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Inside JEB

ALLIGATORS HINT AT WHAT LIFE MAY HAVE BEEN LIKE FOR DINOSAURS

Ruth Elsey

During the last 540 million years, the earth's oxygen levels have fluctuated wildly, probably peaking at 30% about 300 million years ago and bottoming out at 12% 100 million years later. Knowing that the dinosaurs appeared around the time when oxygen levels were at their lowest, Tomasz Owerkowicz, Ruth Elsey and James Hicks wondered how these monsters coped at such low oxygen levels. But without a ready supply of dinosaurs to test their ideas on, Owerkowicz and Hicks turned to a modern relative: the alligator. 'We knew testing the effects of different oxygen levels would work with alligators,' Owerkowicz explains, 'because crocodilians have survived in their basic shape and form for 220 million years. They must be doing something right to have survived the oxygen fluctuations.' Choosing to start at the beginning of alligator development, the trio decided to try incubating alligator eggs at different oxygen levels, to find out how the youngsters grew and developed (p. 1237).

Receiving newly laid alligator eggs from Elsey at the Rockefeller Wildlife Refuge, Owerkowicz divided the eggs into groups incubated at 12% oxygen (hypoxia), 21% oxygen (normoxia) and 30% oxygen (hyperoxia), and waited to see what would happen. After almost 10 weeks of waiting, the eggs began hatching and Owerkowicz could see that there were no obvious differences between the normoxic and hyperoxic alligators.

But he was in for a shock when the hypoxic hatchlings began to emerge. The tiny alligators' bellies were enormously swollen. They had failed to absorb all of the egg yolk food supply, leaving them with huge yolk-distended bellies. In some cases the bellies were so big that the animals' legs could not reach the ground, and the alligators had to sit around until they had burned off the yolk and could begin moving. Owerkowicz suspects that there was not enough oxygen for the developing embryos to consume the yolk. The hypoxic youngsters' organs were much smaller too, all except the heart, which was relatively large; presumably to maximise use of the youngsters' limited oxygen supplies. Owerkowicz admits that he had thought that the hypoxic newborns' lungs would also be enlarged, to compensate for the low oxygen levels, but they were not, probably because the incubating youngsters do not use their lungs and instead obtain their oxygen supply from blood vessels in the egg's membrane.

Next Owerkowicz was curious to see how the alligators performed after 3 months in their respective atmospheres. Checking the reptiles' breathing and metabolic rates, it was clear that the animals in the hyperoxic atmosphere were breathing much less than the normoxic and hypoxic animals, probably because they breathe in more oxygen per lungful, translating into a significant energy saving, which the reptiles could invest in growth. And when Owerkowicz checked the size of the 3 month old hypoxic youngsters' lungs, he could see that they had caught up with his expectations and were larger than those of the normoxic alligators. The alligators' lungs were enlarged to compensate for the low oxygen supply, allowing the alligators to increase their metabolic rates, but not as much as the normoxic or hyperoxic alligators.

Owerkowicz admits that although his results can't tell us what life was like for his alligators' prehistoric predecessors, it is clear that 'their growth and metabolic patterns would have been significantly different,' he says.

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Owerkowicz, T., Elsey, R. M. and Hicks, J. W. (2009). Atmospheric oxygen level affects growth trajectory, cardiopulmonary allometry and metabolic rate in the American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **212**, 1237-1247.

HOW REPTILES DECIDE HOW LONG TO SPEND IN THE EGG

When a mother bird lays her eggs, she settles down on top to keep them warm; but not reptile mums. They usually abandon their eggs after burying them in a nest. 'We know from experimental work that embryos are exquisitely sensitive to external conditions in the nest,' explains Rick Shine from the University of Sydney; the incubation length can vary enormously depending on the temperature. But it wasn't clear exactly how temperature affects developing embryos. 'We couldn't measure what was happening inside the egg, we had to treat it as a "black box",' explains Shine. But all that changed when Shine's postdoc, Rajkumar (Raju) Radder, stumbled across a piece of equipment called 'Buddy' on the internet. Buddy measures the tiny heartbeat of developing chicks in the egg. Shine and Radder realised that it may also work on reptile eggs, and if it did, they could begin to find out more about what was going on inside (p. 1302).

