Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

From tadpole to adult frog locomotion

Keith T. Sillar[1], John Simmers[2] and Denis Combes[2]

Abstract

The transition from larval to adult locomotion in the anuran, *Xenopus laevis*, involves a dramatic switch from axial to appendicular swimming including intermediate stages when the tail and hindlimbs co-exist and contribute to propulsion. Hatchling tadpole swimming is generated by an axial central pattern generator (CPG) which matures rapidly during early larval life. During metamorphosis, the developing limbs are controlled by a *de novo* appendicular CPG driven initially by the axial system before segregating to allow both systems to operate together or independently. Neuromodulation plays important roles throughout, but key modulators switch their effects from early inhibitory influences to facilitating locomotion. Temperature affects the construction and operation of locomotor networks and global changes in environmental temperature place aquatic poikilotherms, like amphibians, at risk. The locomotor control strategy of anurans differs from other amphibian groups such as salamanders, where evolution has acted upon the thyroid hormone pathway to sculpt different developmental outcomes.

Addresses

[1] School of Psychology and Neuroscience, University of St Andrews, St Marys Quad., St Andrews, Fife KY16 9AP, Scotland

[2] Institut des Neurosciences Cognitives et Intégratives d'Aquitaine, CNRS UMR 5287, Université de Bordeaux, 33706 Bordeaux, France

Corresponding author: Sillar, Keith T. (kts1@st-andrews.ac.uk)

Current Opinion in Neurobiology 2023, **82:**102753

Edited by **Dawn Blitz** and **Sten Grillner**

This review comes from a themed issue on **Motor circuits in action 2023**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.conb.2023.102753

Introduction

Swimming in tadpoles, as in fish, requires contractions of axial muscles (myotomes) and neural circuitry to generate left/right tail bending and coordinate a rostrocaudal sequence of contractions to produce forward propulsion. However, whilst still within the egg, amphibian embryos have already developed this neuromuscular machinery [1]. The neural circuitry comprising the swim CPG is thus constructed *in ovo*, partly through an intrinsic programme of gene expression, but with shaping by extrinsic influences. Understanding how this network operates and adapts to environmental and organismal demands during subsequent metamorphosis from tadpole to frog derives mainly from experiments on the clawed frog *Xenopus laevis*, which is the centrepiece of this review. However, the diversity of amphibian developmental programmes, body formats and locomotor strategies also provides fruitful avenues for probing the evolution of vertebrate locomotor control strategies in general, and the transition from the aquatic to terrestrial ecosystems.

Early tadpole locomotor development

Xenopus tadpoles hatch 2–3 days post-fertilization at developmental stage 37/38 [2], by which time well-coordinated swimming occurs in response to stimulation. The tadpole swim CPG is among the most completely described vertebrate locomotor circuits (reviewed in the study by Roberts et al. [3] and Sillar and Li [4]). The core network, which involves descending ipsilateral excitation and reciprocal cross-cord inhibition, is phylogenetically conserved from fish to mammals [5]. The same transcriptional coding system described in fish and mice likely specifies neuron subtypes during *Xenopus* tadpole development. The basic CPG network extends into the hindbrain and comprises ipsilaterally projecting descending interneurons (dINs) [6], interneurons with crossing projections (cINs) [7], ascending ipsilateral interneurons (aINs) [8], homologues of V3 interneurons [9], and myotomal motor neurons (MNs) [10]. The dINs provide the excitatory drive for swimming and appear to be $Chx10^{+ve}$ suggesting they are homologues of V2a interneurons in fish and mammals [11]. Glycinergic cINs mediate cross-cord reciprocal inhibition, like the V0d′s in fish and mice. The dINs are rapidly adapting, firing reliably but only once per swim cycle. cIN inhibition of contralateral dINs at mid-cycle is, therefore, critical to rhythm generation because it removes Na^+ channel inactivation allowing dINs to fire on rebound. If the inhibition is weak and dINs cannot fire, swimming abruptly ceases [12]. Increases in CPG output cycle frequency are controlled by the recruitment of MNs, cINs and aINs. Interestingly, cIN and aIN recruitments have recently been shown to correlate inversely with input resistance [13], the opposite to that predicted by Henneman's size principle [14].

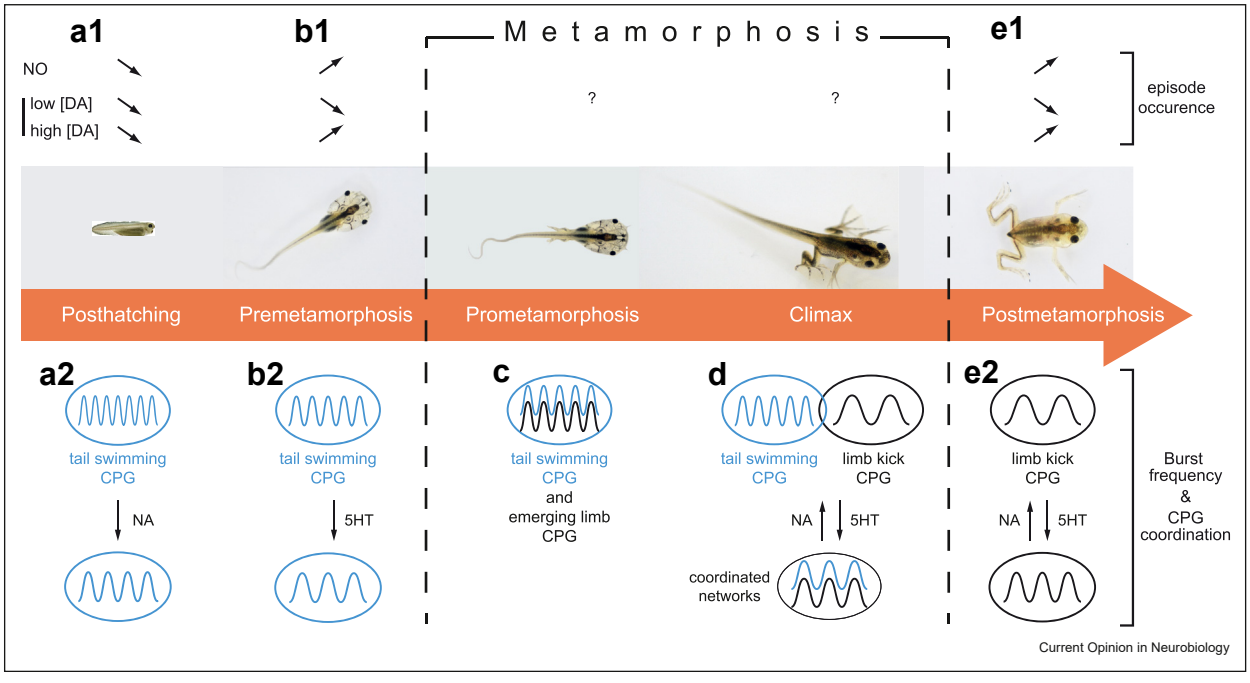
From hatching, the duration and variability of axial MN rhythmic output increases progressively along the spinal cord [15], in correspondence with the growing tadpole's need for greater swimming strength and flexibility. This early transition to more typical vertebrate 'burstiness' reflects changes in the firing properties of spinal MNs [16,17] which can now discharge multiply in each half-cycle. This change is accompanied by a decrease in electrical coupling within individual motor pools, thereby enabling de-synchronized firing [18].

