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**ACTHA Inc. News**

**June - July '20**

*Newsletter of the  
ACT Herpetological  
Association Inc.*

**Diary date - 23 June '20  
ACTHA Zoom meeting**

Due to the current Covid19 physical distancing rules we shall bring you this month's Guest Speaker presentation via Zoom on-line technology, in the comfort of your own home!

The presentation will start at **7.30pm SHARP**.

If you are interested in being part of the Zoom group meeting **please email Margaret Ning at:** [margaretning1@gmail.com](mailto:margaretning1@gmail.com) **ASAP**, and you will be sent an invitation to the Zoom meeting.

You **MUST** get Margaret's email invite to take part!

If this is your first Zoom meeting, and you've received Margaret's invitation to the session, you'll be prompted to download the Zoom App - this may take a few minutes.

Once you've downloaded the App you will automatically be directed to the session. You'll then be prompted to select your audio and video settings, you need to do this before you can hear and be heard and seen. (You can leave the video turned off if you prefer.)



**Gavin Smith**, left, is our guest speaker this month: a snake-catching sociologist, Deputy Head of the ANU School of Sociology'.

During the warmer months, Gavin is a licensed snake catcher in the ACT and in NSW. He has been the owner/operator of ACT Snake Removals since 2017. Gavin got into snake catching mainly due to his love of 'wild' creatures, but also to complement his work as a volunteer for ACT Wildlife. He very quickly fell in love with the *Pseudonaja textilis* species, much to the displeasure of his family...In his other life, he is an Associate Professor in Sociology at the ANU where, among things, he studies socio-ecological and multi-species relations, and has a specific interest in researching human-snake encounters. In this way, he has recently brought two of his passions together: justice and reptiles.

**Talk outline**

In this interactive talk, Gavin will draw on some sociological ideas to explain the visceral feelings of fear and hostility that typically define how many members of the public perceive and interact with elapid snakes when they are encountered in the course of everyday life. The intensity of these reactions seems to be at odds with the threat these creatures pose statistically, or harm they actually leverage. He suggests that we need to look to how these creatures have been historically scripted and represented in all manner of texts as exotic and dangerous contaminants, or as moral hazards to be avoided or eliminated as they slither their way into domestic spaces. Gavin also reflects on some of his experiences - and research - working with our local elapids (and other reptiles) in the field, and some of the key motivations, pleasures, challenges and implications of doing snake rescue work.

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## ACTHA Reptile Keepers Club - 26 May 2020 ZOOM meeting - at home with Gerry Marantelli, ARC

This meeting was an outstanding success! Gerry talked his audience through the history of ARC in its earliest state, before moving on to the new location of the Centre, which now houses a bigger and better breeding and research complex. Education on all things frog for visiting school children can now also be catered for.

There will be another opportunity for a zoom meeting with Gerry, this time for a virtual tour and Q&A session: stay tuned!

Gerry prefaced this meeting by saying:

*'I've spent a lifetime amazed by amphibians, I've had the great privilege to see hundreds of species across dozens of countries and to work with the world's most endangered amphibians while seeking to understand the greatest threats they face. My home is among the frogs - in renovated shipping containers just like those I've used to help save species from extinction. ACTHA has supported my work for many years, and so I thought now in lock-down, it might be a good time to take you on a tour of the amphibians, my work and the frogs I share my home and life with.'*

*Gerry Marantelli"*



The **Amphibian Research Centre** was established in August 1994 as a centre dedicated to research and conservation of Australia's unique frogs. The ARC is self funding and provides for its valuable work through sales of amphibian supplies and sponsorships.

### Education

'With the move to the vastly bigger and better Amphibian Research Centre in Werribee, a "school visit" can now mean a visit by your school to the ARC rather than the other way around. Have a look at the educational tours section for full details.

'Our tadpole kits allow you to turn your classroom into your very own amphibian research centre. These kits are available on their own or as an extension of some of the educational tours operated by the ARC.'