Wondering whether the amount of work that an embryo does in the egg determines incubation duration, Shine, Radder and Wei-Guo Du collected freshly laid three-lined skink eggs from fields near Canberra, and took them back to the lab where they could measure the youngsters' heart rates during incubation at temperatures ranging from 20 to 35°C. The skinks took anything from 30 days to hatch at 30°C, up to 60 days at a chilly 22°C, so temperature certainly affected the duration, but how many heartbeats did the babies take? Measuring the embryos' heart rates, Radder found that they varied from 60 beats min^{-1} at 20°C up to over 120 beats min^{-1} at 33°C. The warmer lizards' hearts were racing and only took 5×10^6 heartbeats before hatching, while the cold lizards' hearts beat more slowly, and they took 6×10^6 heartbeats over their 2 month gestation.

But how would other reptile embryos fare? Travelling to Zhejiang, eastern China, with the Buddy, Du repeated the experiments with Bo Sun, from Hangzhou Normal University, on another lizard species, *Takydromus septentrionalis*, and a turtle, *Pelodiscus sinensis*. Again they found that the coolest lizards used more heartbeats to develop fully, but the turtle seemed to take many more: 6.72×10^6 heartbeats compared with the lizard's 4.79×10^6 heartbeats at 30°C.

Shine suspects that one of the reasons for the difference is that lizard mums allow their young to begin developing *in utero* before laying their eggs, while turtle mums lay their eggs directly after fertilisation. So lizard embryos are already partially developed before their eggs are laid. When the team took this into account the heartbeat numbers began to look more similar.

Shine and Du also knew that the embryos allocate some of their energy to simply surviving, so they wondered how many heartbeats were allotted by the reptiles to growth and development? Shine and Du subtracted the number of heartbeats required for survival from the total number of heartbeats and found that the numbers were also similar, with the Australian and Chinese lizards using 4.04×10^6 and 3.98×10^6 heartbeats to grow, while the turtle used 5.56×10^6.

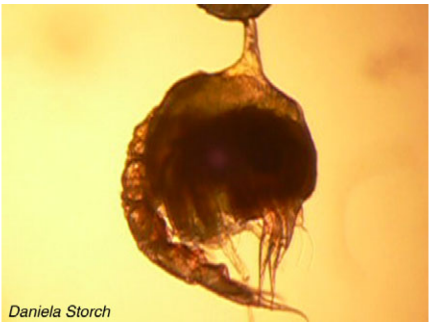
So although the nest's temperature has a dramatic effect on the number of heartbeats that a developing embryo takes, with colder embryos putting more energy and heartbeats into staying alive than warmer embryos, it seems that lizards and turtles use a similar number of heartbeats to grow and develop, and only hatch once each of those heartbeats has been taken.

Tragically, Raju Radder did not live to see this project's completion. He died unexpectedly in May 2008 at the age of 34 from a massive heart attack while home for his sister's wedding in India. 'Raju was a superbly gifted young biologist; he was intelligent, creative, hard working and a joy to collaborate with,' remembers Shine. He adds, 'It is truly bittersweet to see papers coming out with Raju's name on the list of authors.'

10.1242/jeb.032037

Du, W.-G., Radder, R. S., Sun, B. and Shine, R. (2009). Determinants of incubation period: do reptilian embryos hatch after a fixed total number of heart beats? *J. Exp. Biol.* **212**, 1302-1306.

HEART RATE INDICATES LARVAL THERMAL TOLERANCE



Daniela Storch

While global warming is becoming an uncomfortable reality for many terrestrial species, it also poses a significant threat to marine creatures. Daniela Storch from the Alfred Wegener Institute in Germany explains that many studies have aimed at understanding how aquatic adults cope in hot water, but very little is known about the ways that their larvae deal with temperature fluctuations. Knowing that surviving the larval stage is critical for any population, Storch and her colleagues Hans-Otto Pörtner and Miriam Fernández decided to find out how kelp crab first stage larvae manage as waters cool and warm (p. 1371).