The initial period post-hatching also witnesses changes in the sensory systems that influence locomotor behaviour. Some systems operate as interim structures appropriate for a predominantly sessile lifestyle, including the cement gland (CG) pathway that inhibits swimming [19,20] and head skin mechanoreceptors that induce a concussion-like response [21] to keep the tadpole motionless. Skin mechanosensory Rohon-Beard neurons, which trigger escape if the tadpole is touched [22], will gradually disappear [23] once free swimming occurs, as does the CG pathway. A functional lateral line (LL) system is also present after hatching [24] and remains throughout life. The activation of the LL afferent pathway triggers an abrupt turn and rapid swim away from potential danger, mediated by the recruitment of a sub-population of sensory interneurons located in the hindbrain LL nucleus [25].

Roles of neuromodulation

Neuromodulation plays important roles in tadpole CPG development and the eventual emergence of the appendicular system during metamorphosis [26]. Initially, between hatching and continuous free-swimming, the tadpole is especially vulnerable to predation. Whilst rapid changes are occurring to equip the animal for an independent existence, the *modus operandi* is to remain motionless, presumably as an anti-predatory strategy since many predators respond to movement. In association, various neuromodulatory signalling pathways become established, including dopamine (DA), serotonin (5-HT), noradrenaline (NA) and nitric oxide (NO), which alter particular facets of CPG output (Figure 1). Immediately after hatching, they mainly promote a sessile existence (Figure 1a), but then switch later in development to swim facilitating roles [27]. For example, the effects of D1-like and D2-like DA receptor (R) activation on spontaneous bouts of swimming at pre-metamorphic *Xenopus* tadpole stages are dose-dependent. High affinity D2Rs inhibit swimming, an

Figure 1



Multiple and opposing modulation of *Xenopus* locomotor CPG network output from hatching through metamorphosis. In larvae (**a**), exogenous NO and DA, acting on D2-like receptors, reduce (downward arrows) the occurrence of spontaneous fictive swim episodes (a1), whilst NA decreases swim cycle frequencies (a2). In premetamorphic larvae (**b**), both NO and DA switch modulatory effects to promote (upward arrows) episode occurrences (b1). Whereas low DA still activates inhibitory D2-like receptors, high DA levels activate low-affinity, newly incorporated excitatory D1-like receptors that facilitate swimming activity. 5-HT now decreases cycle frequencies (b2). During prometamorphosis (**c**), limb CPG network output is initially coordinated with the axial CPG rhythm. At metamorphic climax (**d**), co-existing axial and limb CPGs can operate independently at different rhythm frequencies. 5-HT can coordinate the two rhythms, by decelerating and accelerating the axial and limb CPGs, respectively. In contrast, NA, can uncouple already coupled rhythms. In the postmetamorphic frog (**e**), locomotion is solely limb-based. NO and DA exert similar effects on the limb CPG as the tadpole's axial CPG (E1), whilst 5-HT and NA maintain their opposing effects as during metamorphosis (E2). *Figure adapted from* the study by Sillar et al. [26].

effect that is overcome by higher DA concentrations that activate low affinity D1Rs to exert the opposite, excitatory effect (Figure 1a1, b1, e1) [28]. Interestingly, at early stages (37/38 to 42), only D2Rs are expressed in the swim circuit so the effects of DA are inhibitory regardless of dose, consistent with promoting non-motility [29]. NO signalling undergoes a similar developmental switch, inhibiting swimming after hatching (by potentiating inhibitory transmitter release within spinal circuitry [30]), but producing the opposite effect of increasing spontaneous swim episode occurrences once free swimming begins.

In hatchling tadpoles, exogenously applied 5-HT increases axial MN bursts during fictive swimming, in part via presynaptic inhibition of glycine release from cINs [31], while reducing episode durations, but without affecting swim cycle frequency. NA exerts opposite effects in that it strengthens glycinergic transmission presynaptically and reduces swim frequency [29]. Subsequently, the amines' effects mirror, in a stage-specific manner, the normal rostrocaudal emergence of MN bursting that occurs during early larval development. Coincident with, but just preceding, the arrival of descending serotonergic innervation from the brainstem raphe nucleus [32], the burst-inducing effects of exogenous 5-HT extend progressively after hatching from rostral-most only to more caudal cord regions [16]. Evidence supporting a causal link between the ingrowth of brainstem raphespinal projections and swimming rhythm development was provided by neurotoxic ablation of serotonergic neurons during pre-hatching development with 5,7 DHT leading to stage 42 larvae restricted to embryonic-like swimming [32]. These findings, therefore, indicate that 5-HT, as for other neuromodulators (DA [28,29] and NO [30,33]), plays a developmental role in the maturation of the tadpole's spinal CPG circuitry, in addition to exerting acute modulatory influences.

