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REVIEW

The phylogeny and ontogeny of autonomic control of the heart and cardiorespiratory interactions in vertebrates

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ABSTRACT

Heart rate in vertebrates is controlled by activity in the autonomic nervous system. In spontaneously active or experimentally prepared animals, inhibitory parasympathetic control is predominant and is responsible for instantaneous changes in heart rate, such as occur at the first air breath following a period of apnoea in discontinuous breathers like inactive reptiles or species that surface to air breathe after a period of submersion. Parasympathetic control, exerted via fast-conducting, myelinated efferent fibres in the vagus nerve, is also responsible for beat-to-beat changes in heart rate such as the high frequency components observed in spectral analysis of heart rate variability. These include respiratory modulation of the heartbeat that can generate cardiorespiratory synchrony in fish and respiratory sinus arrhythmia in mammals. Both may increase the effectiveness of respiratory gas exchange. Although the central interactions generating respiratory modulation of the heartbeat seem to be highly conserved through vertebrate phylogeny, they are different in kind and location, and in most species are as yet little understood. The heart in vertebrate embryos possesses both muscarinic cholinergic and β-adrenergic receptors very early in development. Adrenergic control by circulating catecholamines seems important throughout development. However, innervation of the cardiac receptors is delayed and first evidence of a functional cholinergic tonus on the heart, exerted via the vagus nerve, is often seen shortly before or immediately after hatching or birth, suggesting that it may be coordinated with the onset of central respiratory rhythmicity and subsequent breathing.

KEY WORDS: Autonomic nervous system, Parasympathetic tonus, Cardiorespiratory interaction, Heart rate variability, Respiratory sinus arrhythmia, Ontogeny, Vertebrate

Introduction

Each contraction of the vertebrate heart is initiated by a myogenic pacemaker, but the prevailing heart rate (f_H) is determined from within the central nervous system (CNS) via the autonomic nervous system (ANS), which exerts inhibitory parasympathetic and excitatory sympathetic influences on the heart. These mechanisms allow for gross matching of cardiac output to metabolism, but the effectiveness of gas exchange is also improved by tight beat-to-beat coordination of f_H to ventilation of the respiratory organs, whether they are gills or lungs. Animals with a discontinuous breathing pattern such as air-breathing fish or diving tetrapods typically display bradycardia during apnoea and a pronounced cardiac acceleration immediately upon the first air breath. In animals with regular and rhythmic ventilation, cardiorespiratory interactions are typically revealed as a rise in f_H during inspiration (Taylor et al., 1999). It has long been recognized that the overall rates of flow of air or water and of blood over the respiratory surfaces are matched according to their respective capacities for oxygen so that the ventilation-to-perfusion ratio varies from 1 in air breathers to 10 or more in water breathers, with the bimodal, air/water breathers among the lungfishes and amphibians showing variable ratios (Piiper and Scheid, 1977). As both ventilation and perfusion are typically pulsatile and sometimes intermittent, close co-ordination between the two rhythms would seem essential in order to optimize the effectiveness of respiratory gas exchange and its metabolic costs. Control of these cardiorespiratory interactions resides in the CNS. The cell bodies of the preganglionic neurones in both parts of the ANS are located within the brainstem or spinal cord where their activity can be influenced by afferent input from peripheral baroreceptors and mechanoreceptors as well as higher brain centres. These integrate afferent inputs from a range of central and peripheral receptors and coordinate central interactions between pools of neurones generating the respiratory rhythm and determining f_H variability (Taylor et al., 1999).

This review explores the central coordination of the cardiovascular and respiratory systems in vertebrates, and we will argue that, despite major differences in the construction and mode of operation of these systems, vertebrates share some fundamental similarities in the neural control of f_H and its coordination with the respiratory rhythm (Table 1). We introduce the review with a short description of the origin and nature of tonic nervous control to the heart and of cardiorespiratory interactions (CRI) in mammals and then describe and interpret our current knowledge of the other vertebrate groups.

While adrenergic influences on the heart are important in relation to activity levels, including fight or flight responses, the heart in routinely active or experimentally prepared vertebrates chiefly operates under a variable degree of inhibitory tonus, imposed by the parasympathetic arm of the ANS via the Xth cranial nerve, the vagus (Taylor et al., 1999). In a representative collection of mammals, while adrenergic tone varied from 4 to 30% (Fig. 1), inhibitory vagal tone varied between 15 and 102% (Fig. 2). The vagus is also responsible for instantaneous, beat-to-beat control of f_H (Bootsma et al., 1994; Taylor et al., 1999). The heart accelerates during inspiration in mammals and slows during exhalation. Power spectral analysis of f_H variability (HRV) (Grossman and Taylor, 2007) reveals a peak at a relatively high frequency that is respiration related and this relationship is termed respiratory sinus arrhythmia (RSA). In humans, RSA is normally well developed in juveniles

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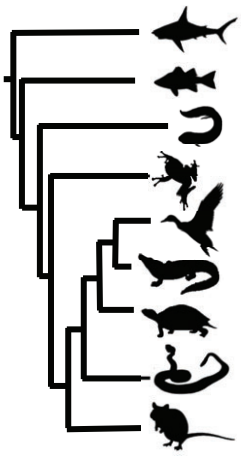
List of abbreviations

ABO	air-breathing organ
ACh	acetylcholine
ANS	autonomic nervous system
CNS	central nervous system
CRI	cardiorespiratory interactions
CRS	cardiorespiratory synchrony
CVPN	cardiac VPN
DVN	dorsal motor nucleus of the vagus
ECG	electrocardiogram
f_H	heart rate
FHRV	fetal heart rate variability
HRV	heart rate variability
NA	nucleus ambiguus
RMN	respiratory motor neurone
RSA	respiratory sinus arrhythmia
VPN	vagal preganglionic neurone

then declines with age but this decline is alleviated in physically fit individuals, such as high-performance athletes (Giardino et al., 2003). It has been suggested that RSA serves to provide temporal matching of ventilation with increased perfusion of the lung, increasing the effectiveness of gas exchange (Yasuma and Hayano, 2004). The lack of high frequency components in spectral analysis of HRV has been suggested as a clinical indicator of cardiovascular disease in infants and adults (Camm et al., 1996; Thompson et al., 1993; Janszky et al., 2004).

In mammals, RSA is mediated by fluctuations in inhibitory input from the brain to the heart through the vagus nerve in response to both centrally generated (feed-forward) influences from respiratory neurones and afferent input from pulmonary stretch receptors (feedback) that gate baroreceptor inputs (Spyer, 1994). While 70% of vagal preganglionic neurones (VPNs) are located within the dorsal motor nucleus of the vagus (DVN) (Ranson et al., 1993), around 80% of the cell bodies of cardiac VPNs (CVPNs) are located outside of the DVN in the ventro-lateral nucleus ambiguus (NA) (Jordan et al., 1986) (Fig. 3). CVPN within the DVN are activated by stimulation of pulmonary C-fibres, but are unaffected by stimulation of arterial baroreceptors or the respiratory cycle (Jones et al., 1994; Jones et al., 1995; Jones et al., 1998). The ongoing activity of these neurones is rather regular but not rhythmic. By contrast, CVPNs located in the NA fire with respiratory-related and cardiac-related rhythms. This respiratory rhythmicity in CVPNs in the NA arises because they receive an inhibitory input from inspiratory neurones in the neighbouring ventral respiratory group, plus inputs from lung stretch receptors that inhibit activity in baroreceptor afferents entering via the nucleus of the solitary tract (Spyer, 1994; Jordan, 1995). Respiratory activity during inspiration accordingly silences or 'gates' the discrete population of CVPNs in the NA, so that they are inactive during inspiration, being no longer responsive to baroreceptor stimulation. Their inhibitory input on the heart is therefore withdrawn, causing f_H to rise during inspiration (Jordan and Spyer, 1987; Jordan, 1995; Taylor et al., 1999). The responses are instantaneous because they are relayed by rapidly conducting myelinated efferent axons leaving the CVPNs in the NA (Table 1), classified as B-fibres on the basis of their conduction velocities, which differ from the slow conducting C-fibres coming from the CVPNs in the DVN. This high conduction velocity is essential for the beat-to-beat control of the heart that generates RSA. In a sophisticated neurophysiological study, Jones et al. (Jones et al., 1995) clearly showed the enhanced degree of control exerted on the heart by activity in myelinated fibres from CVPNs located in the NA when compared with the activity in slow fibres from CVPNs in the DVN (Table 1). So there are two populations of CVPN that have different functional properties, including their afferent connections, the conduction velocity of their efferent axons, their efferent activity and the consequent effects on f_H.

