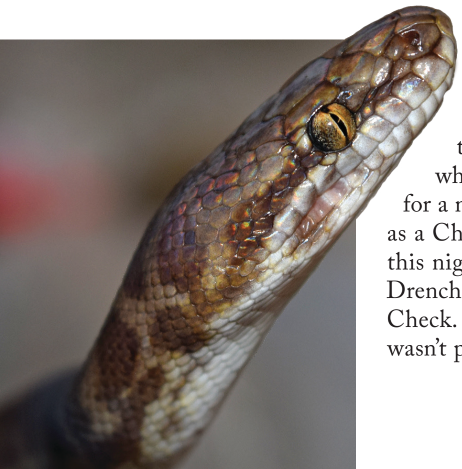
# A thirst for *knowledge*

Children's pythons are nocturnal and prey on other reptiles, birds, small mammals and even bats in Top End caves. Like all pythons, they use constriction to subdue and suffocate prey and then swallow it whole. *Photo: Stephiny Delamare*

A gravid Children's python captured towards the end of the dry season outside Darwin, NT. Gravid females refuse food until after the breeding season, when they gorge to restore fat reserves. *Photo: Dr George Brusch*



## How arid-adapted snakes thrive with limited water

Children's pythons are an ideal 'model' organism for scientific study into water consumption because they are naturally adapted to desert environments where water is scarce during the Austral dry season. They also reproduce and lay their eggs during the dry, when the mercury rises and the ground cracks with thirst. Although seemingly ill-fated, this timing allows their offspring to hatch with the best chance of survival, right as the monsoons roll across northern Australia and replenish the land. Without anything to drink, reproductive female pythons are burdened with transferring massive amounts of water into their developing eggs, giving Environmental Physiologist **Dr George Brusch** an opportunity to examine how dehydration impacts physiology from multiple angles.

It was the first of July and I, a Yank in Australia, marched through the bush, eyes on the ground, watchful for any sign of snake. The night air was hot and pregnant with moisture. A cacophony of frog and insect calls drowned out my thoughts. Off in the distance, a bushfire lit up the sky, giving the illusion of a large sports stadium just over a nearby rise. I'd been working the same area every evening for nearly a month. It was a long way from home in Arizona, USA, but Darwin, NT, was to be my temporary home for ten weeks while I conducted research. I was searching for a medium-sized, non-venomous snake known as a Children's python (*Antaresia childreni*), and this night was proving no different to any other. Drenched in sweat? Check. Deafening wildlife? Check. Inferno in the distance? Check. What I wasn't prepared for were the explosions…

They started off slowly, like the first brave trill of a lone cricket at dusk. But they soon grew until, like rolling thunder, they were all I could hear. I didn't have time to second-guess why any army would choose sleepy Humpty Doo (the closest town) as ground zero for an invasion, but it felt like the only obvious explanation. Never mind that the assailants' munitions had a strange colour palette that included purples, greens, and blues. Doing what any sane person would do, I rushed as quickly as I could back to the University of Sydney's field station at Middle Point, where I was staying. I arrived at the station with an urgent list of things to do: grab my passport, call the US embassy, find the nearest bunker! It was all much scarier than any snake could hope to be – until a grinning colleague stopped me as I sprinted through the door, saying, 'Happy Territory Day, mate!' I'd been terrified by fireworks, and I doubt I've ever felt so foolish in my entire life.

## Wild times

Like a snarky status on social media, my relationship with Children's pythons is best classified as: it's complicated. Part of my dissertation involved laboratory-based experiments with these reptiles; however, it was crucial that I travel to the Territory to see whether wild animals showed the same trends as those studied in the lab. Time after time, my results defied what I always counted as logic and scientific reasoning. It's not that there's anything inherently irregular about Children's pythons: they just provide an excellent model for asking biological questions that sometimes have perplexing answers. Most of the questions I ask have already been answered through a different lens. However, much of the previous work asking similar questions has focused on energetic resources. Energy is a vital commodity, but I'm primarily interested in how animals survive in environments with fluctuating resources; specifically how they survive without another fundamental resource: water.

## Not rattled by drought

My dissertation initially focused on studying rattlesnakes in the Sonoran Desert of Arizona, USA. These animals are naturally adapted to life in arid environments and go for months without water during seasonal droughts. A human would be lucky to make it 12 hours without water in the scorching deserts that rattlesnakes inhabit! Remarkably, in the lab, rattlesnakes survive for up to 16 weeks without food or water, showing no signs of clinical dehydration. They're not entirely resistant to water loss, though. As they reach mild levels of dehydration, portions of their immune system are enhanced, a paradox when compared with most other physiological systems, which crash-and-burn during bouts of dehydration. These results are in stark contrast to previous work. Lack of energy typically dampens immune function, yet somehow a lack of water enhances it in these snakes. Enter Children's pythons.