Acute neuromodulation in the tadpole takes place on multiple time scales, from milliseconds to minutes, via actions on diverse targets. These include a sub-type of 'dynamic' Na^+/K^+ pumps that are only recruited following intense firing, leading to a prolonged post-swim hyperpolarization, the ultra-slow AHP. The usAHP is expressed in all CPG neurons, including excitatory dINs, where it is normally masked by a counteracting, hyperpolarization-activated inward current, Ih [34]. The usAHP encodes the intensity and duration of swim bouts in a swim interval-dependent manner and underlies a form of short-term motor memory (STMM) that links past to future locomotor behaviour. This pump-mediated hyperpolarization can be increased or decreased by the endogenous activation of specific modulatory receptors to strengthen (5HT7) or weaken (5HT2a, likely sGC for NO) STMM [27]. Such complexity is not often acknowledged for vertebrate networks but is reminiscent of many invertebrate neural systems [35]. In *Xenopus* tadpoles, the usAHP also undergoes developmental changes that parallel the increase in spontaneous swimming behaviour leading up to metamorphosis, becoming expressed in a higher proportion of spinal CPG neurons [36]. Although seemingly counterintuitive to the emergence of continuous swimming, it is relevant that NO's reduction of the usAHP may contribute to the modulator's switch to overall excitation at free swimming stages.

Acquisition of limb-based locomotion

The fascinating transition from axial to limb-based locomotion during metamorphosis in amphibians is a highly species-specific process. In fully aquatic *Xenopus*, for example, the two locomotor systems appear sequentially during development (Figure 1), necessitating a critical period when they co-exist and must be coordinated simultaneously (Figures 1c,d); reviewed in the study by Combes et al. [37]). As in earlier stages, swimming of the pre-metamorphic tadpole is generated by left/right alternating ventral root bursts with a rostrocaudal phase-delay [38]. During pro-metamorphosis, motor output to each new limb bud consists of synchronous flexor-extensor motor bursts that alternate between the left and right sides, similar to, and in strict coordination with, the axial rhythm (Figure 1c). Later, around the metamorphic climax, the tail and the now more developed limbs both contribute to locomotor propulsion, with the limb CPG activity having switched coordination such that intralimb extensor and flexor bursts now alternate and occur in synchrony across the two sides. Now the two co-existing CPGs are fully functional, but they can operate with different cadences (Figure 1d). The lower frequency limb CPG rhythm can occur either completely independently or accelerate to become transiently coordinated with the faster axial rhythm. Post climax, the tail has disappeared, and the solitary limb rhythm remains to drive left/right synchronized appendicular swimming, akin to human breaststroke (Figure 1e). How is the coupling between the two systems regulated when they co-exist at mid-metamorphosis? Exogenous 5-HT and NA can couple or uncouple the axial and limb CPG outputs, respectively (Figure 1d) [39], but how this is achieved remains a mystery since the coupling mechanism itself is unknown. Presumably, there are connections between secondary limb and primary axial CPG neurons, but the nature of these interactions, whether they are chemical or electrical and how they are modulated at different points along the metamorphosis timeline have yet to be investigated.

Further progress in understanding changes in spinal CPG function during metamorphosis awaits the availability of techniques suitable for cellular and molecular level analyses. At the MN level, investigating the temporal expression of Hox genes that define the specification of motor pools for the limb and axial systems [40]

would be very illuminating. Patch clamp recordings from isolated preparations [36] have allowed intriguing initial insights, for example on the incorporation of functional D1Rs, the persistence of a usAHP and its negative modulation by NO. However, more advanced methods are now needed, such as calcium imaging that should allow real-time mapping of groups of neurons associated with the two CPGs when they are active. Associated neuromodulatory control mechanisms may be more difficult to explore given the additional complexities anticipated as the metamorphic transition proceeds. A conceptual framework is also needed that considers the changing physical distances over which modulation takes place. This is especially relevant to NO signalling whose neuronal sources in the early stages are restricted to the brainstem, in close proximity to rostral swim CPG neurons, but as the tadpole grows, nitrergic neurons appear within the future spinal appendicular CPG centres [41].

Evo-devo of metamorphosis

Amphibian metamorphosis effectively compresses into a few weeks transitional events that spanned ~300 million years in evolutionary time to allow aquatic ancestors to exploit terrestrial habitats. Metamorphosis is driven by increasing plasma concentrations of thyroid hormone (TH) and remarkably, without sufficient TH, or if TH receptors are compromised, metamorphosis is arrested. A key step in metamorphosis is the transport of circulating THs across cell membranes in target tissues, with a key transporter thought to be monocarboxylate transporter 8 (MCT8). However, a recent study found that mutant frogs devoid of MCT8 did not have impaired metamorphosis, suggesting the involvement of additional transport mechanisms [42].

Comparative studies on metamorphosis have shed light on the evolution and development of vertebrate locomotor movement strategies [43] and on how the forces of natural selection have acted on the TH pathway to shape amphibian diversity. In some species, such as salamanders, metamorphosis is incomplete with the two systems co-existing in adulthood; the axial system drives undulatory aquatic swimming while the limbs are used for terrestrial quadrupedal walking [44]. However, in contrast to *Xenopus*, left/right limb alternation is employed in both behaviours, perhaps indicative of a single axial CPG capable of driving the two modes of locomotion. During urodele evolution, changes in the TH pathway underpin the retention of neotenous characteristics. In axolotls, for example, the thyroid gland produces insufficient TH for metamorphosis, but it can be induced in key tissues by exogenous TH [45], so the cellular machinery, including intracellular TH receptors, must be present. Other salamanders like the mudpuppy, *Necturus*, are paedomorphic because although the thyroid gland produces TH, target tissues are unresponsive, even though functional TRs are expressed [46]. This suggests that genes linked to metamorphosis but downstream of TR receptor activation are compromised. Among some anurans, metamorphosis is completed directly *in ovo*, as in the Puerto Rican coqui tree frog which emerges from the egg having never previously generated axial locomotor behaviour [47]. The challenges this animal faces during its early development include the need for extremely large eggs (~3 mm versus ~1 mm in *Xenopus*) to provide sufficient yolk-derived energy for *in ovo* metamorphosis. The emerging froglet is also tiny as a result, easily fitting on a US penny. Similarly, in marsupial frogs, which have the largest of all (up 10 mm) amphibian eggs, the animals develop in and hatch from special brood pouches on their mothers back as froglets or developmentally advanced tadpoles [48].