Table 1. A schematic diagram of the putative influences determining autonomic control of heart rate in vertebrates, revealing the huge gaps in our present knowledge



	Location of VPN (✓) and CVPN (★)			Heart innervation		
	DVN	NA	Respiration-related HRV	Parasympathetic innervation	Myelinated vagal fibers	Sympathetic innervation
Elasmobranchii	✓★ (+)	✓★	CRS	✓	✓	–
Teleostii	✓★ (+)	✓★	CRS	✓	?	✓
Dipnoi	? ?	? ?	CRI	✓	?	–
Amphibia	✓★	✓★	CRI	✓	?	✓
Aves	✓★	✓★	RSA	✓	?	✓
Crocodilia	✓?	✓?	CRI?	✓	?	✓
Testudinata	✓?	✓?	CRI	✓	?	✓
Squamata	✓?	✓?	CRI/RSA?	✓	?	✓
Mammalia	✓★	✓★ (–)	RSA	✓	✓	✓

We have evidence that the central cardiorespiratory interactions generating respiration-related heart rate variability (HRV) (including cardiorespiratory synchrony, CRS) are due to stimulation of cardiac vagal preganglionic neurones (CVPNs) in the dorsal motor nucleus of the vagus (DVN) in fish and hypothesize that this may be the case in amphibian tadpoles. Mammals have HRV (as respiratory sinus arrhythmia, RSA) generated by the inhibition of CVPNs in the nucleus ambiguus (NA). Air-breathing adult amphibians, reptiles and birds may share this characteristic. CRI, cardio-respiratory interactions. The symbols (✓ and ★) denote the presence of vagal preganglionic neurones (VPNs) and CVPNs, respectively in the DVN or the ventro-lateral NA. The dashed rectangles indicate the putative location of central interactions generating respiration-related HRV. The plus and minus signs indicate stimulation or inhibition of CVPNs by respiratory drive. The question marks indicate where data were not available.

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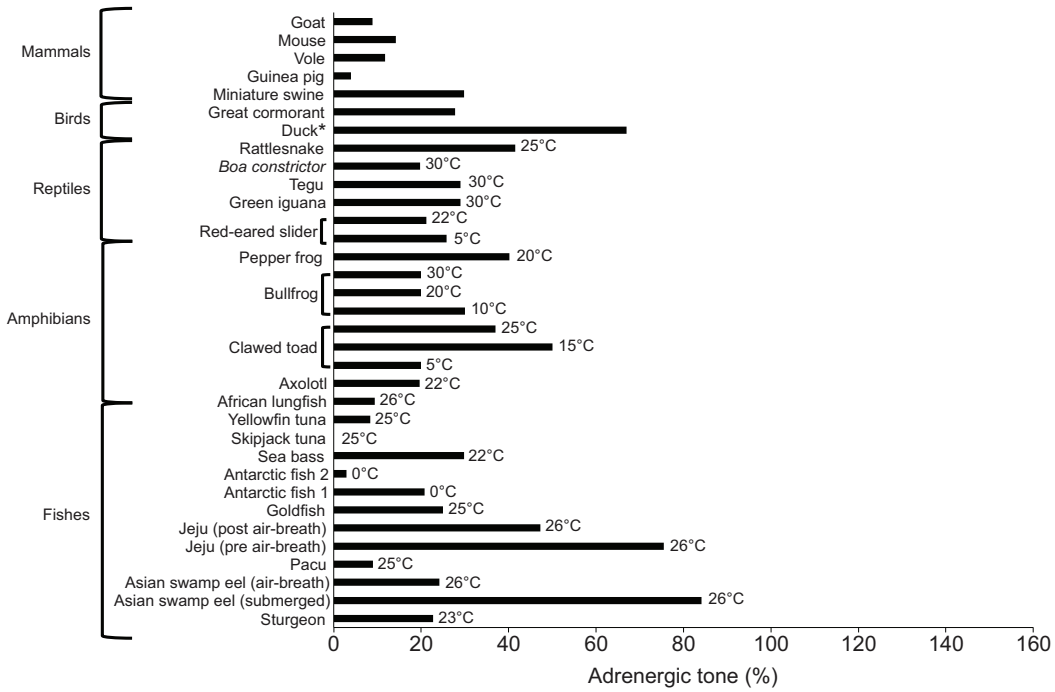

Fig. 1. Adrenergic tone (%) on the heart determined in representative species across different vertebrate groups and at different temperatures. Tones were calculated as detailed elsewhere (Altimiras et al., 1997). Exceptions, denoted by a single asterisk, were calculated as the proportional change in relation to resting values following pharmacological blockade. Fishes: sturgeon, *Acipenser naccarii* (McKenzie et al., 1995); asian swamp eel, *Monopterus albus* (Iversen et al., 2011); pacu, *Piaractus mesopotamicus* (Leite et al., 2009); jeju, *Hoplerythrinus unitaeniatus* (McKenzie et al., 2007); goldfish, *Carassius auratus* (Cameron, 1979); antarctic fish 1, *Pagothenia bernacchii*, and antarctic fish 2, *Pagothenia borchgrevinki* (Axelsson et al., 1992); sea bass, *Dicentrarchus labrax* (Iversen et al., 2010); skipjack tuna, *Katsuwonus pelamis*, and yellowfin tuna, *Thunnus albacores* (Keen et al., 1995); African lungfish, *Protopterus annectens* (Sandblom et al., 2010). Amphibians: axolotl, *Ambystoma mexicanum* (McKenzie and Taylor, 1996); African clawed toad, *Xenopus laevis* (Taylor and Ihmied, 1995); bullfrog, *Lithobates catesbeianus* (Taylor et al., 2012); pepper frog, *Leptodactylus labyrinthicus* (M.R.S., A.S.A. and E.W.T., unpublished). Reptiles: red-eared slider, *Trachemys scripta* (Overgaard et al., 2002); green iguana, *Iguana iguana* (M.R.S., A.S.A. and E.W.T., unpublished); tegu, *Tupinambis merianae* (M.R.S., A.S.A. and E.W.T., unpublished); *Boa constrictor* (Wang et al., 2001a); rattlesnake, *Crotalus durissus terrificus* (Campbell et al., 2006). Birds: duck, *Anas boscas* (Johansen and Reite, 1964); great cormorant, *Phalacrocorax carbo hanedae* (Yamamoto et al., 2009). Mammals: Gottinger miniature swine, *Sus scrofa domestica* (Kuwahara et al., 1999); Hartley guinea pig, *Cavia porcellus* (Akita et al., 2002); vole, *Microtus arvalis* (Ishii et al., 1996); mouse, *Mus musculus* (Ishii et al., 1996); goat, *Capra aegagrus hircus* (Matsui and Sugano, 1989).

The separation of VPNs arises during embryological development as neurones that form the NA migrate ventro-laterally from a more dorso-medial position, possibly the equivalent of the DVN, in the fetal brainstem (Windle, 1933). Although f_H in fetal and newborn sheep was unvarying at around 185 beats min^{-1}, there was a progressive increase in parasympathetic tone, as revealed by injection of the cholinergic antagonist atropine during fetal maturation (Walker et al., 1978). Time–frequency analysis of the beat-to-beat variation in fetal heart rate (FHRV) of human babies revealed that a high frequency component, indicative of neural organization, appeared in the third trimester of pregnancy (David et al., 2007). Similar power spectral analysis of recordings of f_H [as an electrocardiogram (ECG)] and breathing movements detected a clear peak at the ventilation rate in the spectrum for HRV in healthy human neonates, demonstrating that RSA is a major contributor to HRV in healthy term (38–40 week gestation) newborn infants (Thompson et al., 1993). However, in premature human neonates, a high frequency peak at the recorded rate of ventilation, indicative of RSA, was absent at 30 weeks then developed at 33–35 weeks or about 85% gestation (see Taylor et al., 2010). Babies that experienced an asphyxic episode during birth often did not subsequently develop RSA and showed poor outcomes, when assessed as infants by application of standard clinical criteria. In fetal sheep, chronic suppression of FHRV, following induced asphyxia, was a strong predictor of severe brainstem injury (George et al., 2004) (Table 2).

It has been suggested that beat-to-beat modulation of f_H that generates RSA is necessarily restricted to mammals that have the discrete population of CVPNs in the NA that possess fast conducting, myelinated efferent nerve fibres. This is the basis for the ‘polyvagal theory’ propounded by Porges (Porges, 1995). However, the exclusivity of this mechanism has been contested by Grossman and Taylor (Grossman and Taylor, 2007). Many aspects of the brain circuitry of the mammalian system seem to have been highly conserved throughout evolution. Thus the cardio-regulatory mechanisms that operate in the CNS of elasmobranch fishes show a remarkable degree of homology with those that operate in mammals, including humans (Taylor, 1989). This consideration underpins our comparative survey of the other vertebrate groups, considered in turn from fish, through amphibians and reptiles to birds, in relation to the more thorough understanding of the mammalian pattern. The treatment of each group is necessarily uneven because of the limitations on our knowledge and it must be emphasized here that, unlike mammals and birds, the so-called ‘lower vertebrate’ groups have a complex phylogeny; that is to say that fish, amphibian or reptile is an umbrella term describing very diverse groups of animals, some relatively little studied. The review also considers the ontogeny of autonomic control of the heart in each of the vertebrate groups and ends with a summary of the apparent evolutionary changes in their control systems, which attempts to identify areas that merit the attention of comparative physiologists. Such studies

