body Temp (°C)	[O_2] Control	[O_2] Hypoxia	(A) Body mass		(B) Heart mass		(C) Heart-body weight ratio	(D) Heart rate		(E) O_2 carrying capacity	(F) Mito capacity		(G) Cardiac Fibrosis		(H) Sympathetic activity	
					Emb	Juv or Adult	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Emb	Juv or Adult	Emb	Juv or Adult	Emb	Juv or Adult
Florida red-bellied turtle (Pseudemys Nelson): 45-80 days	0-90%	30	21%O_2	10%O_2	↓ [49]		— [49]		↑ [49]			↑ [49]						
Leopard gecko (Eublepharis macularius): 45-53 days	0-70%	34	21%O_2	Regional hypoxia	↓ [50]		↓ [50]		↑ [50]					↑ [38]				
	0-70%	28	21%O_2	Regional hypoxia	↓ [50]		↓ [50]		↑ [50]									
Banded red snake (Lycodon rufozonatu): 50 days	10-100%	28	21%O_2	Regional hypoxia	— [51]				↑ [51]									
Chinese softshell turtle (Pelodiscus sinensis): 60 days	0-100%	28	21%O_2	Regional hypoxia	— [51]				— [51]									
Common wall lizard (Podarcis muralis): 42-77 days	0-100%	24	21%O_2	HB 15%O_2	↓ [52] — [53]		↑ [52]			↓ [52,53]								
	0-100%	24	21%O_2	HB 17%O_2	— [53]					↓ [53]								

Viperine snake (*Natrix maura*)	0-100%	24	21%O_2	HB 15%O_2	— [54]					— [54]								
	0-100%	28	21%O_2	HB 15%O_2	↓ [55]					↑ [55]								
	0-100%	32	21%O_2	HB 15%O_2	↓ [54]					↓ [54]								
Class and species	**Stage**	**Body Temp (°C)**	**[O_2] Control**	**[O_2] Hypoxia**	**(A) Body mass**		**(B) Heart mass**		**(C) Heart-body weight ratio**	**(D) Heart rate**		**(E) O_2 carrying capacity**	**(F) Mito capacity**		**(G) Cardiac Fibrosis**		**(H) Sympathetic activity**	
FISH					**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**	**Emb**	**Emb**	**Juv or Adult**	**Emb**	**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**
Zebrafish (*Danio rerio*): 3-4 days	0-2 dpf	25	6.5 mg/L	0.8 mg/L						↓ [56]								
	1-5 dpf	25	7.5 mg/L	3.8 mg/L	— [57]		↑ [57]			↑ [57]								
	0-1 dpf	28	7.5 mg /L	4.3 mg /L	— [58]	↓ [58]				— [58]								
	0-1 dpf	28	6.5 mg/L	0.6 mg /L	↓ [59]													
	2-4 dpf	28	100%	5%									↓ [60]					
	0-4 dpf	28	6 mg/L	1-2 mg/L			↓ [61,62]			↓ [61,62]								
	1-15 dpf	28	7.5 mg/L	3.3 mg/L	↓ [63]							↑ [63]						
	1-5 dpf	28	7.5 mg/L	3.8 mg/L	— [57]		↑ [57]			↑ [57]								
	5-9 dpf	28	7.5 mg/L	1.5 mg/L						↓ [64]							↑ [64]	
	1-5 dpf	28	7.5 mg/L	1.5 mg/L			↑ [65]			↓ [65]		↑ [65]						
	1-10 dpf	28	7.5 mg/L	1.5 mg/L			↑ [65]			↑ [65]		↑ [65]						
	0-10 dpf	28	7.5 mg/L	1.5 – 1.9 mg/L						↓ [66]								
	0-12 dpf	25	6.5 mg/L	0.8 mg/L						↓ [56]								

Class and species	Stage	Body Temp (°C)	$[O_2]$ Control	$[O_2]$ Hypoxia	(A) Body mass		(B) Heart mass		(C) Heart-body weight ratio	(D) Heart rate		(E) O_2 carrying capacity	(F) Mito capacity		(G) Cardiac Fibrosis		(H) Sympathetic activity	
FISH					**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**	**Emb**	**Emb**	**Juv or Adult**	**Emb**	**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**
	0-3 dpf	28	7.5 mg/L	1.5 – 1.9 mg/L						↓ 66								
	0-30 dpf	28	7.5 mg /L	4.3 mg /L	— 58	↓ 58				↑ 58								
	1-5 dpf	31	7.5 mg/L	3.8 mg/L	— 57		↑ 57			↑ 57								
Rainbow trout *(Oncorhynchus mykiss)*: 60-90 days	25-36 dpf	10	10 mg /L	5 mg /L	↓ 67													
	0-57 dpf	11	100%	34%	↓ 68	↑ 68												
	0-45 dpf	10	100%	30%	↓ 69							↑ 69						
Chinook salmon *(Oncorhynchus tshawytsch)*: 90-150 days	0-1 dph	10	10 mg /L	5.5 mg /L		↓ 70												
	0-1 dph	15	10 mg /L	5.5 mg /L		↓ 70												
***Small-spotted catshark* (*Scyliorhinus canicula*): 240-270 days**	0-28 wpf	15 and 20	100% air sat	50% air sat	— 71													
Grass carp (*Ctenopharyngodon idellus*): 1-3 days	0-1 dpf	22	7.0 mg/L	1.0 mg/L	↓ 72													