### Frog food

'The ARC operates an online and mail-order insect service to allow trouble free access to essential frog food. No need to drive miles to a pet store or spend hours chasing bugs in the garden. A range of different insects, supplements and advice are also available to ensure your frog receives the most appropriate diet.'

Head to <https://frogs.org.au/index.html> to read all about the ARC's history, current activities and projects as well as supplies for home froggies!



### 'Australian Water Dragons' by Lisa Mitchell

- book launched 13 September 2019 at ANBG

ACTHA member Rosemary Blemings recently came across this little gem of a book, and has taken delivery of a number of them for distribution around the ACT.

The book provides a readable overview of the scientific background, history, identification, communication, lifecycle, predators and conservation of these reptiles. Well illustrated with colour photographs, it is aimed at 9+ years, but suitable for young and older readers alike.

The A5 book can be purchased for \$10 from Margaret at any ACTHA meeting, or \$12.50 if postage required.

Please contact Margaret Ning at [margaretning1@gmail.com](mailto:margaretning1@gmail.com) to secure your copy.



## Maternal nest site selection and consequences of sex reversal on hatchling Central Bearded Dragons

*This summary by Phil Pearson.*

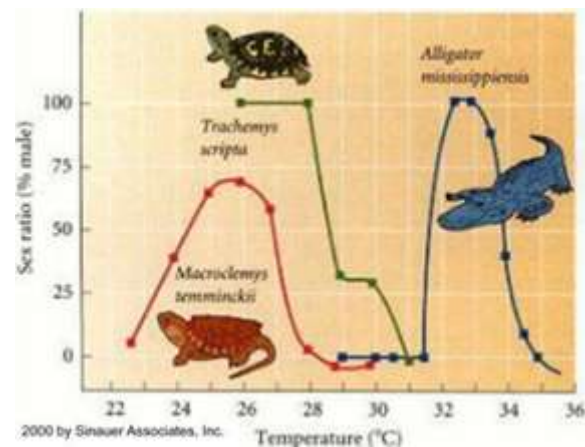
The presenter and host of the Zoom meeting was **Phil Pearson**, a PhD candidate from the University of Canberra, who is originally from Alabama, USA.

Phil Pearson is a PhD Candidate from the University of Canberra's Centre for Conservation, Ecology, and Genetics originally from Alabama, USA. He has a background in evolutionary ecology primarily in reptiles. His previous work in the US focused on the effects of seasonal incubation temperatures on offspring fitness in the invasive Brown Anole lizard in Florida. Phil's PhD work has focused on how sex reversal in the Central Bearded Dragon is affecting their nesting ecology and offspring's fitness-related phenotypes. His prior work greatly influenced questions he has sought to answer while working with the Central Bearded Dragons.

The ecology of the dragons has been mostly unstudied until recently when Phil and Kris Wild (UC) began their doctoral research following the discovery of sex reversed individuals occurring in wild populations. With sex reversal happening in nature, it is important to understand the nesting ecology of the dragons to determine if nesting behaviours are affecting the rate of sex reversal in their wild populations. Following nesting, Phil is interested in how those chosen nests may affect the phenotypes of their offspring. His primary question for his thesis is "How is sex reversal affecting phenotypes that could perpetuate sex reversal?" He is looking at this in at the nesting level and at the offspring level.

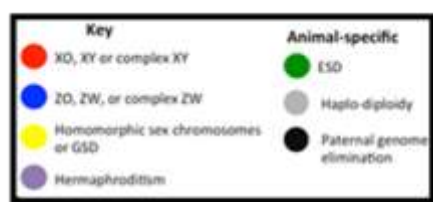
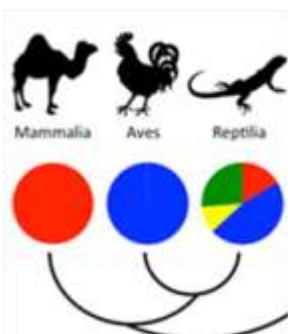


Sex determination is varied throughout the animal kingdom, but the most familiar in amniotic vertebrates (mammals, birds, and reptiles) is typically Genotypic Sex Determination (GSD) where animal has sex chromosomes (XX/XY or ZZ/ZW) that determine if an individual is male or female. However, some reptiles have Temperature-dependent Sex Determination (TSD), where the temperatures experience early in incubation determine the sex of the individual. TSD occurs in all crocodilians, most turtles, tuatara, and many lizards.