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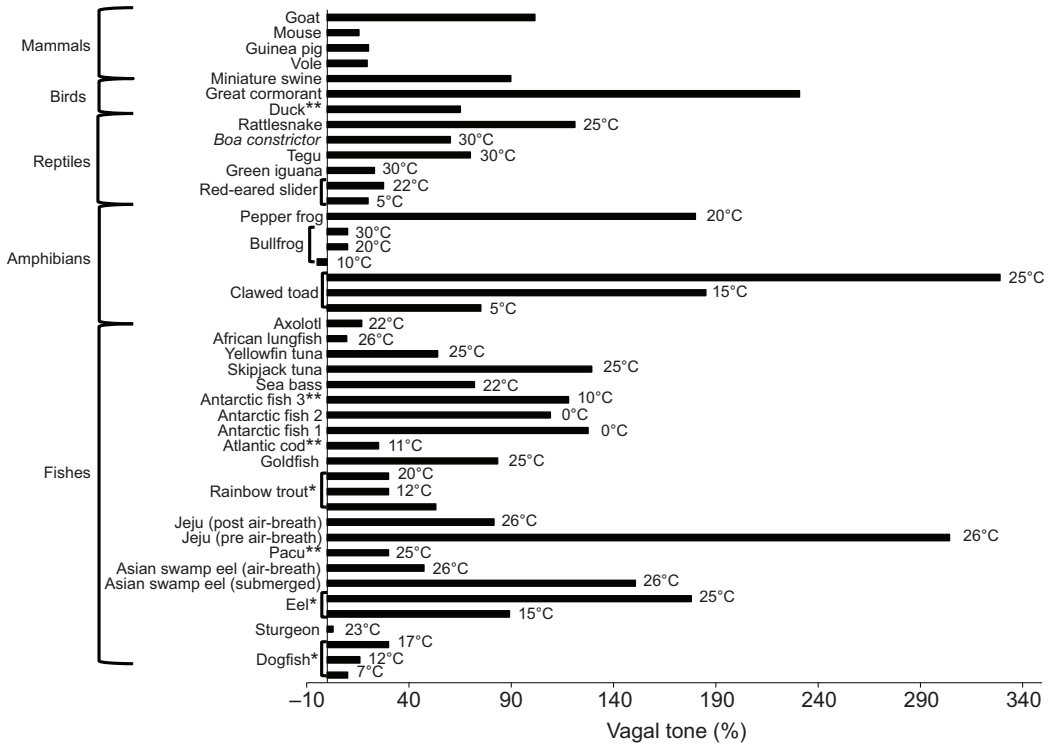

Fig. 2. Vagal tone (%) on the heart determined in representative species across different vertebrate groups and at different temperatures. When possible, tone was recalculated as detailed elsewhere (Altimiras et al., 1997). Exceptions, denoted by a single asterisk, were calculated as proportional change in relation to resting values following pharmacological blockade. Double asterisk denotes proportional changes observed after vagotomy rather than pharmacological blockade. Fishes: dogfish, *Scyliorhinus canicula* (Taylor et al., 1977); sturgeon, *Acipenser naccarii* (McKenzie et al., 1995); eel, *Anguilla anguilla* (Seibert, 1979); Asian swamp eel, *Monopterus albus* (Iversen et al., 2011); pacu, *Piaractus mesopotamicus* (Leite et al., 2009); jeju, *Hoplerythrinus unitaeniatus* (McKenzie et al., 2007); rainbow trout, *Oncorhynchus mykiss* (Wood and Shelton, 1980); goldfish, *Carassius auratus* (Cameron, 1979); Atlantic cod, *Gadus morhua* (McKenzie et al., 2009); Antarctic fish 1, *Pagothenia bernacchii*, and Antarctic fish 2, *Pagothenia borchgrevinki* (Axelsson et al., 1992); Antarctic fish 3, *Paranotothenia angustata* (Campbell and Egginton, 2007); sea bass, *Dicentrarchus labrax* (Iversen et al., 2010); skipjack tuna, *Katsuwonus pelamis*, and yellowfin tuna, *Thunnus albacores* (Keen et al., 1995); African lungfish, *Protopterus annectens* (Sandblom et al., 2010). Amphibians: axolotl, *Ambystoma mexicanum* (McKenzie and Taylor, 1996); African clawed toad, *Xenopus laevis* (Taylor and Ihmied, 1995); bullfrog, *Lithobates catesbeianus* (Taylor et al., 2012); pepper frog, *Leptodactylus labyrinthicus* (M.R.S., A.S.A. and E.W.T., unpublished). Reptiles: red-eared slider, *Trachemys scripta* (Overgaard et al., 2002); green iguana, *Iguana iguana* (M.R.S., A.S.A. and E.W.T., unpublished); tegu, *Tupinambis merianae* (M.R.S., A.S.A. and E.W.T., unpublished); *Boa constrictor* (Wang et al., 2001a); rattlesnake, *Crotalus durissus terrificus* (Campbell et al., 2006). Birds: duck, *Anas boscas* (Johansen and Reite, 1964); great cormorant, *Phalacrocorax carbo hanedae* (Yamamoto et al., 2009). Mammals: Gottinger miniature swine, *Sus scrofa domestica* (Kuwahara et al., 1999); vole, *Microtus arvalis* (Ishii et al., 1996); Hartley guinea pig, *Cavia porcellus* (Akita et al., 2002); mouse, *Mus musculus* (Ishii et al., 1996); goat, *Capra aegagrus hircus* (Matsui and Sugano, 1989).

are not only of great intrinsic interest but also can further illuminate our understanding of mammalian, and therefore human, systems.

Fish

Cyclostomes

The heart of myxinoids is aneural whereas the lamprey receives vagal innervation. In contrast to all other chordates, vagal stimulation in lampetroids accelerates the heart through activation of nicotinic rather than muscarinic receptors (Augustinsson at el., 1956) (reviewed by Taylor, 1992).

Chondrichthyes (cartilaginous fishes – the Elasmobranchs)

Ancestors of contemporary sharks were present during the Silurian period over 300 million years ago and are thought to represent the earliest vertebrates with a well-developed ANS (Young, 1958). Although they have an inhibitory vagal innervation of the heart, the sympathetic nervous system does not extend rostrally into the cephalic and pharyngeal regions so there is no direct sympathetic innervation of the heart or the branchial circulation (Taylor, 1992). This condition is unique amongst vertebrates and may be either an ancestral trait or the result of secondary loss (Young, 1958). Circulating catecholamines have a direct effect on f_H (Short et al., 1977) (see Table 1) and also modulate vagal control of the heart (Agnisola et al., 2003). Despite these adrenergic influences, the most important modulator of f_H in elasmobranchs is the degree of cholinergic vagal tonus on the heart. The dogfish, *Scyliorhinus canicula*, shows a pronounced reflex bradycardia during hypoxia whereas vagal tone is released during hyperoxia, although extreme hyperoxia induces a secondary reflex bradycardia, possibly from stimulation of venous receptors (Barrett and Taylor, 1984; Taylor, 1992). In addition, cholinergic vagal tone, assessed as the proportional change in f_H following atropinization, increases with increasing temperature (Fig. 1). Collectively, these studies show that variations in the overall degree of cholinergic vagal tonus serve as the predominant mode of nervous cardio-regulation in dogfish (Butler and Taylor, 1975; Taylor et al., 1977; Taylor, 1992; Taylor et al., 1999).

The cardiac vagus is also responsible for the generation of cardiorespiratory synchrony (CRS) in dogfish. Unrestrained and normoxic fish exhibited long periods of CRS, which is under vagal control, as it can be abolished by cardiac vagotomy or injection of atropine (Taylor, 1992). Injection of neural tracers to identify VPNs

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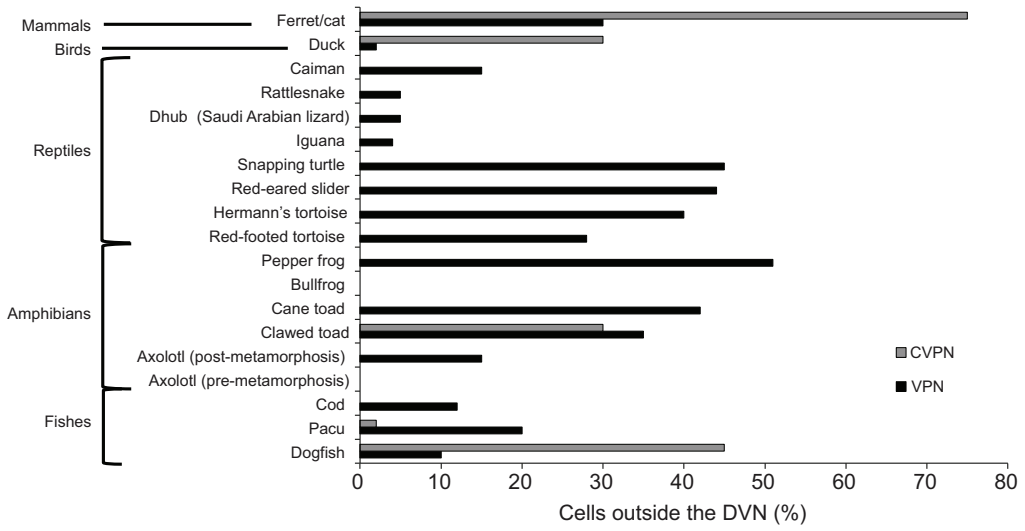

Fig. 3. Distribution of vagal preganglionic neurones (VPNs) and cardiac vagal preganglionic neurones (CVPNs) in representative species. Fishes: dogfish, *Scyliorhinus canicula* (Taylor, 1992); pacu, *Piaractus mesopotamicus* (Leite et al., 2009); Atlantic cod, *Gadus morhua* (Withington-Wray et al., 1986). Amphibians: axolotl, *Ambystoma mexicanum* (Taylor et al., 1999); African clawed toad, *Xenopus laevis* (Wang et al., 1999); cane toad, *Bufo marinus* (Innes et al., 1986) (M.R.S., A.S.A. and E.W.T., unpublished); bullfrog, *Lithobates catesbeianus* (Taylor et al., 2012); pepper frog, *Leptodactylus labyrinthicus* (M.R.S., A.S.A. and E.W.T., unpublished). Reptiles: red-footed tortoise, *Chelonoidis carbonaria* (M.R.S., A.S.A. and E.W.T., unpublished), Hermann's tortoise, *Testudo hermanni* (Cruce and Nieuwenhuys, 1974); red-eared slider, *Trachemys scripta* (M.R.S., A.S.A. and E.W.T., unpublished); snapping turtle, *Chelydra serpentina* (M.R.S., A.S.A. and E.W.T., unpublished); iguana, *Iguana iguana* (M.R.S., A.S.A. and E.W.T., unpublished), dhub, *Uromastyx aegyptius microlepis* (Taylor et al., 2001); rattlesnake, *Crotalus durissus terrificus* (Campbell et al., 2006); caiman, *Caiman latirostris* (M.R.S., A.S.A. and E.W.T., unpublished). Bird: duck, *Anas platyrhynchos* (Taylor et al., 2001); Mammals: ferret, *Mustela putorius furo* and *M. vison* (Ranson et al., 1993); cat, *Felis catus* (Jordan et al., 1986).