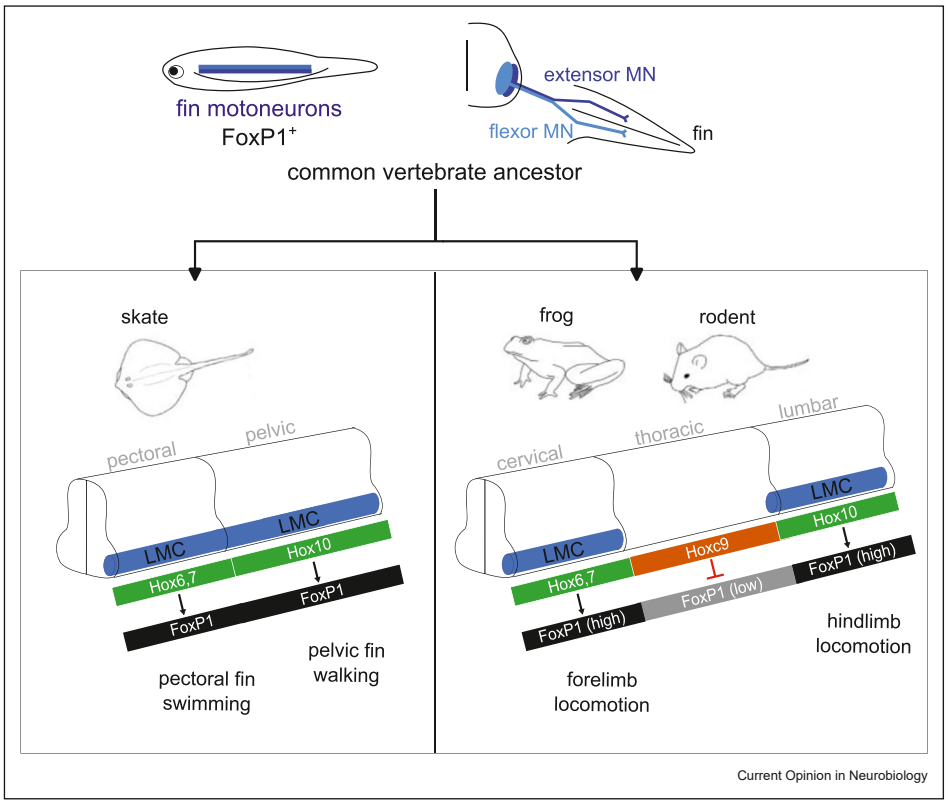
While comparative studies of different amphibian species offer clues regarding the transition from water to land, recent research on fish has also provided fascinating insights into the evolution of the vertebrate limb. The little skate *Leucoraja* uses its pectoral fins to swim in the familiar skate-like fashion but uses its pelvic fins to walk bipedally along the seafloor [49]. Interestingly, the use of molecular approaches to examine transcription factor expression revealed the same Hox genes as used to construct the mammalian hindlimb [50,51], indicating a conservation of gene regulatory machinery across vertebrates with paired lateral appendages (Figure 2).

Environmental impact on tadpole development

As ectotherms, amphibians are exposed to the elements throughout development which, for fully aquatic species, spans the entire larval phase from hatching to metamorphosis. In *Xenopus*, the effects of temperature on metamorphosis are dramatic, taking 12 days at 22 °C but 36 days at 15 °C [52]. Correspondingly, the Q10 effect on the proteins and pathways involved, including the TH system, are slowed and can even arrest metamorphosis with cooler temperatures. In addition, because locomotor activity itself contributes to motor system development, the developmental programmes that generate and adapt movements are also temperature-dependent. Accordingly, if movements are slower in colder conditions, there will be negative consequences for the developmental progression of the animal's behavioural repertoire.

Early tadpole development is especially sensitive to changes in ambient temperature, and even *in ovo*, locomotor circuit construction is profoundly affected. A recent study [53] explored how abiotic stress regulates myotomal MN development by rearing embryos from early blastula stages (stages 7–8) in relatively cool (14.5 °C) or warm (22.5 °C) environments and then examining their locomotor behaviour from hatching up to stage 40. Somewhat counterintuitively, cold-reared

