INSIDE JEB

Pipefish fathers do not boost embryos' oxygen

Male pipefish. Photo credit: Anders Bergland.

Many aquatic species have a reputation for negligent parenting. Having cast their gametes to the currents, they abandon their offspring to their fate. However, hands-on parenting is taken to a whole new dimension in the Syngnathidae fish family. Instead of leaving the responsibility to the females, seahorse and pipefish males take the pledge to care for their young even before the eggs are fertilized. The females depart soon after placing their eggs directly into the male's brood pouch, leaving the soon-to-be fathers to incubate the developing embryos. Ines Braga Goncalves from the University of Zurich, Switzerland, explains that the pregnant males support their offspring by removing the youngsters' waste and supplementing their nutrition, but it wasn't clear whether the males also provided the youngsters with an ample oxygen supply. Braga Goncalves explains that the lower availability of oxygen in water was believed to naturally limit the size of fish eggs and, as pipefish eggs are relatively large for the fish's size, it was assumed that the males somehow provided an abundant supply of oxygen, but no one had checked.

Intrigued, Braga Goncalves and her colleagues Ingrid Ahnesjö from Uppsala University, Sweden, and Charlotta Kvarnemo from the University of Gothenburg, Sweden, went trawling for broadnosed pipefish in a fjord just off the quay of the Sven Loven Centre for Marine Sciences at the start of the mating season. Keeping the pipefish in large storage tanks with ribbons to simulate seaweed, Braga Goncalves recalls that the females were happy to mate with the males in captivity. Next she transferred half of the pregnant males to aquaria supplied with well-oxygenated water (100% O_2 saturation) and the other males to tanks that had low oxygen levels (40% O_2 saturation) before beginning the tricky process of measuring how much oxygen was available to the precious broods tucked safely away inside their fathers' pouches.

Gently securing the wriggling fish in a silicon sleeve and inserting a thin electrode, which precisely measured oxygen concentration, into the brood pouch of each male every 6 days during the first 24 days of the pregnancy, Braga Goncalves was surprised to see that even in the fully saturated water, the oxygen saturation in the fluid bathing the incubating embryos was relatively low and only averaged 51%. And the situation was even worse for the youngsters whose fathers were in the poorly oxygenated water: the oxygen saturation only averaged 32%. Tracking brood pouch oxygenation over the first half of the pregnancy, Braga Goncalves was also concerned to see that the oxygen saturation fell by almost 50% in the well-oxygenated fish, reaching 40%; although it plummeted below 30% in the oxygen-deprived fish. The embryos didn't seem to be too badly affected by their breathless start – they were smaller and shorter, but survived as well as the better oxygenated embryos – however, the out-of-breath fathers suffered for their young, losing weight and body condition.

So, contrary to popular belief, male pipefish are unable to boost the oxygen supply that they provide to their precious cargo and the mystery of why pipefish eggs are so large remains. However, Braga Goncalves stresses that the fathers still give their young a fantastic start in life, protecting them from predators when they are at their most vulnerable and providing them with many of life's essentials.

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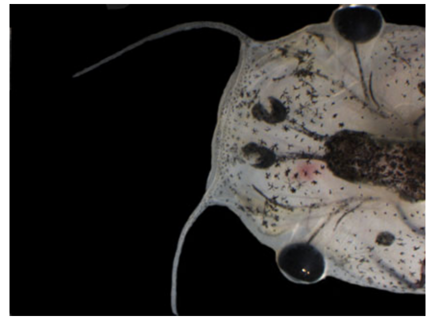
Braga Goncalves, I., Ahnesjö, I. and Kvarnemo, C. (2015). Embryo oxygenation in pipefish brood pouches: novel insights. *J. Exp. Biol.* **218**, 1639-1646.

Kathryn Knight

Corollary signals coordinate swinging tentacles with swimming



Xenopus laevis tadpole. Photo credit: Boris Chagnaud.

Keeping track of the sensory signals generated by the world around us is challenging enough without having to disentangle the nerve signals generated by our own movements. But this is exactly what we would have to do if the neurons that control our every move didn't send simultaneous signals to our sensory systems, telling them to disregard confounding information generated by our own bodies. In addition, these so-called 'corollary discharges' may also contribute to other aspects of behaviour, such as providing stabilisation when senses may be overwhelmed by other inputs. However, unscrambling the impact of corollary signals on movements in the intricate nervous systems of multi-limbed animals is too complex, so Hans Straka and Boris Chagnaud from the Ludwig-Maximilians-University Munich, Germany, decided to focus on a much simpler animal: African clawed frog tadpoles. The nerve signals that control the tail beats of tadpoles and their associated corollary discharges are much simpler to interpret. Reasoning that a pair of touch-sensitive tentacles on the jaws of the tadpoles could be over-stimulated as the tiny swimmers wriggle through the water, Chagnaud, Straka, Sara Hänzi and Roberto Banchi began to investigate how corollary signals from spinal locomotor centres that control swimming might protect the tentacles from sensory overload.