in the brainstem of dogfish revealed that about 90% are located in the DVN. However, there is a clearly distinguishable group of cells in scattered ventro-lateral locations outside of DVN that comprise 8% of the total population of VPNs (Fig. 3). These are solely CVPNs, innervating the heart via the branchial cardiac nerve. They constitute about 45% of CVPNs with the rest located in the DVN, where they have an overlapping rostro-caudal distribution with neurones supplying respiratory muscles in the gill arches (Barrett and Taylor, 1985b; Taylor, 1992; Taylor et al., 2009b). Thus, this primitive vertebrate is endowed with two locations for CVPNs supplying the heart via efferent cardiac nerves (Table 1).

Recordings from the central cut end of a branchial cardiac branch of the vagus in decerebrate, paralysed dogfish revealed high levels of spontaneous, centrally generated efferent activity that could be attributed to two types of unit (Taylor and Butler, 1982; Barrett and Taylor, 1985a; Barrett and Taylor, 1985c). Some units fired sporadically and increased their firing rate during hypoxia. Injection of capsaicin into the ventilatory stream of the dogfish, which was accompanied by a marked bradycardia, powerfully stimulated these units (Jones et al., 1995), suggesting they initiate reflex changes in f_H, as well as contributing to the overall level of vagal tone on the heart. Other, typically larger units fired in rhythmical bursts, which were synchronous with ventilatory movements (Taylor and Butler, 1982; Barrett and Taylor, 1985a). These units, showing respiration-related activity which was unaffected by hypoxia, may serve to synchronize heartbeat with ventilation (Taylor, 1992). The cardiac vagal branches contain myelinated fibres (Table 1) with high conduction velocities that resemble mammalian B-fibres (Short et al., 1977; Barrett and Taylor, 1985c; Grossman and Taylor, 2007) (Table 1) so that the respiration-related efferent activity can affect instantaneous f_H. Central recordings from CVPNs, identified by antidromic stimulation of the cardiac vagus, identified this respiration-related activity as originating in CVPNs located in the DVN (Barrett and Taylor, 1985c). Thus, there is clear evidence for the generation of phasic efferent activity in cardiac vagi generated by central interactions between respiratory neurones and CVPNs located together in the DVN (Taylor et al., 2009b; Taylor, 2011). As the bursts are synchronous, the innervation of CVPNs is likely to be excitatory rather than inhibitory as described for mammals and it is equally possible that a direct drive from a central pattern generator operates both on respiratory motor neurones (RMNs) and the CVPNs (Taylor, 1992; Taylor, 2011). Neurones located ventro-laterally outside the DVN were either spontaneously active, firing regularly or sporadically but never rhythmically, or were silent. This activity resembles that described for CVPNs in the DVN of mammals. Thus, the two types of efferent activity recorded from the cardiac nerve in dogfish arise from separate groups of CVPNs, as identified by neuroanatomical studies (Taylor, 1992). This again resembles the situation described in mammals, except that the characteristics of CVPNs within the DVN and those outside the DVN are reversed. Activity recorded from the central cut end of the cardiac vagus or centrally from CVPNs was generated centrally in the decerebrated, paralysed dogfish but the same units fired in response to mechanical stimulation of gill septa (Barrett and Taylor, 1985a; Taylor, 1992). Phasic electrical stimulation of cardiac vagi drove the denervated heart of dogfish at rates both slower and faster than its intrinsic rate (Taylor et al., 2006), identifying a role for the phasic activity recorded from these nerves in determining f_H. Also, central stimulation of a branchial branch of the vagus affected activity in the cardiac vagus (Young et al., 1993). Thus, in the intact fish, normal breathing movements that stimulate peripheral mechanoreceptors on the gills may generate activity in CVPNs and consequently in the cardiac vagi, affecting f_H. This implies that CRS arises from central interactions between CVPNs and RMNs in the DVN and from stimulation of mechanoreceptors, and that the typical reflex bradycardia in response to hypoxia may arise from stimulation of peripheral chemoreceptors plus, via increased ventilation, branchial mechanoreceptors. This is reminiscent of, but

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Table 2. A schematic diagram of the putative development of autonomic influences on heart rate in vertebrates

		ACh receptor	Vagal innervation	Cholinergic tonus	Ventilatory drive	Adrenergic receptor	Sympathetic innervation	Adrenergic tonus
Early stage embryos		✓	–	–	–	✓	–	✓
Late stage embryos		✓	✓	–	–	✓	✓	✓
Embryos close to hatching/birth		✓	✓	✓	✓	✓	✓	✓
Adult vertebrate		✓	✓	✓	✓	✓	✓	✓

Cholinergic and adrenergic receptors appear on the heart early in development but their innervation is delayed. Adrenergic tone is established early but is attributable to circulating catecholamines. In many species an inhibitory cholinergic tonus on the heart appears close to hatching/birth, possibly related to the onset of respiratory drive.

opposite in kind to, the hypoxic response in mammals, where stimulation of lung stretch receptors causes an increase in f_H (De Burgh Daly and Scott, 1962).

Osteichthyes (bony fishes – chiefly the Teleosts)

The teleost heart receives dual autonomic innervation, although the degree of sympathetic control varies considerably (Taylor, 1992; Taylor et al., 1999; Perry et al., 2004) (Table 1). Thus, the degree of excitatory adrenergic tone on the heart of submerged, inactive, normoxic fish varies from 5 to 30% amongst species and with temperature within species, but identifying the neural component of this control is complicated by the role of circulating catecholamines (Fig. 1). Also, cardiac vagal tone in submerged fish varies with values from 2 to 180% depending on oxygen levels, temperature and other intrinsic and extrinsic factors (Fig. 2). The low f_H recorded in Antarctic fishes is attributable to high levels of vagal tonus rather than to temperature per se (Fig. 2). As well as largely determining overall f_H, the vagal efferent supply to the heart also generates HRV. In the sculpin, *Myoxocephalus scorpius*, cardiac vagotomy abolished HRV that included CRI (Campbell et al., 2004). Work on teleosts has stressed the importance of inputs from peripheral receptors in the genesis of CRS. In the trout, hypoxic bradycardia resulted in the development of an exact synchrony between breathing and heart beat (Randall and Smith, 1967). Both the bradycardia and synchrony were abolished by atropine. In addition, Randall and Smith (Randall and Smith, 1967) were able to demonstrate 1:1 synchronization of hypoxic f_H with pulsatile forced ventilation, which was clearly generated by reflex pathways, presumably arising from mechanoreceptors on the gills. In pacu, *Piaractus mesopotamicus*, the cardiac vagus was silent in lightly anaesthetized, normoxic fish. Spontaneous coughs generated a burst of efferent activity in the cardiac vagus and an associated bradycardia. Hypoxia caused an increase in ventilatory amplitude accompanied by respiration-related bursts of activity in the cardiac vagus, which appeared to recruit the heart (Leite et al., 2009). As in the dogfish, peripheral, phasic stimulation of the cardiac vagus or central stimulation of respiratory branches of cranial nerves VII, IX and X entrained the heart over a wide range of frequencies, both below and above the intrinsic rate (Taylor et al., 2009a). Pacu has CVPNs distributed in topologically separate nuclei within the DVN, a ventral group containing about 60% of cell bodies and a dorsal group containing about 40% of cell bodies. In addition, a small number of CVPNs (about 2%) are scattered laterally outside of the DVN. They constitute only 10% of VPNs in this location (Fig. 3). In cod, *Gadus morhua*, 12% of VPNs are located in a ventro-lateral nucleus outside the DVN. Although many of these are CVPNs, others innervate gill arches, possibly supplying the branchial arteries (Taylor, 1992) (Fig. 3).

Thus, we are left with an apparent conflict of evidence on the mode of generation of CRS, which in elasmobranchs may be primarily generated by central interactions in inactive, normoxic or hyperoxic fish when cardiac vagal tone is low; while in teleosts, it appears during hypoxia, perhaps as a result of stimulation of branchial mechanoreceptors during forced ventilation, and is generated reflexly by increased vagal tone. There is, however, some intriguing data from our current studies suggesting that respiration-related activity in the cardiac vagi of both groups of fish is synchronous with activity in the Vth cranial nerve that innervates respiratory muscles in the jaw, suggesting that central interactions may be implicated in the generation of CRS in both teleosts and elasmobranchs (Taylor et al., 2009a). However, a role for different conduction rates between these nerves cannot yet be ruled out.