Figure 2

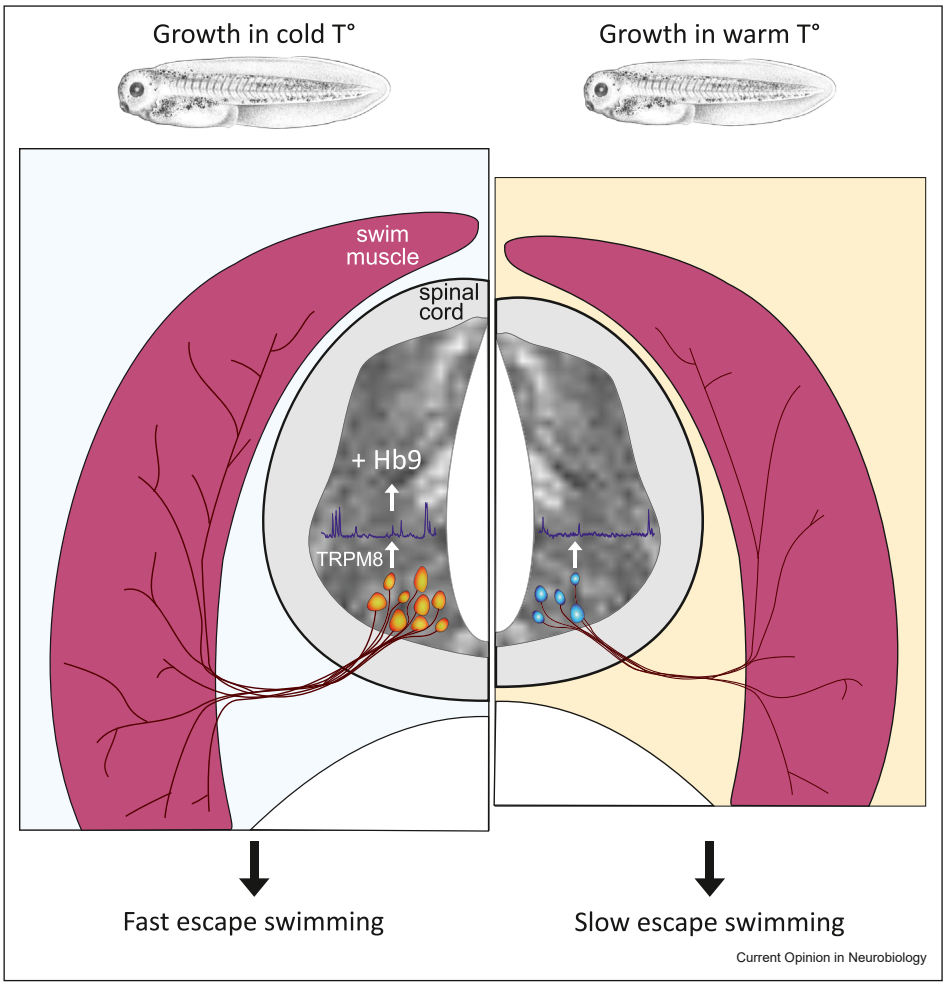


Proposed phylogenetic conservation of motor neuron (MN) subtypes subserving ambulatory movements in vertebrates. In ancestral vertebrates, fin MNs, defined by expression of the transcription factor FoxP1, are distributed longitudinally in the Lateral Motor Column (LMC; top left) and divided into adductor (flexor homologue) and abductor (extensor homologue) motor pools (top right). In the little skate, LMC MNs innervating the adjacent pectoral and pelvic fin muscles also express specific Hox genes and FoxP1 to control swimming and pelvic fin walking. Similarly, in quadrupeds like frogs and mice, fore- and hindlimb locomotor control involves flexor and extensor muscles innervated by LMC MNs in cervical and lumbar cord regions, respectively. In inter-limb (thoracic) regions, FoxP1 expression is repressed by Hoxc9 so that thoracic segments are innervated only by axial MNs. In skates, a natural deletion of the HoxC cluster led to a lack of Hoxc9 along the spinal cord and thus to an inability for LMC Hoxc9-related specification. Together these findings suggest that the gene regulatory system required for appendicular motor control evolved from a common ancestral vertebrate possessing paired appendages. Figure adapted from the studies by Gillis et al. [49] and Jung et al. [50].

larvae displayed more forceful escape swimming responses when stimulated, correlated with an increased number of spinal MNs (Figure 3). Thus, the response to a more challenging environment appears to be the development of a more robust motor control system. Mechanisms underlying this cold hardening response involves activation of a cold receptor, TRPM8, which increases Ca^{2+} waves during development, facilitating expression of the transcription factor Hb9 resulting in increased MN numbers. Whether there are additional effects on the motor circuitry is unknown but seems likely.

The effects of raised temperature on the developing ectothermic brain also have important consequences for locomotor behaviour and hence survival, so aquatic animals like amphibians and fish may be particularly vulnerable to the impact of global warming [54]. In principle, rising water temperatures could have the opposite effect to cold hardening, producing tadpoles with fewer MNs that are less able to escape from predators and transition to adulthood. With respect to metamorphosis, warmer temperatures profoundly affect its duration; *Xenopus* tadpoles exposed to higher temperatures tend to metamorphose quicker (the drying pond effect), but consequently, they are smaller [55]. Whilst this strategy can help to avoid desiccation and death, there are adverse outcomes for an individual's fitness. In *Rana chensinensis*, warm temperatures not only accelerate the time to metamorphosis but they also impair skeletal development and ossification such that froglets have reduced bone length, affecting their jumping capability [56]. Furthermore, the thyroid gland is significantly smaller in warm-reared tadpoles and larger in cold-reared animals. Thus, multiple factors influencing the growth rate and size of the newly metamorphosed froglet can be affected, impacting the animal's survival. A recent study demonstrated in the

Figure 3



Developmental adaptation to cold temperature. In animals grown in cold temperatures (left), cold-sensitive TRPM8 channels are activated and trigger higher frequency Ca^{2+} spikes (blue insert), which increases Hb9-dependent MN differentiation and survival. Consequently, MN numbers are greater than in animals grown in warm temperature (right). This developmental temperature-dependent adaptation allows tadpoles to escape more quickly in cold water than if they had been reared in warm water, increasing their chances of escaping predators. Figure adapted from the study by Spencer et al. [53].

tropical Pacific horned frog *Ceratophrys stolzmanni* that locomotor performance is negatively affected by animals being relatively small after metamorphosis [57]. Jumping distances post-metamorphosis were >50% greater in larger juveniles than smaller ones, an outcome associated with hindlimb length and affecting the ability to evade predators and access food resources. Moreover, even with no risk from predators and ample food available, smaller froglets still showed a higher mortality. The ultimate consequence of rising temperatures is, therefore, a lower survival rate, suggesting that amphibians are especially susceptible to climate change, making them highly relevant indicators of global warming.

Conclusions and future prospects

The *Xenopus* locomotor system has been an influential model in motor control research, providing many insights of general importance in neuroscience. In this organism, the underlying CPG mechanisms can be studied in detail from embryos and hatchling tadpoles through the various stages of metamorphosis. Locomotion can be investigated on a broad range of levels (molecular, cellular and synaptic, neuromodulatory and behavioural), using a wide variety of methods. The studies on how tadpoles are transformed into frogs also afford an important evolutionary perspective, providing clues about the evolution of the vertebrate limb and appendicular locomotion.

On hatching, the stage 37/38 axial swim CPG is understood in remarkable detail, but in contrast, only the surface has been scratched regarding later stages from pre-metamorphosis through the climax to the froglet, and despite some tantalising glimpses, progress has temporarily stalled. We know little about how the limb

control systems are constructed within the framework of a pre-existing axial CPG circuit. The future application of molecular genetics and cellular-resolution population imaging techniques will enable initial observations to mature into a more detailed understanding of the mechanisms underpinning the development of amphibian locomotion, especially during the dramatic process of metamorphosis.

Declaration of competing interest

The authors declare no competing interests that could have appeared to influence the work reported in this paper.

Data availability

This review article does not report unpublished data.

Acknowledgements

Work in the Sillar lab is currently funded by BBSRC grant ref: BB/T015705/1. We thank Lamia Hachoumi for helpful comments on the manuscript.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Kahn JA, Roberts A, Kashin SM: **The neuromuscular basis of swimming movements in embryos of the amphibian Xenopus laevis**. *J Exp Biol* 1982, **99**:175–184.

2. Nieuwkoop P, Faber B. In *Normal tables for Xenopus laevis*. Edited by Daudin, Amsterdam: North Holland Publishing Company; 1956.

3. Roberts A, Li WC, Soffe SR: **How neurons generate behavior in a hatchling amphibian tadpole: an outline**. *Front Behav Neurosci* 2010, **4**:16.