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Recording electrical signals from the nerves that indicated when the tadpole was swimming, the team saw that when the corollary signals were generated, the touch-sensitive tentacles swung back; and as soon as the animal stopped swimming, the tentacles moved forward again. Next, the team identified which nerve controlled the tentacles' movements by painstakingly disconnecting different portions of the muscle that pulled the tentacle back, until they confirmed that the mandibular branch of the trigeminal nerve, which controls the tadpole's jaw movements, controlled the swinging movement. Then, the team tested how many of the motoneurons in the trigeminal nerve triggered the tentacles' movements by measuring changes in calcium ion concentration in the cell bodies of trigeminal motoneurons as the tadpole swam, and they found that the calcium signals harmonised with the animal's swimming activity. Finally, the team compared the pattern of nerve signals generated in the spinal cord of the swimming tadpole with the nerve signals in the tentacle and found that the nerve discharge in the spinal ventral roots associated with swimming closely matched the discharge in the section of the trigeminal nerve that innervated the tentacle. And when the tadpoles swam hard, increasing the ventral root discharge, the tentacles retracted even further. 'This corollary discharge encodes duration and strength of locomotor activity, thereby ensuring a reliable coupling between locomotion and tentacle motion', the team says.

Having provided convincing evidence that corollary discharges from the spinal cord of swimming tadpoles control the movements of touch-sensitive tentacles either side of the tadpole's mouth in coordination with swimming, the team is more confident that the tadpoles retract the tentacles to avoid over stimulation while swimming through sticky water. In addition, they suggest that folding the tentacles back could make the animals more streamlined to speed them on their way. Either way, the team has unravelled the mechanism uniting the tadpole's swimming wriggle with its swinging touch-sensitive tentacles.

10.1242/jeb.125211

Hänzi, S., Banchi, R., Straka, H. and Chagnaud, B. P. (2015). Locomotor corollary activation of trigeminal motoneurons: coupling of discrete motor behaviors. *J. Exp. Biol.* **218**, 1748-1758.

Kathryn Knight

Silk moth larvae suffer more than pupae in heat

Silk moth larva. Photo credit: Leigh Boardman.

Striking a deal with the environment, ectotherms have come up with a low-cost lifestyle compromise by hitching their body temperature to that of their surroundings. However, as climate change takes a grip and the equilibrium begins shifting, this compromise may come unstuck if conditions become uncomfortably hot. Leigh Boardman and John Terblanche from Stellenbosch University, South Africa, explain that as temperatures rise, aquatic ectotherms are unable to meet the oxygen demands of their warmer bodies, but it wasn't clear whether insects suffered the same fate. Knowing that oxygen availability should alter the thermal tolerance of animals if it is the factor limiting their ability to adapt to hotter conditions, Boardman and Terblanche decided to test the impact of altering the oxygen supply on the metabolism and activity of silk moth larvae and sluggish pupae as the mercury rises.

Gently increasing the environmental temperature from 25 to 60°C over 2 h 20 min, the duo measured the metabolic rates and breathing activity of silk moth larvae and pupae in air ranging from hypoxic (with ~2.5% O_2) to normoxic (21% O_2) and hyperoxic (40% O_2) to find out whether oxygen availability affected the insects' thermal tolerance. They also tested the effects of changing the density of normoxic air (replacing some of the nitrogen with low density helium) and the air humidity to see whether physical changes to the air altered how hard the insects had to work to breathe in order to alter their thermal tolerance.

Surprisingly, the team found that the effects of oxygen availability depended on the insects' life stage. While the pupae seemed unaffected by oxygen availability – maintaining a critical thermal maximum of 50°C at all oxygen levels – the larvae's critical thermal maximum fell from 53°C in normoxic air to 51°C in hypoxic air: the larvae were oxygen limited while the pupae, with a lower metabolic rate, were not. And the larvae required more oxygen to sustain metabolism than pupae at the same temperature, as well as having a higher critical thermal maximum (53°C) than pupae (50°C) in normoxic air. Next, the team analysed the impact of humidity and density on the insects' thermal tolerance, and found that humidified hypoxic air increased the larval critical thermal limit, suggesting that the water vapour may have somehow increased delivery of oxygen through the larvae's tracheal system. However, altering the air density did not seem to affect how hard the insects had to breathe and did not affect their thermal tolerance.