Even though respiratory gas exchange can be satisfied by diffusion directly over the body surface, a beating heart appears early during development in larval fish (Pelster and Burggren, 1996). Typically, there is a progressive rise in f_H throughout embryogenesis, followed by a decline immediately prior to hatching (Rombough, 1997; Pelster and Bemis, 1991). The role of the ANS in determining these changes has recently been studied. f_H in larval trout, *Oncorhynchus mykiss*, was reduced by a β-adrenergic antagonist early in development and the adrenergic tone increased throughout development as a result of increasing levels of circulating catecholamines. In contrast, cholinergic inhibition of the heart by the addition of acetylcholine (ACh) was ineffective until after hatching (Miller et al., 2011), so that slowing of the heart upon hatching is likely to stem from the appearance of muscarinic cholinoceptors and the establishment of inhibitory nervous control of the heart via the vagus nerve, as speculated by Holeton (Holeton, 1971). If that assumption is correct, establishment of a cholinergic tonus on the heart may correspond to the onset of gill ventilation.

Air-breathing fish

Air breathing has evolved independently on numerous occasions within fishes. Even though the structures used for air breathing differ enormously, all air-breathing fish show a marked rise in f_H after taking an air breath. For example, f_H increases during air breathing in the electric eel and two related species of synbranchid eels that use the buccopharyngeal cavity for gas exchange (Johansen, 1966; Graham et al., 1995; Skals et al., 2006). Manual inflation of the buccopharyngeal cavity elicits changes in f_H that are similar to those occurring during spontaneous air breathing (e.g. Graham et al., 1995). This evidence for stretch receptor feedback inducing the increase in f_H associated with an air breath does not exclude a role for central interactions between respiratory and cardiac motor neurones. Air breathing in the Asian swamp eel, *Monopterus albus*,

caused an instantaneous rise in f_H from 28 to 41 bpm, doubling cardiac output, that was primarily mediated by withdrawal of a cholinergic vagal tonus (Iversen et al., 2011) (Figs 1, 2). In jeju, *Hoplerythrinus unitaeniatus*, a teleost fish that uses a modified swim-bladder as an air-breathing organ (ABO), the frequency of air breathing increased tenfold in deep hypoxia and this was associated with a significant increase in HRV. Each air breath was preceded by a brief bradycardia then followed by a brief tachycardia (McKenzie et al., 2007). These f_H changes were qualitatively similar to those associated with breathing in unimodal air-breathing vertebrates. Within 20 heartbeats of each air breath, a beat-to-beat variability in f_H typical of water-breathing fish (see Campbell et al., 2004) was re-established. Pharmacological blockade revealed that following an air-breath both adrenergic and cholinergic tone fell, with the latter reduced from 300% to 80% (Figs 1, 2). Furthermore, modulation of inhibitory cholinergic tone was responsible for the major proportion of HRV, including the precise beat-to-beat modulation of f_H around each air breath, as described for mammals. The lungfishes (Dipnoi) do not possess sympathetic innervation of the heart (Table 1). Changes in cholinergic tone seem to dominate the efferent regulation of the f_H changes associated with air breathing in both primitive and derived fishes.

At present very little is known about the central control of air breathing in fish. In a facultative air breather, the bowfin, *Amia calva*, VPNs were distributed in the DVN and in ventro-lateral locations outside the DVN. The ABO was supplied by the hypobranchial nerve (i.e. occipital and anterior spinal nerves) plus VPNs in the DVN that may provide efferent axons to smooth muscle in the swimbladder wall, comparable with the vagal efferents controlling reflex broncho-constriction in the mammalian lung (Taylor et al., 1996).

Amphibians – the transition to air breathing

Adult amphibians use a buccal pump to force-ventilate their lungs often in intermittent patterns, where lung inflation is associated with increased f_H and a rise in pulmonary blood flow (reviewed by Wang et al., 1999). These cardiovascular changes are primarily due to a release of vagal tone on the heart and pulmonary artery as cardiac vagotomy or injection of atropine reduces or abolishes the cardiorespiratory coupling. f_H and pulmonary blood flow increased during bouts of fictive breathing in decerebrate, paralysed and through-ventilated toads, indicating central control of cardiorespiratory interactions (Wang et al., 1999; Wang et al., 2004). However, artificial inflation of the lungs in anaesthetized frogs and toads elicited cardiovascular responses similar to those observed in normally breathing animals, which were abolished by deep anaesthesia or injection of atropine. So, stimulation of lung stretch receptors during bouts of breathing may result in the release of vagal tone on the heart and pulmonary artery. Although the mechanisms generating these responses are unknown, the major respiratory muscles in amphibians, as well as their airways, are innervated by cranial nerves, including the vagus, which have their cell bodies in the brainstem where they are in close proximity to CVPNs (Wang et al., 1999).

There is an apparent correlation between the distribution of VPNs and the degree of parasympathetic tonus on the heart of some amphibians. All branches of the vagus nerve including the cardiac branch in *Xenopus* are supplied with efferent axons by neurones with their cell bodies either in a medial nucleus, within the central grey, equivalent to the mammalian DVN, or in a ventro-lateral nucleus, outside the central grey, which may be the amphibian equivalent of the NA and contains about 30% of VPNs (Wang et al., 1999) (Fig. 3). Cardiac and pulmonary vagal motoneurones show a largely overlapping distribution. This amphibian exhibited high levels of inhibitory vagal tone on the heart in normoxia that varied with temperature and was 4–6 times higher than adrenergic tone (Taylor and Ihmied, 1995; Taylor et al., 2012) (Figs 1, 2). In contrast, the bullfrog, which has negligible normoxic vagal tone on the heart and moderate levels of adrenergic tone, has all of its VPNs located in a single nucleus in the medial DVN (Taylor et al., 2012) (Figs 1–3).

The amphibians are a key group for studying the functional changes associated with the development and evolution of air breathing in vertebrates because their transition at metamorphosis from a larval water-breathing form with gills to an air-breathing adult ventilating and perfusing lungs enables the associated cardiorespiratory adaptations to be studied, including the mechanisms for the modulation of f_H. There are detailed descriptions of embryological development in amphibians, extending from the earliest cell divisions to the formation of the CNS and other major organ systems, including development of the cardiovascular system and its control. However, there has been little advancement in our knowledge of the changes in f_H during development in amphibians since previous reviews (Burggren and Doyle, 1986; Burggren, 1995; Burggren and Fritsche, 1997; Fritsche, 1997). The course of changes in f_H during ontogeny is species specific. Patterns include a progressive decline, a progressive increase or an initial increase followed by a decline (Burggren and Fritsche, 1997). Studies of the ontogeny of cardiac control have been conducted in bullfrogs, *Lithobates catesbeianus* (Burggren and Fritsche, 1997), and African clawed toads, *Xenopus laevis* (Jacobsson and Fritsche, 1999). These investigations revealed a dichotomy, with the bullfrog exhibiting cholinergic tone on the heart during larval development while *Xenopus* lacks it (Burggren and Fritsche, 1997; Jacobsson and Fritsche, 1999). However, the utility of the bullfrog as a model for the study of the development of cardiac vagal tone is compromised by the fact that it has negligible resting tone on the adult heart whereas *Xenopus* has high levels of vagal tone that increase with temperature, ameliorating the temperature dependency of f_H (Taylor et al., 2012) (Fig. 2).

Work on the neotenous axolotl, *Ambystoma mexicanum*, has revealed changes in the location of VPNs, possibly including CVPNs, associated with the switch to air breathing at metamorphosis. When axolotls were induced to metamorphose by injection of thyroid hormone, this led to loss of larval gills and voluntary migration onto land (Taylor et al., 1999; Taylor et al., 2001). These overt changes were accompanied by a doubling of the number of VPNs in the DVN, with 15% now located in a novel area in the white matter outside the DVN that could be designated as an amphibian NA (Taylor et al., 2001) (Fig. 3). This change was associated with an increase in apparent variability of f_H (A. Narshi and E.W.T., unpublished). Two specific rhythmogenic sites, which appear to be involved in the generation of endogenous respiratory activity, have been identified within the ventral medullary reticular formation of bullfrogs. One was located between the origins of the VIIth and IXth cranial nerves, and the other at the level of the vagus nerve root (McLean et al., 1995; Wilson et al., 2002). It may be this latter group that is co-located with CVPNs in the putative NA following metamorphosis. Further detailed study of neural control of the heart and in particular the possibility of a separation of roles for CVPNs in dual locations in the brainstem of amphibians that occurs around metamorphosis is clearly merited.

Reptiles

Reptiles ventilate their lungs using an aspiratory thoracic pump and have a rib cage but no diaphragm. The thoracic respiratory muscles

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are innervated by spinal nerves with rhythmicity relayed from the brainstem by descending fibres. Some species reinforce the thoracic pump with a buccal pump that further inflates the lungs and responds to experimental lung inflation (Al-Ghamdi et al., 2001). They typically exhibit intermittent breathing patterns accompanied by very pronounced changes in f_H and cardiac shunt patterns. In some species, such as turtles, f_H remains elevated throughout ventilatory bouts consisting of numerous breaths, whereas f_H also increases during single breaths in other species of reptiles (Burggren, 1975; Wang and Hicks, 1996a; Wang and Hicks, 1996b; Wang et al., 2001b; Campbell et al., 2006). Reptiles show clear examples of cardiorespiratory coupling. In the free diving turtle, *Trachemys scripta*, pulmonary blood flow increased more than threefold at the onset of breathing (Wang and Hicks, 1996a). These cardiovascular changes associated with intermittent lung ventilation in discontinuous breathers were termed CRS but this is not the one-to-one synchrony described in fish. In the turtle, *Pseudemys scripta*, and the tortoise, *Testudo graeca*, the onset of lung ventilation was accompanied by an immediate tachycardia (Burggren, 1975). As stimulation of pulmonary stretch receptors, arterial chemoreceptors and baroreceptors or water receptors was without effect on f_H; it was concluded that this ventilation tachycardia resulted from central interactions between respiratory and cardiac neurones in the medulla. Further experimental evidence indicated that the response was mediated by withdrawal of a cardiac vagal tonus. In the tortoise and in rattlesnakes *Crotalus durissus* the tachycardia during ventilation was abolished by vagotomy (Burggren, 1975; Wang et al., 2001b). Overall, cholinergic tone predominates in determining f_H in reptiles though the prepared but unrestrained green iguana has similar levels of cholinergic and adrenergic tone on the heart (Figs 1, 2).