Though recent studies have shown there are special cases, such as the Central Bearded Dragon, where typically GSD species experience sex reversal under extreme temperatures shifting the next generation to TSD.

In the Central Bearded Dragon, the sex of the individual is typically determined by ZW/ZZ chromosome system, where ZW are males and ZZ are females. However, when eggs are incubated under higher temperatures (>32°C), ZZ individuals undergo sex reversal to become fully functional females. In the lab, previous studies have shown that these sex-reversed animals produce more offspring and exhibit "male-like" traits (boldness and aggression). They are also occurring in wild populations. With these characteristics and warming



Adapted from Bachtrog et al. (2014) PLOS Biology



conditions, sex-reversed animals may become more prevalent in populations. TSD species are reliant on their nest temperatures to produce appropriate sex ratios. As global temperatures continue to rise, nest temperatures are susceptible to warmer temperatures, which may increase the chances of sex reversal in bearded dragons. We've seen record breaking temperatures in the past few years. However, mother dragons may choose nests that may choose nests that can offset the warmer temperatures for example digging deeper nests or choosing areas with less sun exposure. Phil's research thus far has tried to quantify the nesting behaviours of wild dragons.

#### Key questions of Phil's research:

How is sex reversal affecting phenotypes that could perpetuate sex reversal?

At the Nesting level

Where are wild female Central Bearded Dragons nesting?

Are soil temperatures across the range of the dragons hot enough to induce sex reversal?

At the offspring level

Are there fitness-related differences between offspring of sex reversed and normal mothers?

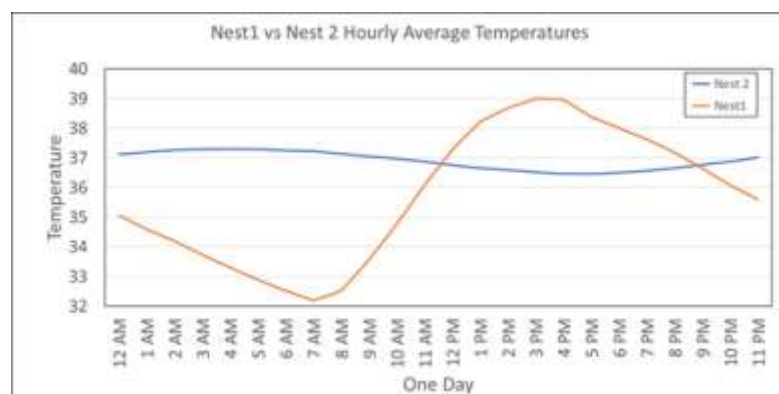
Are females differentially allocating resources to their offspring?

#### Where are wild female dragons nesting?

To do study these behaviours, Phil teamed up with Kris Wild to use radio telemetry to track and monitor female lizards while they were gravid. After females had nested, Phil would carefully excavate the eggs, weigh them, and replace them along with three temperature dataloggers (iButtons) that record the temperatures every hour. He also took soil samples and hemispherical photos to quantify nest moisture levels and solar exposure.