Lack of water during the dry season should lead to dehydration. Reproduction, especially pumping water into the eggs, should also lead to dehydration. A combination where animals must do both simultaneously should lead to additive levels of dehydration. With these presumptions in mind, our first experiment used four groups of female Children's pythons: reproductive or non-reproductive and with water or without water during gravidity (a ~3-week period after mating when eggs develop). We predicted that non-reproductive snakes with water would be the most hydrated – they have plenty to drink and don't need to give any precious water to their offspring. Conversely, reproductive snakes without water would be the most dehydrated – they have a combined 'hydric cost' of lacking drinking water and giving what little internal water they have to their offspring. In the mid-range would be the two other groups. This design allowed us to explore how dehydration, reproduction, and a combination of the two, impacted immune function, which we expected would be enhanced – as found in rattlesnakes. Complementing this lab-based study was fieldwork in the NT, where I collected data from wild pythons during the dry.

## Dry July

Results from my fieldwork in the Northern Territory were as we had expected. Animals became more dehydrated as the dry season progressed, and with dehydration came enhanced immune function. In the lab, non-reproductive females with water were as expected: they did not dehydrate and did not have elevated immune scores. The two groups in the mid-range were similarly what we expected: mildly dehydrated with elevated immune scores. Our results thus far painted a seemingly clear picture. Reproductive females who had water were dehydrated, which was due to the hydric cost of reproduction. Similarly, non-reproductive females without water were dehydrated due to the hydric cost of living without water. But what about the reproductive females without water, the group we predicted would be the most dehydrated? They had both the hydric cost of reproduction and the cost of living without water. However, their levels of dehydration and immune scores were comparable to the two groups in the mid-range. It was promising to see a clear link between dehydration and immune function in an additional species. However, reproduction seemed to muddy the waters. I couldn't shake the feeling there was more to the story. ▶

^ A juvenile Children's python. Clutches of 10–15 eggs hatch during the Top End wet season, when resources are plentiful, to give juveniles the best chance of survival. Those that survive to adulthood may live two decades or more in the wild. *Photo: Dr George Brusch*

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**Fast facts**

**This non-venomous snake's common name and species epithet (*childreni*) make it a favourite with kids, and it is a common pet-shop species, but the name actually honours John George Children, the curator of zoology at the British Museum when this snake first came to European attention.**


^ Found across the Top End from northern WA to north-western Qld, Children's pythons inhabit various habitats, from savanna woodlands and monsoon vine thicket to grasslands and rocky outcrops. *Photo: Jasmine Zeleny*

˅ Despite being capable climbers, Children's pythons are most often found on the ground. *Photo: Michael J Barritt*

## Capital breeders

All organisms fall somewhere on a spectrum of income to capital breeder. The former must constantly consume resources to fund reproduction; the latter, where Children's pythons lie, use stored, internal resources to fuel reproduction. Energy is the primary focus of most questions about reproduction, but again, I wanted to know about water. In seasonal environments like the Top End and Arizona, a common strategy is to have babies born, hatch, or emerge at the start of the wet season, when resources are plentiful. That means females must invest considerable resources, including water, during the driest times of the year when few resources are available. How then, do animals provide water for their own needs and their developing offspring?

A few animals have internal water reservoirs. Desert tortoises store water in their bladders, and camels in the rumen of their stomachs. Most, however, rely on drinking water, water from food, or water as a by-product of metabolism. We knew the animals in our study weren't drinking water. We also knew they weren't getting any from their food (Children's pythons don't eat in the wild during the dry season, and even if we try to feed them in the lab, they refuse food during reproduction). With relatively low metabolic demands, water from metabolism seemed unlikely. But we knew they were getting water from somewhere. So where?

My colleagues and I decided to shift our focus to metabolism. Specifically, we wanted to see if reproductive females without water were changing the type of internal resources they used, which might explain our bewildering results. We measured a variety of physical and biochemical characteristics and compared them between the same four treatment groups as in our previous experiment. As with before, at first glance our results didn't make sense. Fat is an amazing energy source. Gram for gram, burning fat provides more energy and more water than burning protein (muscles) or carbohydrates (sugars). We expected that the mysterious source of water in our reproductive snakes was from increased fat metabolism. I should have known better, because a cursory glance at a sinewy python winding itself around a tree branch provides a telltale clue about what was really happening.