4. Sillar KT, Li W: **Neural control of swimming in hatchling Xenopus frog tadpoles**. In *The neural control of movement*. Edited by Whelan PJ, Sharples SA, Cambridge, MA: Academic Press; 2020:153–174.

5. Kiehn O: **Decoding the organization of spinal circuits that control locomotion**. *Nat Rev Neurosci* 2016, **17**:224–238.

6. Soffe SR, Roberts A, Li WC: **Defining the excitatory neurons that drive the locomotor rhythm in a simple vertebrate: insights into the origin of reticulospinal control**. *J Physiol Lond* 2009, **587**:4829–4844.

7. Dale N: **Reciprocal inhibitory interneurones in the Xenopus embryo spinal cord**. *J Physiol Lond* 1985, **363**:61–70.

8. Li WC, Soffe SR, Roberts A: **Spinal inhibitory neurons that modulate cutaneous sensory pathways during locomotion in a simple vertebrate**. *J Neurosci* 2002, **22**:10924–10934.

9. Li WC, Higashijima S, Parry DM, Roberts A, Soffe SR: **Primitive roles for inhibitory interneurons in developing frog spinal cord**. *J Neurosci* 2004, **24**:5840–5848.

10. Perrins R, Roberts A: **Cholinergic and electrical motoneuron-to-motoneuron synapses contribute to on-cycle excitation during swimming in *Xenopus* embryos**. *J Neurophysiol* 1995, **73**:1005–1012.

11. * Li WC, Soffe SR: **Stimulation of single, possible CHX10 hindbrain neurons turns swimming on and off in young Xenopus tadpoles**. *Front Cell Neurosci* 2019, **13**:47.
Glutamatergic dIN interneurons in the spinal cord and hindbrain are responsible for the maintenance of swimming activity generated by the *Xenopus* tadpole locomotor circuit. They bear many similarities to the V2a interneurons of fish and mice, but evidence for homology was not previously available. In this paper, evidence is presented that suggests the rostral dINs, distinct from other excitatory interneurons in similar positions, are immuno-positive for the transcription factor ChX10.

12. Moult PR, Cottrell GA, Li WC: **Fast silencing reveals a lost role for reciprocal inhibition in locomotion**. *Neuron* 2013, **77**:129–140.

13. * Ferrario A, Saccomanno V, Zhang HY, Borisyuk R, Li WC: **Mechanisms underlying the recruitment of inhibitory interneurons in fictive swimming in developing Xenopus laevis tadpoles**. *J Neurosci* 2023, **43**:1387–1404.
Motor neuron recruitment is important for adjusting the force required for most movements. According to Henneman's size principle, when greater forces are required, larger motor neurons are recruited. This study investigated the recruitment of inhibitory interneurons during fictive swimming in hatchling *Xenopus* tadpoles. In contrast to current dogma, the recruitment of inhibitory interneurons was found to be negatively correlated with their input resistances (a proxy for cell size). This is partly because the amplitudes of the synaptic inputs to inhibitory interneurons scaled inversely with input resistance. Using computer simulations of the swim CPG it was also shown that this inverse relationship is required for the maintenance of the locomotor rhythm.

14. Henneman E: **Relation between size of neurons and their susceptibility to discharge**. *Science* 1957, **126**:1345–1347.

15. Sillar KT, Wedderburn JF, Simmers AJ: **The development of swimming rhythmicity in post-embryonic Xenopus laevis**. *Proc Biol Sci* 1991, **246**:147–153.

16. Sillar KT, Wedderburn JFS, Simmers AJ: **Modulation of swimming rhythmicity by 5-hydroxytriptamine during post-embryonic development in *Xenopus laevis***. *Proc Roy Soc Lond* 1992, **250**:107–114.

17. Zhang HY, Issberner J, Sillar KT: **Development of a spinal locomotor rheostat**. *Proc Natl Acad Sci USA* 2011, **108**:11674–11679.

18. Zhang HY, Li WC, Heitler WJ, Sillar KT: **Electrical coupling synchronises spinal motoneuron activity during swimming in hatchling Xenopus tadpoles**. *J Physiol Lond* 2009, **587**:4455–4466.

19. Boothby KM, Roberts A: **The stopping response of Xenopus laevis embryos: behaviour, development and physiology**. *J Comp Physiol* 1992, **170**:171–180.

20. Perrins R, Walford A, Roberts A: **Sensory activation and role of inhibitory reticulospinal neurons that stop swimming in hatchling frog tadpoles**. *J Neurosci* 2002, **22**:4229–4240.

21. Li WC, Zhu XY, Ritson E: **Mechanosensory stimulation evokes acute concussion-like behavior by activating GIRKs coupled to muscarinic receptors in a simple vertebrate**. *eNeuro* 2017, **4**.

22. Clarke JD, Hayes BP, Hunt SP, Roberts A: **Sensory physiology, anatomy and immunohistochemistry of Rohon-Beard neurones in embryos of *Xenopus laevis***. *J Physiol Lond* 1984, **348**:511–525.

23. Lamborghini JE: **Disappearance of Rohon-Beard neurons from the spinal cord of larval Xenopus laevis**. *J Comp Neurol* 1987, **264**:47–55.

24. Roberts A, Feetham B, Pajak M, Teare T: **Responses of hatchling Xenopus tadpoles to water currents: first function of lateral line receptors without cupulae**. *J Exp Biol* 2009, **212**:914–921.

25. * Saccomanno V, Love H, Sylvester A, Li WC: **The early development and physiology of Xenopus laevis tadpole lateral line system**. *J Neurophysiol* 2021, **126**:1814–1830.
Hatchling *Xenopus laevis* tadpoles are already endowed with a functional lateral line (LL) mechanosensory system that can be activated by water movements, as demonstrated in this article. Activating LL afferents with suction initiates escape swimming behaviour away from the direction of suction, a response that disappears when the LL neuromasts are ablated with neomycin. Fictive versions of responses to similar stimuli were less reliable, however. The paper revealed the nature of the LL afferents with DASPEI staining and also used calcium imaging to locate the sensory interneurons in the hindbrain that are activated by stimulation of LL afferents in the anterior LL nerve.