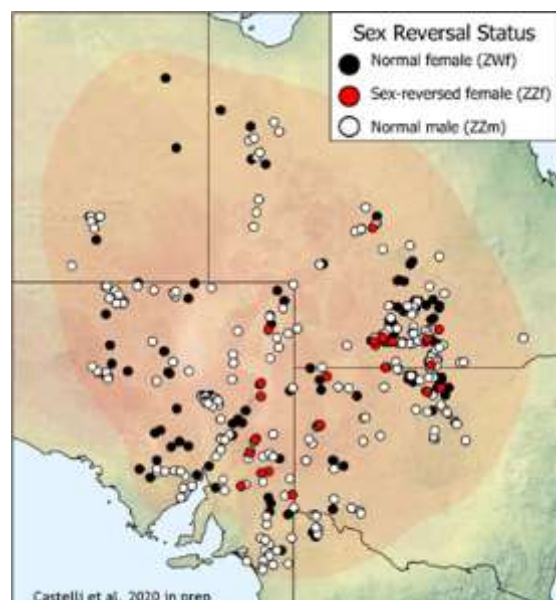


However, finding gravid females is not easy. Phil was only able to acquire two nests in 2018 and four in 2019. All nests had high egg mortality as well, which after looking at nest temperatures is understandable. Late season nests (January; Nest 2 blue line in figure below) had very high average temperatures (36.6-36.9°C) even at a depth of 41cm. This shows that late season nests are getting hot enough to induce sex reversal, but the high temperatures may be lethal.



#### Are soil temperatures across the range of the dragons hot enough to induce sex reversal?

Central Bearded dragons have a very wide distribution across Australia from northern Victoria to Mount Isa and from St. George, QLD to just west of Alice Springs, NT. However, sex reversal has only been documented in a few places across their range and no cases reported in areas that should have the highest temperatures.



*(Maternal nest site selection and consequences of sex reversal on hatchling Central Bearded Dragons, cont'd...)*

Phil has deployed iButton temperature loggers across the range of the Central bearded dragon (blue dots on the map below). These iButtons are attached to a 60cm dowel and buried to collect temperatures at given depths across the range to gain a better understanding of what temperatures are available for nesting dragons during their reproductive season.



Due to COVID-19 restrictions, these will have to be collected and analysed at a later date. The data from this study will allow for predictions to be made about the potential for sex reversal in populations.

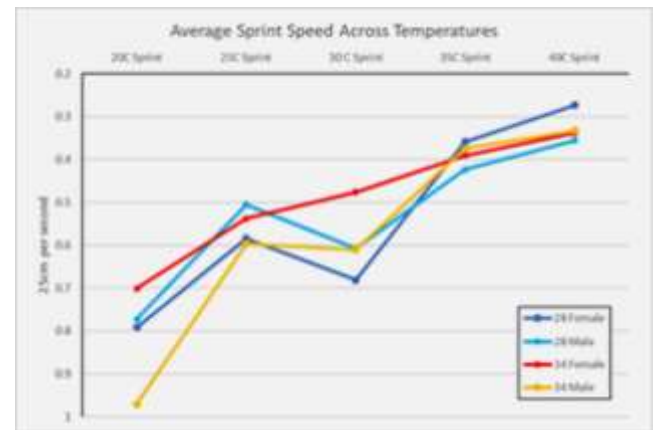
**Are there fitness-related differences between offspring of sex reversed and normal mothers?**

For the reproductive season of 2019-2020, Phil has conducted incubation experiments using the captive breeding colony of dragons at UC to answer the following questions:

How do fitness characteristics differ between sex reversed and non?

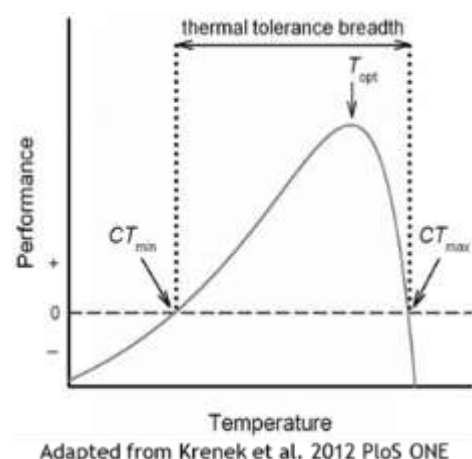
Do incubation temperatures also play a role?

Do the two types of mothers allocate resources to their offspring differently?



To test the hatchlings fitness, they were weighed and measured, and at five weeks old raced along a one metre track with infrared sensors. Hatchlings were acclimated to varying temperatures prior to racing to create performance curves. Sprint speed is a common measure of fitness as faster individuals may have advantages of slower ones. Overall, 467 eggs were incubated, and 326 hatchlings were produced over the two incubation treatments. Phil presented some “very preliminary” results, showing that hatchling dragons are running slower at cooler temperatures than warmer temperatures. However, more statistics are to come.