Travelling to Fernandez's lab at the Pontificia Universidad Católica de Chile, Storch collected female crabs with eggs from a warm northern site on the central Chilean coast and a cooler site at Melinka in the south of the country. Back in the lab, Storch waited for each female's eggs to hatch before tethering individual microscopic larvae to glass rods in small chambers midway through their first larval stage and recording their behaviour.

Storch first cooled the larvae's water from 11°C to 7°C and then further down to 3°C. Allowing the larvae to adjust at each temperature for an hour, she filmed them through a microscope and also measured their oxygen consumption to see how their activity and metabolism changed with the cold. Having cooled the larvae, Storch tethered others and repeated the process while she slowly warmed their water from 11°C to 27°C in 4°C steps.

After completing the experiments, Storch, Jessica Barria and Pedro Santelices were able to correlate the larvae's oxygen consumption with their temperature and activity, to find out how the temperature changes affected the larvae. They found that as they cooled the northern and southern larvae, both populations began swimming enthusiastically until it became too cold, when they became inactive. Storch admits that this was a surprise, as cooling the larvae should have slowed them down, but suspects that the animals were trying to swim away from the uncomfortable water and find more pleasant conditions. However, when she warmed the two populations, the southern population began struggling and were unable to swim at higher temperatures where the northern population were quite content. The warm adapted northern larvae coped better with the warm water than the cold adapted southern population.

The temperature changes also affected the larvae's metabolism, with their oxygen consumption rising, although the cold adapted southern population's metabolic rate was higher than that of the warmer northern population, reflecting their adaptation to the cold climate. And when Storch monitored the larvae's cardiac activity, she saw that the crustaceans' heart rates increased as the temperature rose, but levelled off at the highest and lowest temperatures.

Summarising the larvae's reactions to the changing water temperatures, Storch says that the larvae's activity decreases first as the temperature changes, then their oxygen consumption levels off before finally the heart fails. 'Swimming activity is a good indicator of thermal tolerance,' says Storch. She concludes that both populations seem well adapted to their own locations' thermal windows, but they could be forced to find new locations if temperatures continue to rise.

10.1242/jeb.032045

Storch, D., Santelices, P., Barria, J., Cabeza, K., Pörtner, H.-O. and Fernández, M. (2009). Thermal tolerance of crustacean larvae (zoea I) in two different populations of the kelp crab *Taliepus dentatus* (Milne-Edwards). *J. Exp. Biol.* **212**, 1371-1376.

DIET CHANGE INFLUENCES HATCHLINGS' DIGESTION

Neil Smith is an illustrator living in Salisbury

It's in every newborn's job description: eat lots and grow fast. In their early days, freshly hatched house sparrows dine on a high protein diet of insects before their parents begin delivering starchy seeds to their voracious offspring. Paweł Brzęk and his colleagues from the University of Wisconsin and Universidad Nacional de San Luis wondered how the youngsters' digestive tracts respond to the drastic diet change while working full-out to keep pace with the youngsters' growth (p. 1284).

Feeding young sparrows on either a high-protein starch-free diet or a diet supplemented with starch, the team tracked the youngsters' growth, digestive tract enzyme levels and their ability to maintain their own body temperature until the birds were close to fledging.

The team found that although the birds on a starch-free diet were slightly smaller and took a little longer to maintain their own temperature, removing starch from the diet didn't seem to compromise the youngsters' growth. And when they checked the birds' intestines, the team also found that the levels of a carbohydrate-digesting enzyme, maltase, doubled when the birds were on a starchy diet; their intestines were definitely responding. Brzęk and his colleagues suspect that the hatchlings' maltase increase is 'important for maintaining digestive efficiency and rate at the whole animal level,' allowing the youngster to process the enormous amounts of starch they consume. They believe that although some of the changes in the youngsters' intestines are 'hard wired' by a genetic programme, others are governed by the diet change itself.

10.1242/jeb.032060

Brzęk, P., Kohl, K., Caviedes-Vidal, E. and Karasov, W. H. (2009). Developmental adjustments of house sparrow (*Passer domesticus*) nestlings to diet composition. *J. Exp. Biol.* **212**, 1284-1293.

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