26. Sillar KT, Combes D, Simmers J: **Neuromodulation in developing motor microcircuits**. *Curr Opin Neurobiol* 2014, **29C**: 73–81.

27. ** Hachoumi L, Rensner R, Richmond C, Picton L, Zhang H, Sillar KT: **Bimodal modulation of short-term motor memory via dynamic sodium pumps in a vertebrate spinal cord**. *Curr Biol* 2022, **32**:1038–1048 e1032.

During swimming in *Xenopus* tadpoles, a subtype of "dynamic" sodium pumps in spinal neurons are only recruited during intense neuronal firing due to their low sensitivity to intracellular sodium concentrations. Dynamic pump activation creates a prolonged post-swim hyperpolarization, the usAHP, that underlies a form of short-term motor memory (STMM) in which swim episodes are weakened if in close temporal proximity to a preceding episode. Both the usAHP and STMM are subject to regulation by endogenous neuromodulators. This article reports positive modulation by serotonin 5-HT7 receptors and negative modulation by nitric oxide signalling and 5-HT2a receptors.

28. Clemens S, Belin-Rauscent A, Simmers J, Combes D: **Opposing modulatory effects of D1- and D2-like receptor activation on a spinal central pattern generator**. *J Neurophysiol* 2012, **107**: 2250–2259.

29. Picton LD, Sillar KT: **Mechanisms underlying the endogenous dopaminergic inhibition of spinal locomotor circuit function in Xenopus tadpoles**. *Sci Rep* 2016, **6**:35749.

30. McLean DL, Sillar KT: **Nitric oxide selectively tunes inhibitory synapses to modulate vertebrate locomotion**. *J Neurosci* 2002, **22**:4175–4184.

31. McDearmid JR, Scrymgeour-Wedderburn JF, Sillar KT: **Aminergic modulation of glycine release in a spinal network controlling swimming in *Xenopus laevis***. *J Physiol Lond* 1997, **503**:111–117.

32. Sillar KT, Woolston AM, Wedderburn JF: **Involvement of brainstem serotonergic interneurons in the development of a vertebrate spinal locomotor circuit**. *Proc Roy Soc Lond* 1995, **259**:65–70.

33. Currie SP, Combes D, Scott NW, Simmers J, Sillar KT: **A behaviorally related developmental switch in nitrergic modulation of locomotor rhythmogenesis in larval Xenopus tadpoles**. *J Neurophysiol* 2016, **115**:1446–1457.

34. ** Picton LD, Sillar KT, Zhang HY: **Control of Xenopus tadpole locomotion via selective expression of Ih in excitatory interneurons**. *Curr Biol* 2018, **24**:3911–3923.

A proportion of hatchling *Xenopus* tadpole swim CPG neurons display a post-swim usAHP, although normally, never excitatory dIN interneurons. dINs are unusual in having a depolarized resting membrane potential (RMP) and low input resistance (IR). They are also the only swim CPG neurons i that express an Ih sag potential when hyperpolarized. The Ih blocker, ZD7288, increases dIN IR and hyperpolarizes their RMPs suggesting that Ih is active at rest and this current explains their atypical properties. Importantly, under ZD7288, dINs now generate a usAHP indicating that the normal absence of this property is not due to an actual lack of dynamic pumps, but instead Ih represses usAHP function during intense activity. It is proposed that this mechanism protects dIN from becoming refractory so that tadpoles can always generate escape swimming, even at very short inter-swim intervals.

35. Williams AH, Hamood AW, Marder E: **Neuromodulation in small networks**. In *Encyclopedia of computational neuroscience*. New York NSNY; 2014:1–15.

36. * Currie SP, Sillar KT: **Developmental changes in spinal neuronal properties, motor network configuration, and neuromodulation at free-swimming stages of Xenopus tadpoles**. *J Neurophysiol* 2018, **119**:786–795.

In this article, a new isolated brainstem/spinal cord preparation is described allowing patch clamp recordings to be made from spinal neurons in pre-metamorphic stages during spontaneous episodes of fictive swimming. These recordings revealed that the usAHP described at early stages not only remains but occurs in a higher percentage of rhythmically active spinal neurons and is reduced or abolished by NO. In contrast to earlier stages, the paper also reports the presence of functional D1-like excitatory dopamine receptors.

37. Combes D, Sillar KT, Simmers J: **Xenopus frog metamorphosis: a model for studying locomotor network development and neuromodulation**. In *Neural control of movement: model systems for examining motor function*. Edited by Whelan P, Academic Press/Elsevier; 2020.

38. Combes D, Merrywest SD, Simmers J, Sillar KT: **Developmental segregation of spinal networks driving axial- and hindlimb-based locomotion in metamorphosing *Xenopus laevis***. *J Physiol Lond* 2004, **559**:17–24.

39. Rauscent A, Einum J, Le Ray D, Simmers J, Combes D: **Opposing aminergic modulation of distinct spinal locomotor circuits and their functional coupling during amphibian metamorphosis**. *J Neurosci* 2009, **29**:1163–1174.

40. Miller A, Dasen JS: **Establishing and maintaining Hox profiles during spinal cord development**. *Semin Cell Dev Biol* 2023, https://doi.org/10.1016/j.semcdb.2023.03.014.

41. Ramanathan S, Combes D, Molinari M, Simmers J, Sillar KT: **Developmental and regional expression of NADPH-diaphorase/nitric oxide synthase in spinal cord neurons correlates with the emergence of limb motor networks in metamorphosing *Xenopus laevis***. *Eur J Neurosci* 2006, **24**: 1907–1922.

42. * Sterner ZR, Jabrah A, Shaidani NI, Horb ME, Dockery R, Paul B, Buchholz DR: **Development and metamorphosis in frogs deficient in the thyroid hormone transporter MCT8**. *Gen Comp Endocrinol* 2023:331.

Movement of thyroid hormone across plasma membranes during amphibian metamorphosis occurs via a highly specific transporter termed MCT8. This study hypothesized that an absence of functional MCT8 would compromise sensitivity to TH and the progression of metamorphosis. However, using CRISPR/Cas9 to mutate the gene encoding MCT8 in *Xenopus laevis*, the authors found that metamorphosis was unaffected. This surprising finding suggests the presence of compensatory transport mechanisms that facilitate the tissue-specific progression of the transition from tadpole to frog.