To answer question 3, Phil will be looking at how mothers allocate nutrients and hormones via egg yolk. Egg laying reptiles must provide all the nutrients and compounds needed for proper embryo development. Given sex reversed females are genetically male and exhibit “male-like” behaviours, they may be allocating different levels of steroid hormones to their offspring (testosterone for instance). Phil will use Enzyme-linked Immunoassays (ELISAs) to measure the levels of steroid hormones in mothers and their offspring. Phil expressed gratitude for all the volunteers and funding sources including ACTHA saying without them none of his work would be possible, and he looks forward to speaking to ACTHA with more results in the future. Hopefully in person next time!



To answer questions 1 and 2, Phil incubated eggs from regular and sex reversed females under 28° and 34°C, which produced a mixture of male, female, and sex reversed females at different temperatures.



## The Australian & International Scene

### Snake handlers 'completely baffled' after Canberra family find 19 venomous Eastern Brown Snake hatchlings in their home

*By Peta Doherty, ABC News, 20 April 2020*

Many Canberrans will recall the hail storm that swept through the capital in January, but for one family, it brought some unexpected and unwelcome visitors into their home.

A total of 19 eastern brown hatchlings were found over the course of a few weeks in a house in north Canberra following the extreme weather event.

Snake handlers believe the downpour swept the hatchlings into a drainage shaft of the house, and they settled in.

The first two eastern brown hatchlings were discovered when they slithered their way into the family rumpus room, nestling into a towel that was being used to block bushfire smoke.



*Above: Eleven of the Eastern Brown Snake hatchlings. Image: Gavin Smith.*

The snakes, which are venomous, were discovered when a woman in the house shook the towel out. She was bitten when she tried to stop one of the snakes from attacking her cat. "I didn't recognise these two little things all twisted up, I thought it was a millipede," the woman, who did not wish to be named, told the ABC.

Luckily, the woman's mother recognised the deadly species thanks to a snake awareness course she had completed just a few months earlier.

"Mum is the real hero in this," the woman said, explaining that her mum went straight for the



*Above: Two of the hatchlings were found under a concrete step. Image: Gavin Smith.*

compression bandage she had given the family after the course, which slowed the spread of venom.

ACT Snake Removals owner Gavin Smith, who ran the snake awareness course and responded to the family's call for help, said the mother had potentially saved her daughter from serious health implications.

"The person administering the first aid had only recently attended a training session and knew exactly what to do," he said.

"They kept calm and kept them completely still and called triple zero and got the person to hospital."

But the story did not end there — in fact, ACT Snake Removals were called to the house an additional five times.

Mr Smith was in disbelief when the woman called a couple of weeks later, for two more snakes which she had found in the rumpus room and in the toilet.

"You pinch yourself. It had already been quite an intense event [the first time] because of the fact the lady was bitten and venomated," he said.

During a third callout, snake catchers found another 11 eastern brown hatchlings in a shaft outside a window.

The fourth call out saw a baby eastern brown removed from the rumpus room. It was stuck on some duct tape which the homeowners had

used to seal various gaps, and Mr Smith was able to take it home and remove it safely from the duct tape using cooking oil, before releasing it.

Finally, a day later, a deceased hatching snake was found. In all, 19 hatchlings were collected.

"We suspect they'd been washed into a shaft behind the house and the only way to dig out of that was actually through the house," said Mr Smith, who went on to praise the composure of the family and their openness to learning more about the wild animals throughout the ordeal.

"Even though one of them was actually bitten by one of the little snakes, they still had so much care for the wildlife," he said.

The woman said Mr Smith and his team helped her family transform the experience into a positive.

"We coped really well as a family simply because of [the other handler] Alex and Gavin's care and concern," she said.

"They spent time explaining to the children that the snakes weren't sneaking into the house. That really took away the idea of a predatory thing coming into our home.