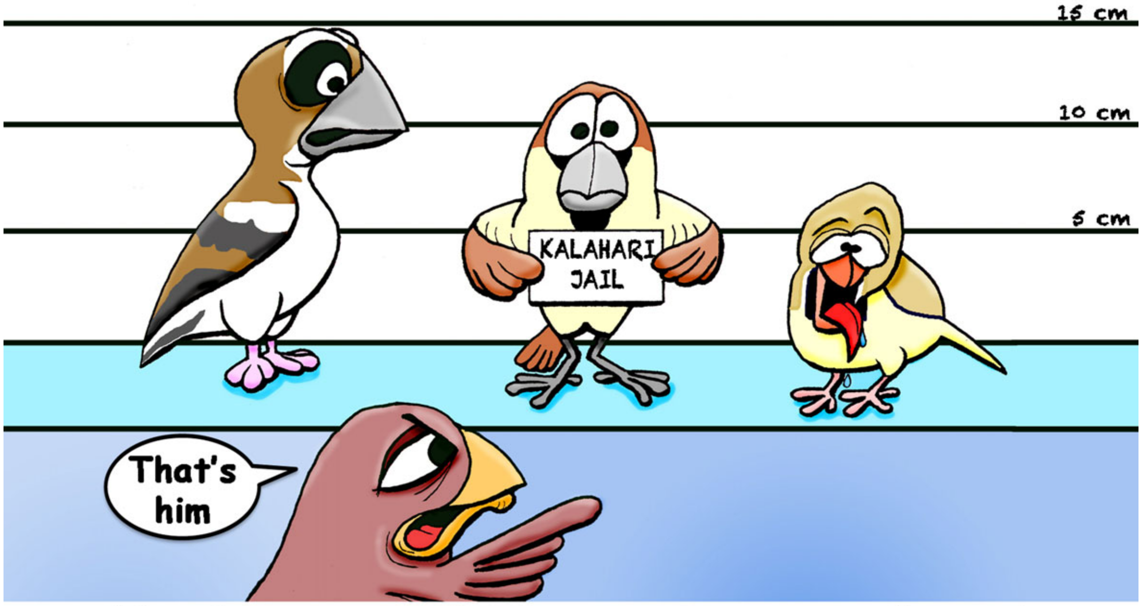
So, different insect life stages respond to thermal stress in different ways, with oxygen supply determining the thermal limits of fifth instar larvae, while it has little impact on the thermal tolerance of metamorphosing pupae. Realising that some insect life stages may therefore be more susceptible to the detrimental effects of climate change than others Boardman and Terblanche say, 'Further investigation into life stage-related oxygen safety margins is urgently needed'.

10.1242/jeb.125187

Boardman, L. and Terblanche, J. S. (2015). Oxygen safety margins set thermal limits in an insect model system. *J. Exp. Biol.* **218**, 1677-1685.

Kathryn Knight

Hot birds pant to keep cooler than air



Although size was a factor, it was easy to pick out the smaller bird, who started to pant at a lower temperature

Staying cool is crucial when the mercury rises, and if you choose to make your home in the desert it is even more essential. Many species rely on the cooling effects of water evaporation to prevent themselves from burning up, but little was known about how birds respond when temperatures rocket: how much heat they could tolerate and how those tolerances vary from small to large animals. Teaming up with Blair Wolf from the University of New Mexico, USA, Andrew McKechnie from the University of Pretoria, South Africa, decided to find out how three desert-dwelling song bird species [the tiny scaly-feathered weaver (10 g), the sociable weaver (25 g) and the large white-browed sparrow-weaver (39g)] cope in scorching conditions.

Carefully trapping birds in the arid southern Kalahari Desert, Wolf, Maxine Whitfield and Ben Smit gently injected a minute thermometer into the abdomen of each of the birds before measuring the animals' body temperature, metabolic rate and water loss as the temperature increased gradually from 25°C to a sweltering 54°C. However, if the birds began to overheat, the team jumped in quickly, getting the animals' temperature back to normal by holding them in front of a chilly air conditioner while wiping them with ethanol.

Although the two larger species coped better than the small scaly-feathered weavers – which struggled to regulate their body temperature at air temperatures ranging from 44 to 50°C – the large white-browed sparrow weavers lost the ability to regulate their body temperature at air temperatures of 54°C and the sociable weavers failed at 52°C. In response to the high temperatures, the birds began panting heavily, with the scaly-feathered weavers increasing their evaporative water loss rates at the highest temperatures by 10.8-fold, while the sociable weavers and the large white-browed sparrow-weavers increased their evaporative water loss rates by 18.4- and 16-fold, respectively. And when the team calculated the birds' heat loss rates, they were impressed to see that the birds were able to dissipate heat at rates that were 41–122% above their normal metabolic heat production. Andrew McKechnie says, 'The birds were able to offload heat to the environment at a rate faster than they were producing it through metabolism', adding that the birds lose heat by evaporation to maintain a body temperature that is lower than the air temperature.

10.1242/jeb.125179

Whitfield, M. C., Smit, B., McKechnie, A. E. and Wolf, B. O. (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol.* **218**, 1705-1714.

Kathryn Knight
Kathryn@biologists.com