43. * Iyer AA, Briggman KL: **Amphibian behavioral diversity offers insights into evolutionary neurobiology**. *Curr Op Neurobiol* 2021, **71**:19–28.

This recent review covers the evolution of the brain, behaviour and ecology in different amphibian groups and argues for the extensive use of amphibian models for the comparative study of neural circuit function and evolution, especially concerning vocalization and in motor behaviour with respect to limb control and development.

44. Chevallier S, Jan Ijspeert A, Ryczko D, Nagy F, Cabelguen JM: **Organisation of the spinal central pattern generators for locomotion in the salamander: biology and modelling**. *Brain Res Rev* 2008, **57**:147–161.

45. Crowner A, Khatri S, Blichmann D, Voss SR: **Rediscovering the axolotl as a model for thyroid hormone dependent development**. *Front Endocrinol* 2019, **10**:237.

46. Safi R, Vlaeminck-Guillem V, Duffraisse M, Seugnet I, Plateroti M, Margotat A, Duterque-Coquillaud M, Crespi EJ, Denver RJ, Demeneix B, *et al.*: **Pedomorphosis revisited: thyroid hormone receptors are functional in Necturus maculosus**. *Evol Dev* 2006, **8**:284–292.

47. Westrick SE, Laslo M, Fischer EK: **The big potential of the small frog Eleutherodactylus coqui**. *Elife* 2022:11.

48. Del Pino EM: **From egg to embryo in marsupial frogs**. *Curr Top Dev Biol* 2021, **145**:91–109.

49. * Gillis JA, Bennett S, Criswell KE, Rees J, Sleight VA, Hirschberger C, Calzarette D, Kerr S, Dasen J: **Big insight from the little skate: Leucoraja erinacea as a developmental model system**. *Curr Top Dev Biol* 2022, **147**:595–630.

This article provides a comprehensive introduction to the biology of the little skate, which will be valuable to scientists interested in what this fascinating animal offers to a wide range of fields related to motor system development and developmental biology more generally.

50. ** Jung H, Baek M, D'Elia KP, Boisvert C, Currie PD, Tay BH, Venkatesh B, Brown SM, Heguy A, Schoppik D, *et al.*: **The ancient origins of neural substrates for land walking**. *Cell* 2018, **172**:667–682 e615.

When did the neural circuits for terrestrial locomotion in vertebrates first appear during evolution? This article provides evidence that the answer lies with marine fish, some of which like the little skate, *Leucoraja erinacea*, walk over the seafloor using their pelvic fins by deploying the

essential features of a tetrapod gate with left-right alternation and extension–flexion cycles. Notably, the walking system is established during development by a programme involving conserved Hox transcription factors.

51. * Yoo D, Park J, Lee C, Song I, Lee YH, Yun T, Lee H, Heguy A, Han JY, Dasen JS, *et al.*: **Little skate genome provides insights into genetic programs essential for limb-based locomotion**. *Elife* 2022:11.

The little skate uses its pelvic fins to walk across marine substrates. This species therefore offers a unique opportunity to explore the evolution and development of the vertebrate limb and is rapidly becoming an influential model for understanding the genetic origins of limb circuit during ontogeny. This important paper documents the genome of this species, *Leucoraja erinacea*, focussing on the characterization of genes responsible for the development of motor neurons and making comparisons with other vertebrate species (mouse and chicken) to determine conserved and divergent molecular mechanisms.

52. Dodd MHI, Dodd GS: **The biology of metamorphosis**. In *Physiology of the Amphibia*. Edited by Lofts, Academic Press; 1976:467–599.

53. ** Spencer KA, Belgacem YH, Visina O, Shim S, Genus H, Borodinsky LN: **Growth at cold temperature increases the number of motor neurons to optimize locomotor function**. *Curr Biol* 2019, **29**:1787–1799.

In this paper the authors describe how rearing temperature affects motor system development using *Xenopus laevis* embryos and young larvae as a model. Embryos reared in cold temperature generate stronger escape responses compared to siblings reared in warmer conditions. This is shown to be due to a cold-sensitive receptor, TRPM8, which increases calcium spike frequency and leads to the generation of more motor neurons in cold reared tadpoles.

54. * Beltran I, Herculano-Houzel S, Sinervo B, Whiting MJ: **Are ectotherm brains vulnerable to global warming?** *Trends Ecol Evol* 2021, **36**:691–699.

This wide-ranging article addresses the profound impacts of environmental temperature on the development of the brain in ectothermic animals, in both aquatic and terrestrial environments, from the perspective of global warming. Within limits, warm temperatures facilitate neural circuit development via excitatory actions on neural activity and synaptic transmission, but when these limits are breached, and despite several protective mechanisms being available, the effects become detrimental.

55. Walsh PT, Downie JR, Monaghan P: **Temperature-mediated morphology changes during metamorphic climax in the African clawed frog, Xenopus laevis**. *J Therm Biol* 2008, **33**: 244–249.

56. * Niu Z, Xue H, Jiang Z, Chai L, Wang H: **Effects of temperature on metamorphosis and endochondral ossification in Rana chensinensis tadpoles**. *Comp Biochem Physiol, Part D: Genomics Proteomics* 2023, **45**, 101057.

Bone deposition during amphibian metamorphosis is fundamentally important to animal fitness and survival. In this report the important influence of environmental rearing temperature on this process was investigated in *Rana chensinensis* tadpoles, a Chinese species that is particularly susceptible to high temperatures. It is shown that rearing tadpoles in warm temperatures (26 °C) reduces ossification and causes damage to the thyroid gland. The resulting froglets have shorter hindlimbs and consequently are unable to jump as far as their cold-reared (16 °C) siblings.

57. * Szekely D, Cogalniceanu D, Szekely P, Armijos-Ojeda D, Espinosa-Mogrovejo V, Denoel M: **How to recover from a bad start: size at metamorphosis affects growth and survival in a tropical amphibian**. *BMC Ecol* 2020, **20**:24.

This article investigated the relationship between the size of pacific horned frogs and their survival chances later in life. The data support the notion that larger froglets display a significantly higher survival rate (95%) compared with individuals that are smaller postmetamorphosis. Furthermore, the larger animals could jump further, and their head width was greater, indicating that they were better able to capture prey. Despite these advantages of being larger, small individuals after metamorphosis displayed higher growth rates, decreasing the size gap in adulthood.