Class and species	Stage	Body Temp (°C)	[O_2] Control	[O_2] Hypoxia	(A) Body mass		(B) Heart mass	(C) Heart-body weight ratio		(D) Heart rate		(E) O_2 carrying capacity	(F) Mito capacity		(G) Cardiac Fibrosis		(H) Sympathetic activity	
FISH					**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**	**Emb**	**Emb**	**Juv or Adult**	**Emb**	**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**	**Emb**	**Juv or Adult**
European seabass (*Dicentrarchus labrax*): 9 days	28-50 dph	15	7.35 mg/L	2.95 mg/L	— [73]	↓ [73]												
	28-50 dph	20	7.35 mg/L	2.95 mg/L	— [73]	— [73]												
	30-38 dph	19	9.3 mg/L	3,7 mg/L		↓ [74]												
Atlantic salmon (*Salmo salar*): 57-75 days	0-100 dpf	8	11.9 mg/L	5.96 mg/L		— [75]												
Abbreviations: HB, hypobaric hypoxic; dpf, days post fertilisation; dph, days post hatch; wpf, weeks post-fertilisation; di, days of incubation; $\%O_2$, % oxygen saturation. Average gestation period for each species is given for each species in column 1.																		

Supplementary Table 2: Effects of chronic developmental hypoxia on mammalian body mass and cardiovascular parameters

MAMMALS	Stage (GD)	Body Temp (°C)	[O_2] Control	[O_2] Hypoxia	(A) Body mass	(B) Heart mass	(C) Heart-body weight ratio	(D) Heart rate	(E) O_2 carrying capacity	(F) Mito capacity	(G) Cardiac Fibrosis	(H) Sympathetic activity

					Fetal	Juv or Adult	Fetal	Juv or Adult	Fetal	Fetal	Juv or Adult	Fetal	Fetal	Juv or Adult	Fetal	Juv or Adult	Fetal	Juv or Adult
Rat (*Rattus norvegicus*): 21-23 days	6-20	37	21%O_2	13%O_2	— 76-78	— 77	—76,77	— 77	—76,77		— 77	↑78	↓ 79 — 79					↑ 77
	10-20	37	21%O_2	12%O_2							—80							↑ 80
	15-20	37	21%O_2	10%O_2	↓ 81,82	— 81	—82	—82	—82		— 81	↑82						
	15-21	37	21%O_2	10.5%O_2	↓ 83		—83		↑83						↑83			
	15-21	37	21%O_2	12%O_2	↓ 84,85		—85			—85					↑85			
	15-21	37	21%O_2	11%O_2	↓ 86	—86	↑86		↑86							↑86		
Mouse (*Mus musculus*): 19-21 days	14-20	37	21%O_2	12%O_2	↓ 87	— 87										↑87		
	6-18	37	21%O_2	14%O_2										↑↓ 88				
Guinea pig (*Cavia porcellus*): 59 – 72 days	58-70	38	21%O_2	12%O_2	— 89													
	49-63	38	21%O_2	10.5%O_2	↓ 90		—90		↑90						↑90			
	35-60	38		Uterine Artery Constriction	— 91	↓ 91												
	25-64	38	21%O_2	10%O_2	↓ 92								↑↓ 92					
	50-64	38	21%O_2	10.5%O_2	↓ 92								— 93 ↓ 92	↓ 94,95 — 95				

Supplementary Table 3: Common pathological cardiovascular signatures in juvenile and adult vertebrates that were exposed to chronic developmental hypoxia

Pathological signatures observed in mammals from hypoxic pregnancies	Birds	Crocodilians	Squamates and testudines	Fish
Catch up growth [81,84,96-100]	Yes [4,24] (2/5)	Yes [35-37] (3/5)	Yes [45-47] (3/4)	Yes [68,73,75] (3/9)

Increased heart/body weight ratio [85]	Yes [3,5,11,13,24] (5/10)	Yes [35-39] (4/4)	Yes [46,47] (2/3)	Unknown
Fibrosis [85,101,102]	Yes [15] (1/2)	Unknown	Unknown	Unknown
Ventricular wall thinning [99]	Yes [15] (1/2)	Unknown	Unknown	Unknown
Ventricular wall thickening [103]	Yes [23,104] (1/3)	Unknown	Unknown	Unknown
Aortic wall thickening [105]	Unknown	Unknown	No [106] (1/1)	Unknown
Systemic hypertension 22,80,100,102,105,107	Yes [23] (1/1)	No [108] (1/1)	No [44,106] (2/2)	Unknown
Enhanced Sympathetic tone 96,98,109	Yes [24] (1/1)	Yes [35,36] (2/2)	Unknown	Unknown
Mitochondrial dysfunction 88,94,95,107	Unknown	No [38] (1/1)	No [45,47] (2/2)	Unknown
Increased sensitivity to hypoxia, anoxia or ischemia 80,81,85,98,109-111	Unknown	No [36,37,108] (3/3)	No [46,112] (2/2)	Unknown
Diastolic dysfunction [85,103,109]	Yes [15] (1/3)	No [36,39] (2/2)	Unknown	Unknown
Systolic dysfunction [107]	Yes [15,24] (2/3)	No [36,39] (2/2)	Unknown	Unknown
Enhanced contractility [96,109]	Yes [23] (1/2)	No [36] (1/1)	Unknown	Unknown
Pulmonary hypertension 103,113,114	Unknown	Unknown	No [106] (1/1)	Unknown

References in first column are from mammalian studies of chronic developmental hypoxia (CDH). Red and green colours indicate the presence or absence of the response, respectively. Grey colour indicates that the parameter has yet to be studied in this vertebrate class. Fractions in brackets indicate the percentage of papers that found the result (e.g. 1/3 = one in three papers found this result).

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