Since the ordeal, one of her children has decided they want to become a vet "after being inspired by the fact Gavin and Alex would put their life on the line to help an animal that could hurt them".

And in sharing her story, the woman hopes other families recognise the importance of snake awareness, and that they aren't to be feared.

"It was freak event on the back of a really difficult summer that came about as a result of an 'unfortunate architectural design', that we are getting fixed," she said.

## **Egg laying or live birth: how evolution chooses**

*By Dana Najjar, Quanta Magazine, 18 May 2020*

A lizard that both lays eggs and gives birth to live young is helping scientists understand how and why these forms of reproduction evolved. The old riddle, "Which came first, the chicken or the egg?" is relatively easy to answer as a question about the evolution of birth in animals.

Egg laying almost certainly came before live birth; the armoured fish that inhabited the oceans half a billion years ago and were ancestral to all land vertebrates seem to have laid eggs. But the rest of the story is far from straightforward.

Over millennia of evolution, nature has come up with only two ways for a newborn animal to come into the world. Either its mother lays it in an egg, where it can continue to grow before hatching, or it stays inside its mother until emerging as a more fully formed squirming newborn. "We have this really fundamental split," said Camilla Whittington, a biologist at the University of Sydney.

Is there some primordial reason for this strict reproductive dichotomy between egg laying (oviparity) and live birth (viviparity)? When and why did live birth evolve? These are just some of the questions that new research — including studies of a remarkable lizard that can lay eggs and bear live young at the same time — is exploring, all the while underscoring the enormous complexity and variability of sexual reproduction.

### **A Strategic Choice**

Early female animals laid eggs in the sense that they released their ova into the world, often thousands at a time. Sperm released by males then fertilized some of these eggs in a hit-or-miss fashion, and the resulting embryos took their chances on surviving in the hostile world until they hatched. Many creatures, particularly small, simple ones, still reproduce this way.

But as animals became more complex, vertebrate species — including many amphibians, reptiles and even some fish, like sharks — turned to a less chancy strategy: internal fertilization. Females could then ensure that a higher percentage of their eggs would be fertilized, and they could get more selective about which males they would breed with. The embryo could develop safely inside its mother until she eventually released it inside a protective shell.

Live birth evolved later — and more than once. In reptiles alone, it has evolved at least 121 separate times. And although scientists don't know exactly when the first live animal emerged from its mother, they do know what

forces may have been driving the transition from egg laying and what evolutionary steps may have preceded it.

Both birth methods get the job done, of course, but they present contrasting advantages and difficulties. Crucially, egg-laying mothers can be physically free of their offspring sooner. Birds, for instance, have never evolved live birth, possibly because the energy cost of flying while pregnant is unsupportable. Egg layers can also generally have more offspring in a single litter, since the size of the mother's body isn't a constraint. This advantage may partially offset the risks of leaving eggs exposed to predation and the elements.

Live-bearing mothers, on the other hand, can house their embryos and protect them from predators and environmental dangers for longer. But they do so at their own peril: Being pregnant exposes them to more predation and puts them at considerable risk from the embryo itself. "The embryo is partially foreign, and its tissues are invading into the tissues of a mom," said Chris Organ, a biologist at Montana State University. "It's wild to think about." For the length of her gestation, the mother balances on a tightrope, diverting resources to a foreign being while keeping herself healthy.



Above: The Australian three-toed skink (*Saiphos equalis*) is doubly remarkable: Not only can it both lay eggs and bear live young, but it can do both within a single litter of offspring.

Image: blickwinkel/Alamy Stock Photo.

The major difference between oviparity and viviparity therefore centers on a strategic evolutionary decision about when the mother

should deposit her embryos. If she deposits them early, she's an egg layer, and if she deposits them late, she's a live bearer. Most reptiles, for instance, deposit their embryos just a third of the way through their development.

"Between true egg laying and live bearing there's a whole range of possible times [to deposit the embryo], but it's probably disadvantageous to do that," Whittington said. "We call it a fitness valley." Animals that try to give birth somewhere in that fitness valley might incur all the risks of egg laying and live bearing without reaping the benefits of either. "We think that, evolutionarily, that's quite disadvantageous," she said.