A series of experiments using power spectral analysis to analyse HRV in the lizard *Gallotia galloti* showed that it included oscillations at low frequency that were abolished by adrenergic antagonists (De Vera and González, 1999). A high frequency peak appeared whenever the lizards exhibited a pronounced regular respiratory pattern (De Vera et al., 2012). Atropine injection decreased the low frequency peaks and abolished the high frequency oscillation (De Vera and Gonzales, 1997). In the South American rattlesnake, *Crotalus durissus*, vagal, parasympathetic tone largely determines resting f_H (Wang et al., 2001b; Campbell et al., 2006) (Figs 1, 2). However, there was clear respiratory modulation of f_H in rattlesnakes, identified in the spectrum of its HRV that was abolished by atropinization (Campbell et al., 2006).

There is presently a paucity of data relating the central integration of patterns of cardiorespiratory interaction in reptiles to their neuranatomy. The somatotopic organization of the vagus in reptiles seems to vary among species though early studies described two divisions (medial and ventro-lateral) of the vagal motor column (Ariens Kappers et al., 1936) (Table 1, Fig. 3). Between 36 and 50% of VPNs were located in the ventro-lateral NA of the turtle *Testudo hermanni* (Cruce and Nieuwenhuys, 1974) while up to 50% of VPNs are reportedly located in the NA of the terrapin *Trionyx sinensis* (Leong et al., 1984) and our current studies on a range of chelonians suggests that this high proportion is characteristic of the group (Fig. 3). In hatchling turtles *Trachemys scripta* we have located two groups of VPN, one medial in the DVN and the other lateral. The cell shapes as well as the separate locations identify them as two separate nuclei. These neuroanatomical observations have been confirmed by electrical stimulation of the brainstem that caused a pronounced bradycardia, as well as vasomotor responses, in spontaneously breathing, anaesthetized pond turtles *Cyclemys flavomarginata*. The cardioinhibitory response depended on the integrity of the vagus nerves and was particularly marked upon stimulation of areas in the caudal medulla corresponding to the NA and DVN (Hsieh et al., 1988).

An exploratory study of the central location of VPNs in a range of reptiles has revealed that in the caiman, *Caiman latirostris*, the majority of VPNs are in the DVN but there is a discrete lateral cell group outside the DVN containing about 12% of VPNs and designated as the NA (Taylor et al., 2010) (Fig. 3). In the agamid lizard, *Uromastyx microlepis*, the majority of VPNs are located in the DVN with a small proportion (2–6%) ventrolaterally located in the NA (Fig. 3). However, there was a clear separation of VPNs in the DVN into two distinct groups with a more lateral group making up about 13% of the whole (Taylor et al., 2001). In the boa, *Boa constrictor*, the majority of VPNs are again concentrated in a discrete DVN rostral of obex with a small number (about 10%) of neurone cell bodies in scattered ventrolateral locations outside the DVN at obex. In the rattlesnake *Crotalus durissus*, 95% of VPNs are in the DVN while ~4% of VPNs are located in scattered ventrolateral locations outside the DVN (Taylor et al., 2001; Campbell et al., 2006) (Fig. 3). Despite the lack of a discrete NA, the rattlesnake showed a clear high frequency component in the power spectrum of HRV that was respiration related, developed at low f_H and was abolished by atropine (Campbell et al., 2006). Whether this relationship is generated centrally or as a result of peripheral reflexes was not determined. Although afferent activity, associated with stimulation of lung stretch receptors, was recorded from the vagus nerve in rattlesnakes (Sundin et al., 2001), this does not rule out a role for central interactions in the generation of respiration-related HRV.

We are currently using fluorescent neural tracers to determine the location of VPNs and pathways of their axons in a range of adult, juvenile and embryonic reptiles in an attempt to reveal the neuroanatomical basis of central integration and its development. In an early embryo of the green iguana, *Iguana iguana*, VPNs were restricted to a discrete DVN close to the fourth ventricle, while in juveniles VPNs were located both in the DVN and in a lateral nucleus, implying that VPNs migrate from the DVN during development. An incomplete plot of the distribution of cells in the brain revealed that the lateral nucleus was restricted to a narrow band in the rostro-caudal extent of the vagal motor nucleus containing about 5% of cell bodies (M.R.S., A.S.A. and E.W.T., unpublished) (Fig. 3). So, in squamate reptiles a relatively sparse distribution of VPNs outside the DVN seems to be the rule (Fig. 3). Specific labelling of CVPNs has remained elusive in reptiles and this will be the focus of future work.

Functional maturation of the reptilian cardiovascular system is poorly understood though the group provides interesting models because of their phylogenetic diversity, with separate lines representing the evolutionary link between the ancestral amphibians, birds and mammals. Changes in f_H during development have been described in representative species from each of the major reptilian clades, excluding Sphenodontia (Fig. 4), but measurements of blood flow have only been conducted in embryonic alligators (Eme et al., 2011a). Embryonic heart mass has been suggested to be an index of stroke volume during reptilian development (Birchard and Reiber, 1996; Crossley and Altimiras, 2000) so that changes in heart mass, in combination with measurement of f_H, may provide an index of the changes in cardiac output accompanying development, enabling study of its control.

The ontogenetic development of regulation of f_H has been investigated primarily in American alligators and common snapping turtles. Two main characteristics have emerged. Embryonic

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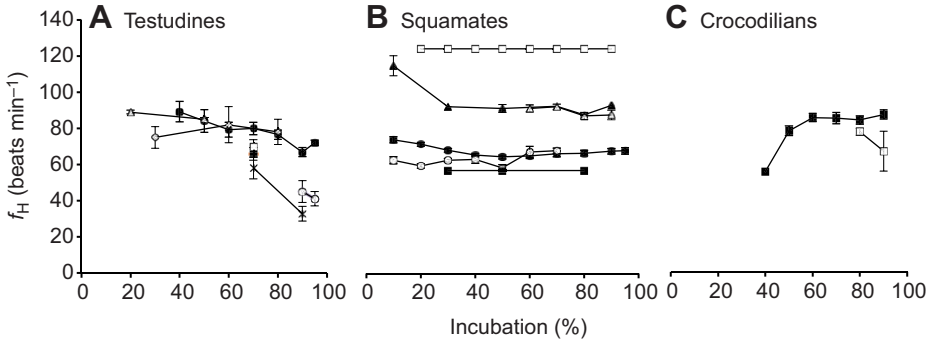

Fig. 4. Heart rate (f_H) at different points of incubation in representative species. (A) Testudine embryos including: *Gopherus agassizi*[a] (filled circles), *Chelonoidis carbonaria*[b] (open circles), *Chelydra serpentina*[c] (crosses), *Trachemys scripta*[d] (filled square), *Trachemys dorbigni*[d] (open square), *Chelodina longicollis*[e] (open diamonds) and *Pelodiscus sinensis*[f] (open triangles). (B) Squamate embryos including *Bassiana duperreyi*[g] (filled squares), *Bassiana duperreyi*[h] (open squares), *Pogona henrylawsoni*[i] (open circles), *Lamprophis fuliginous*[j] (filled circles), *Iguana iguana*[d] (filled triangles) and *Tupinambis merianae*[d] (open triangles). (C) Crocodilian embryos including *Alligator mississippiensis*[k] (filled squares) and *Caiman yacare*[d] (open squares). [a](Crossley and Burggren 2009); [b](Crossley et al., 2013); [c](Eme et al., 2013); [d]M.R.S., unpublished; [e](Spencer, 2012); [f](Zhao et al., 2013); [g](Radder and Shine, 2006); [h](Du et al., 2010); [i](Crossley and Burggren, 2009); [j](Crossley and Burggren, 2009); [k](Crossley and Burggren, 2009).

alligators maintain a constant and pronounced β-adrenergic tone (Fig. 5) throughout the final 40% of incubation that is attributable to circulating catecholamines rather that adrenergic innervation. Cholinergic receptor-mediated regulation is absent until the time of hatching (Eme et al., 2011b) (Fig. 6). Alligator embryos also lack central nervous control of f_H in response to hypoxia and exhibit a limited hypertensive baroreflex response (Crossley et al., 2003a; Crossley and Altimiras, 2005). We have recently obtained similar findings in the Paraguayan caiman (D.A.C., unpublished) (Fig. 5), suggesting this may be a common feature of development in Crocodilians (Figs 5, 6).