## Feel the burn

Our reproductive females without water weren't burning fat; they were burning muscle! Both reproductive groups (with and without water) burned fat throughout the experiment, which wasn't entirely unexpected; the energetic demands of egg production are high, and fat-burning provides a reliable energy source. So, if females need water and burning fat produces water, why would muscle metabolism increase?

It's important to consider how fat and muscle are stored in the body. Think about a 30 g glob of fat. If you put it in the oven for a day, in addition to making a stink, the mass wouldn't change much because there wasn't that much water in it to begin with. Do the same thing with a 30 g chunk of steak, and after a day, the mass would dramatically decrease (think 'roo jerky') because muscle is stored in approximately 70% water. In fact, when you consider this water (called bound-water) and the water released from metabolising muscle, you get about five times more water from muscle than from fat. I initially felt quite foolish not to have recognised that, too. Using Children's pythons and a different perspective showed us, for the first time, that mobilising proteins can support increased water demands during reproduction.

We also examined reproductive output between the females with and without water. We found no difference in the number of eggs each female laid, but individual eggs from dehydrated mothers tended to weigh less, perhaps because they too were dehydrated (mums had less water to give), but we needed to dig a little deeper.

## Dehydrated eggs

Using two groups of reproductive females, hydrated or dehydrated, our next experiment focused on the eggs. As with the previous study, dehydrated mothers laid eggs that weighed less. To explore why, we examined the percentage of water within the eggs compared to other components (shell, yolk proteins, etc.) and found that these eggs weighed less because they had less water content. That isn't a perfect assessment of hydration, so we used the same metric (osmolality: the measure of a solute per kilogram of solvent) for hydration as we used on the adults. We found that the eggs were more dehydrated too.

When we measured water loss across the shell, we found that dehydrated eggs (from dehydrated mothers) lost water at a faster rate, suggesting that the shells were thinner. To verify this, eggshell sections were sent to a colleague at the University of Rennes, France, and incredibly detailed images using a scanning electron microscope were taken. These measurements confirmed that dehydrated eggs had thinner shells. The effects might not be all bad, however. A thinner shell means that water can be lost or gained at a quicker rate. Presumably, females in the wild avoid allowing their eggs to get moist (and therefore rehydrated) due to an increased risk of microbial attack. We also measured immune function in these eggs and found that dehydrated eggs had enhanced immune function, just like in the dehydrated adults, including enhanced microbial killing ability. This suggested that dehydrated eggs might be able to absorb water from the environment and fend off any associated microbes.

In a follow-up study, using a similar experimental design, we answered some crucial remaining questions. Notably, we found that the embryos were independently responding to dehydration, rather than relying on enhanced immune capabilities passed off by their mothers. We also found that thinner-shelled eggs could, in fact, absorb environmental water, rehydrate, and subsequently show decreases in immune function.

I still have an unending list of questions about Children's pythons that remain unanswered. I would never have guessed that such a small, unobtrusive python from Australia could elucidate so much. I plan to return to the Territory eventually – still curious, more prepared, and diligently watchful for any sign of snake – and, next time, aware that Territory Day might be better for fireworks than for fieldwork. ■

‹ Undergraduate researcher Brittany Kaminsky and Dr George Brusch IV give a Children's python a bath in the lab in Arizona, USA. Because Arizona's air is so dry compared to the humidity of northern Australia, bathing these snakes helps them completely shed their skin. *Photo: Sandra Leander/ ASU School of Life Sciences*

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**READING** ■ Brusch IV GA, Heulin B, DeNardo DF. 2019. Dehydration during egg production alters egg composition and yolk immune function. *Comparative Biochemistry and Physiology A* 227:68–74. ■ Brusch IV GA, DeNardo DF. 2019. Egg desiccation leads to dehydration and enhances innate immunity in python embryos. *Developmental and Comparative Immunology* 90:147–51 ■ Brusch IV GA, Lourdais O, Kaminsky B, DeNardo DF. 2018. Muscles provide an internal water reserve for reproduction. *Proceedings of the Royal Society (London) B* 285:20180752 ■ Brusch IV GA, Billy G, Blattman JN, DeNardo DF. 2017. Reproduction alters hydration state but does not impact the positive effects of dehydration on innate immune function in Children's pythons (*Antaresia childreni*). *Physiological and Biochemical Zoology* 90(6):646–54.

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⌄ Seen in daylight, the scales of a Children's python can take on a rainbow sheen. *Photo: Greg Brown*