Like alligators, embryonic turtles possess a marked β-adrenergic tone on f_H at 70% of incubation that does not derive from sympathetic nervous outflow (Fig. 5). f_H fell slightly before and markedly after hatching in the snapping turtle, *Chelydra serpentina*, indicating the establishment of an effective vagal tonus on the heart, coincident with the onset of lung breathing (Birchard and Reiber, 1996). A number of turtles and tortoises lack cholinergic tone on f_H until the time of hatching although cholinergic tonus was verified during the final 30% of embryonic incubation in snapping turtles (Alvine et al., 2013) (Fig. 6; Table 2).

Data from Squamate embryos are limited. β-Adrenergic blockade markedly reduced f_H in all stages of embryological development in green iguanas (Table 2; Fig. 1). The cholinergic receptors seem to be present in the heart of early iguana embryos (10% of incubation time) as they exhibited a clear dose-dependent reduction in f_H upon topical application of ACh, which could be blocked with atropine (M.R.S., A.S.A. and E.W.T., unpublished). However, injection of atropine alone had no effect on f_H of embryos until they had undergone more than 90% of development, indicating that the onset of a cardiac vagal tonus was delayed until immediately before hatching (Table 2; Fig. 6). A similar result was reported for the

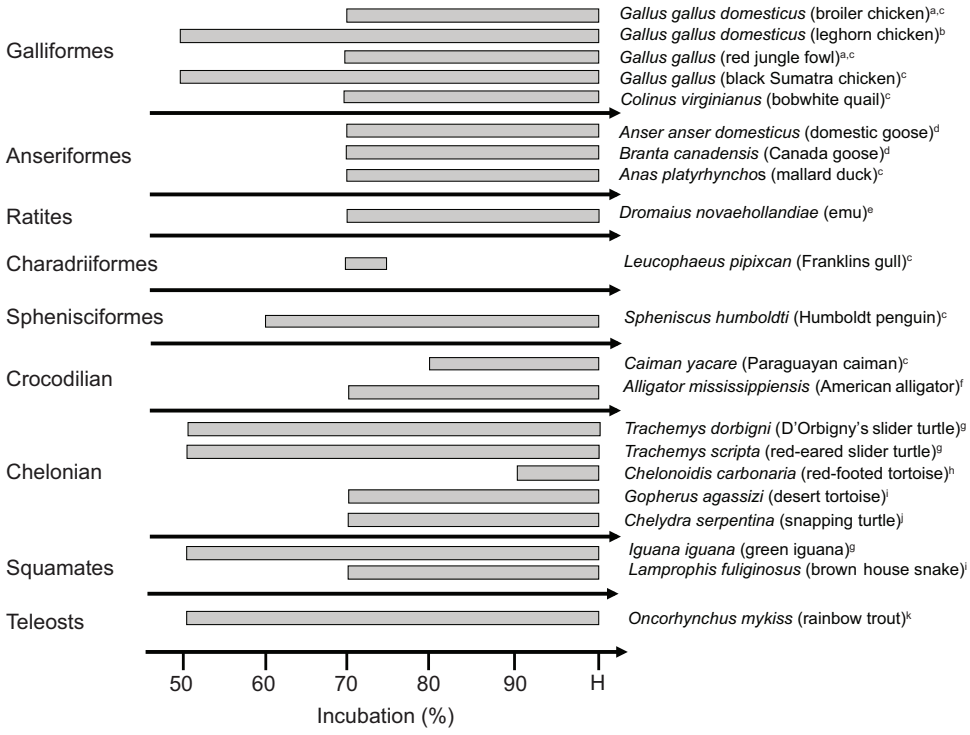

Fig. 5. Timeline indicating the presence of β-adrenergic tone on f_H in avian, reptilian and fish species during the course of embryonic development. Filled bars indicate the presence of β-adrenergic tone. The figure is based on the following: [a](Crossley and Altimiras, 2012); [b](Crossley, 1999); [c]D.A.C., unpublished; [d](Swart et al., 2014); [e](Crossley et al., 2003b); [f](Eme et al., 2011b); [g]M.R.S., unpublished; [h](Crossley et al., 2013); [i](Crossley and Burggren, 2009); [j](Alvine et al., 2013); [k](Miller et al., 2011).

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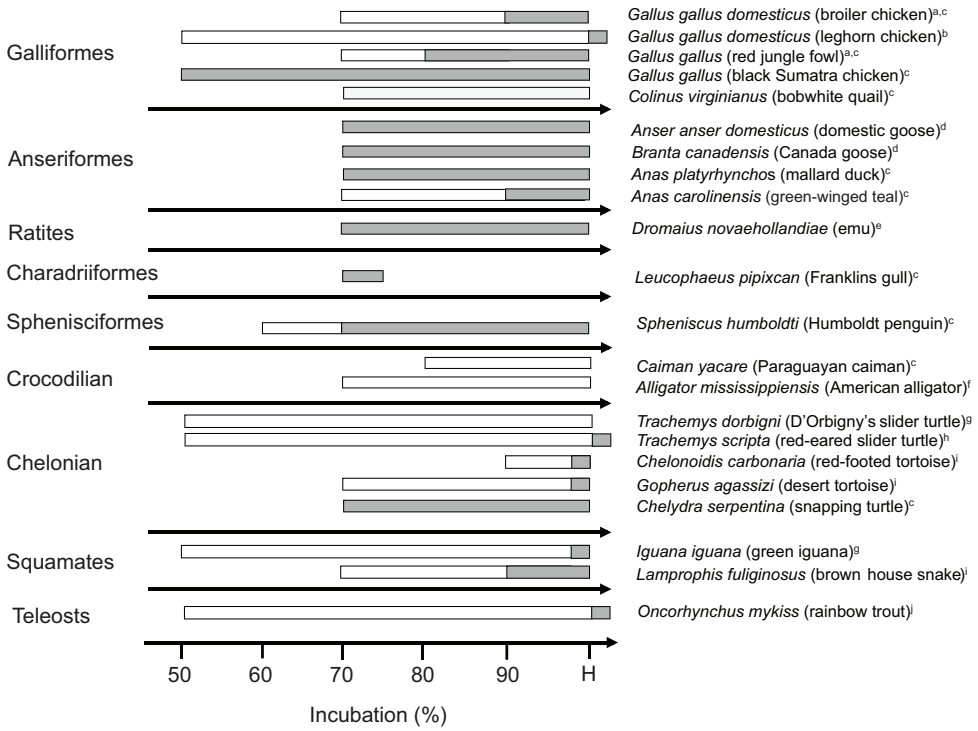

Fig. 6. Timeline indicating the presence of cholinergic tone on f_H in avian, reptilian and fish species during the course of embryonic development. Filled bars indicate the presence of cholinergic tone. The open portion indicates that cholinergic tone was not present. Figure is based on the following: [a](Crossley and Altimiras, 2012); [b](Crossley, 1999); [c]D.A.C., unpublished; [d](Swart et al., 2014); [e](Crossley et al., 2003b); [f](Eme et al., 2011b); [g]M.R.S., unpublished; [h](Crossley et al., 2013); [i](Alvine et al., 2013); [j](Miller et al., 2011).

African brown house snake, *Lamprophis fuliginosus* (Crossley and Burggren, 2009) (Fig. 6). These data imply that the lack of cholinergic control can be attributed to the absence of effective innervation from the CNS of muscarinic receptors on the heart. This may indicate a lack of establishment of appropriate connections within the CNS or peripherally on the heart. It may also relate to a delay in the myelination of efferent fibres supplying the heart, necessary to provide effective conduction velocities (Foster et al., 1982). We suggest that the establishment of a cholinergic cardiac tonus at hatching may be correlated with the onset of central respiratory rhythmicity and active ventilation (Table 2).

Birds

Although birds have dual autonomic innervation of the heart, inhibitory parasympathetic control is often predominant in determining f_H. In the great cormorant, *Phalacrocorax carbo*, f_H increased from 178 to 359 bpm following injection of atropine whereas sympathetic blockade caused a decrease from 178 to 142 bpm (Yamamoto et al., 2009) (Figs 1, 2). HRV has been recorded in several birds. Similar to mammalian HRV, there are low frequency and high frequency components on the power spectrum signal calculated from R–R intervals. Low frequency oscillations appear to be related to thermoregulation and are altered by stress, while the high frequency component relates to ventilation (Khandoker et al., 2004; Cyr et al., 2009; Kjaer and Jørgensen, 2011). The mechanisms generating this latter component in HRV are unknown, and may be affected by the ventilatory mechanics of bird lungs that involve air sacs rather than changes in lung volume (Scheid and Piiper, 1986). In addition, the cardiac chronotropic effects of the relationships between flight and lung ventilation (Boggs, 1997) remain unresolved.

Central control of HRV in birds is relatively poorly studied. Atropine reduced HRV in the cormorant, particularly in the high frequency of the power spectrum that is likely to include RSA (Yamamoto et al., 2009), indicating that it is generated by cholinergic mechanisms. The duck *Aythya fuligula* has less than 5% of VPNs outside the DVN but 30% of CVPNs are in a ventro-lateral nucleus that has been identified as the NA (Blogg et al., 1998; Taylor et al., 2001) (Table 1; Fig. 3). This disproportionate distribution of CVPNs outside the DVN suggests that birds may have evolved a similar functional separation of CVPNs to that described in mammals. Stimulation of water receptors in the upper airways of anaesthetized and force-ventilated ducks, in order to evoke a diving apnoea, induced a bradycardia resulting from abolition of respiration-related oscillations in f_H, suggesting that this bird may show central generation of RSA (Butler and Taylor, 1983; Taylor et al., 2001).

HRV related to respiration is present from hatching in several species (Khandoker et al., 2004). In chicken hatchlings, f_H was substantially more arrhythmic than embryonic f_H, with a high frequency oscillation identified as RSA (Moriya et al., 1999). The onset of RSA was associated with external 'pipping' (the process of breaking through the egg shell during hatching) in chicks (Tazawa et al., 1999). Shah et al. (Shah et al., 2010) reported that both sympathetic and parasympathetic systems exert a tonic influence on f_H in the emu hatchling. Spectral analysis of HRV revealed that the majority of high frequency variability, which constituted an avian RSA, was mediated by the parasympathetic system. Injection of atropine abolished spectral power over the whole frequency range.

Several bird species studied exhibit an excitatory β-adrenergic control of the heart throughout development or for at least the final 30% of development that was observed (Fig. 5). This excitatory tone is derived primarily from humoral control, as a result of high levels of circulating catecholamines in embryonic chickens and possibly other species (Crossley and Altimiras, 2000). Although cardiac muscarinic and adrenergic receptors are found in embryonic chickens during the first quarter of incubation (Barry, 1950), and the mechanisms for production and degradation of ACh and noradrenaline are also present during this time (Zacks, 1954; Ignarro and Shideman, 1968), the inhibitory vagal control of f_H is absent until close to hatching in some species (Table 2; Fig. 6). More extensive cholinergic control has been observed in some chickens but they have been subject to long periods of selective breeding

under artificial conditions (Fig. 6). So, in common with reptiles, bird embryos show a maintained reliance on adrenergic control of the heart throughout development but cholinergic control and in particular the onset of RSA is often delayed until hatching and the onset of air breathing (Table 2).

Conclusions

This review has considered the factors determining autonomic control of f_H in the major groups of vertebrates. There are important factors in the nature of this control that are held in common across the vertebrate groups, many of them summarized in Table 1. Although all but the cyclostomes, the elasmobranchs and the dipnoi possess sympathetic innervation of the heart, its influence on f_H in routinely active or experimentally manipulated animals is proportionally smaller than the influence of the parasympathetic innervation via the vagus nerve. This predominance of parasympathetic inhibitory control extends to its unique role in the instantaneous beat-to-beat control of the heart. We provide examples from all major groups of vertebrates of a role for cholinergic control of the instantaneous changes in f_H that occur in discontinuous breathers when taking their first air breath following a period of apnoea, whether this is at the termination of a respiratory pause or on surfacing after a period of submergence. In addition, the regular fluctuations in the f_H of animals that breathe regularly that can be shown by power spectral analysis to be respiration related have been shown to be controlled by phasic efferent activity in the cardiac vagus in fish and mammals.

The central origins of these fluctuations in vagal tone on the heart, and the consequent variability of f_H in the different vertebrate groups have been a central theme of this review. The central interactions appear to originate partially as a result of the convergence of sensory projections and the specific, and sometimes overlapping, distribution of preganglionic and visceral motor neurones in the brainstem. For example, comparative neuroanatomical studies have revealed that the distribution of VPNs between the DVN and ventro-lateral locations equivalent to the mammalian NA varies between groups. In elasmobranch fish, only 8% of VPNs are in a scattered ventro-lateral location outside the DVN but they are all CVPNs and account for 45% of the total CVPNs, with the rest in the DVN. The ventro-lateral group of cells seem to determine the reflex responses of the heart to external stimuli, such as hypoxia, whereas the CVPNs in the DVN show respiration-related activity that seems to generate CRS due to central, excitatory interactions with RMNs that are also located in the DVN. This topographical and functional separation of CVPNs has also been found in the brainstem of mammals. The majority (70%) of VPNs are found in the DVN of mammals but a high proportion (80%) of CVPNs are found in the NA where they receive inhibitory inputs from the ventral group of respiratory neurones that generate RSA. These topographical separations are fundamental to their control functions. However, there are fundamental functional differences between mammals and elasmobranchs in that the respiration-related activity in CVPNs located in the NA of mammals results from the inhibition exerted by neighbouring respiratory neurones, while in elasmobranchs CVPNs in the DVN show respiration-related activity that is driven by activity in neighbouring respiratory neurones. The resultant respiration-related efferent activity in the cardiac vagi is able to influence instantaneous f_H because the efferent axons are myelinated and have relatively high conduction velocities.

This apparent functional link between primitive elasmobranch fishes and mammals is bridged by an intriguing phylogenetic progression. In some bony fishes 12% of VPNs are located ventrolaterally outside the DVN but only 2% of CVPNs are outside the DVN. Present evidence suggests that bony fishes rely on peripheral inputs from gill chemoreceptors and mechanoreceptors to generate CRS so that it characteristically only occurs during hypoxia. However, there are some intriguing data suggesting that respiration-related activity in the cardiac vagi of fish is synchronous with activity in the Vth cranial nerve and that both may be driven centrally. The possibility that there are central interactions between the motor centres supplying these cranial nerves merits further investigation. The bullfrog has all of its VPNs in the DVN and has negligible vagal tone on the heart. However, this is the exception as the proportion of VPNs outside the DVN rises to 15, 20 and even 30% in different classes of amphibians. Cardiac vagal tone can be as high as 300%. The ventro-lateral relocation of VPNs outside the DVN has been shown to occur at metamorphosis, concurrent with the onset of episodic lung breathing and central chemoreceptor responses. The proportion of VPNs in the NA stabilizes at 30–40% in mammals, but this includes up to 80% of CVPNs. However, this neat progression is confused in the markedly polyphyletic reptiles, with turtles having up to 40% of VPNs in the NA and crocodilians 20%, while squamates (snakes and lizards) have only around 5% of VPNs in this location. Rattlesnakes show CRI that resemble mammalian RSA, despite having negligible numbers of VPNs in scattered locations outside the DVN, and further work on the physiological basis of this relationship is merited. This alternative distribution of VPNs characterizes those near-ancestors of the dinosaurs, the birds, with only 3% of VPNs found in the NA of the duck. Interestingly, though, a large proportion of these VPNs are CVPNs and there is abundant evidence that birds show CRI resembling RSA. We have yet to determine the distribution of CVPNs in our range of reptiles and separate functions have yet to be assigned to the CVPNs found in dual locations in the brainstems of specific amphibians, reptiles and birds. In addition, we need to measure conduction velocities in the cranial nerves of a range of species from these various groups and also examine their ultrastructure to determine whether their axons are myelinated as this is an important feature enabling instantaneous control of the heart.

The ontogeny of autonomic control of the heart is also featured in this review. There is evidence that vertebrate embryos have muscarinic cholinergic and β-adrenergic receptors on the heart at a very early stage of development (10% in iguana). They also show β-adrenergic tone on the heart at a relatively early stage (often earlier than 50%) but this seems dependent on high levels of circulating catecholamines rather than sympathetic innervation. The cardiac receptors are innervated by the ANS later in embryonic development and in many species it is at this stage that cholinergic tone can first be detected by pharmacological blockade (Table 2). As cholinergic tone is often not detectable until close to or immediately after hatching or birth, we hypothesize that this may relate to the onset of respiratory rhythmicity or physical respiratory movements. If this hypothesis proves correct then it ties into the relationship being established between f_H variability and activity in the respiratory system at the onset of an independent existence and in terrestrial species the onset of air breathing. Together, they will generate the cardiorespiratory interactions that may serve to optimize the effectiveness of respiratory gas exchange in the juvenile and adult animal.

Further study of this area seems likely to be of very great interest and of importance to our understanding of the development and evolution of the control systems associated with air breathing. The amphibians should be the primary target for

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these studies because they metamorphose from committed gill breathers to facultative lung breathers, and we already know that this process is accompanied by topographical changes in appropriate regions of the CNS. The oviparous reptiles and birds similarly provide excellent opportunities for work on their embryos and this review has described some of our present work on these groups. Future studies will combine neuroanatomical work, and in particular the study of the central distribution of CVPNs and the number and composition of their efferent axons, with neurophysiological study of the activity in cardiac nerves, their conduction velocities and the origins of any activity in the CNS. These fundamental studies will be extended to the embryos and larvae of chosen species in order to determine the ontogeny of the control systems and in particular their responses during the transition to air breathing at metamorphosis, hatching or birth. The variety of temporal relationships between f_H and respiration and the responses to afferent inputs from peripheral mechanoreceptors and chemoreceptors responsible for reflex respiratory and cardiovascular control will need to be measured. Functional studies will include laboratory-based cardiorespiratory responses of animals to increased metabolic rate engendered by exercise, feeding or temperature change or restriction of oxygen supply by environmental hypoxia or apnoea. Field studies on unrestrained animals will ultimately explore diurnal changes in cardiorespiratory variables and their responses to spontaneous activity such as swimming, running or diving. A combined approach along these lines should uncover new and fascinating insights into this exciting area of study as amphibian and reptilian ontogeny, at least in part, recapitulates their vertebrate phylogeny.

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Competing interests

The authors declare no competing financial interests.

Author contributions

All authors made significant and substantial contributions to the subject matter of this review. It is focused on an overview of data arising from experiments conducted in the laboratories of each of the authors that is discussed in the context of our present understanding of the background to our studies. Much of the unpublished work cited in the review arises from the work of M.R.S. who is a student in the laboratory of A.S.A. and has worked in collaboration with E.W.T., C.A.C.L. and D.A.C. The original manuscript was prepared by E.W.T. and D.A.C. with help in the preparation of figures from M.R.S. Comprehensive revision of the manuscript was conducted by E.W.T. with major contributions from C.A.C.L. and T.W.

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