(Marsupials found a novel solution to balancing these risks: The young they give birth to are practically fetal in their immaturity, but they then finish their development inside their mother's pouch. In this way, the mother can provide the protective advantages of carrying her young to full term without needing to accommodate a full-size newborn inside her body.)

### **The Right Temperature for Males**

Scientists are still learning about the developmental constraints and requirements of these birth strategies. Consider, for instance, the thickness of an eggshell. Because oxygen must make it through a mother's bloodstream and into the egg, a thin shell is advantageous before it is laid. In the outside world, though, a thicker shell is helpful to protect against predators. An egg laid too early, then, might be too thin to survive, and one laid too late might be too thick to meet the exponentially growing oxygen demands of the embryo. It's a finely tuned balance.

In a paper published in *Nature* in 2009, Organ and his colleagues demonstrated that before a species could evolve live birth, it probably had to evolve the ability to determine the sex of its offspring genetically. The sex of many creatures is circumstantial: Environmental factors, particularly temperature, can determine whether the embryo develops as male or female. Organ's team showed a strong statistical association between using genes to determine sex and giving birth to live young. "It's something that biologists knew in an observational way, but hadn't ever been rigorously tested," Organ said.

Consider sea turtles. "They can barely move around on land, but they still come to the beach



to lay eggs," he said. If they laid all their eggs in the water, they would be less likely to get a variety of males and females because the temperature gradient there is much smaller than it is on land.

But once a marine species has evolved the ability to determine sex through genes, it no longer needs to venture onto land and can fully adapt to its aquatic life. As Organ and his co-authors wrote in their paper: "Freed from the need to move and nest on land, extreme physical adaptations to a pelagic lifestyle evolved in each group, such as the fluked tails, dorsal fins and wing-shaped limbs of ichthyosaurs [a group of prehistoric marine



reptiles]."

*Above: The embryo of a three-toed skink just before it is laid in an egg is almost fully formed. Because the commitment to egg laying occurs so late in development, this species has the option for live birth instead. Image: Stephanie Liang.*

At the time of that publication, scientists thought that live birth might have evolved among the reptilian ancestors of ichthyosaurs only after they moved from the land to the sea. But the discovery of a 248-million-year-old fossil changed that. In a paper published in *PLOS ONE* in 2014, researchers describe the fossil of an ichthyosaur that died while giving birth. Amazingly, the fossil captured the precise moment when the newborn emerged from its mother's pelvis headfirst. That position is telling: Most viviparous marine reptiles are born tail first so that they can continue to draw oxygen from their mother during labor. The headfirst birth position indicates the ichthyosaur inherited live birth from an even more ancient land ancestor. Land reptiles may therefore have been giving birth to live young for at least 250 million years, though the oldest fossil of live birth on dry land doesn't date nearly that far back.

## Eggs, Babies or Both

Live birth or egg laying might seem like a definitive either-or choice for a species, but surprisingly, that's not always the case. Whittington and her team study the Australian Three-toed Skink (*Saiphos equalis*), a lizard with the remarkable distinction of being able to both lay eggs and give birth to live young. A couple of other lizard species have been known to do both, usually in different settings, but in Whittington's laboratory, the researchers observed a three-toed skink produce a litter that consisted of three eggs and one live baby. "We were absolutely flabbergasted," Whittington said.

Recently in *Molecular Ecology*, Whittington and her team describe the differences in gene expression — which genes are switched on or off — between a lizard mother that lays eggs and one that gives birth to live young. Within a single species, there are thousands of such differences between a female with an egg and one without. That's because certain genes get switched on when it's time for the uterus to house an egg. The same goes for a uterus that's sheltering an embryo. Crucially, the specific genes that get switched on in these cases are very different.

But in three-toed skinks, a lot of the genes that switch on when a mother makes an egg also get switched on in mothers with embryos. The finding implies that this lizard is in a transitional state between egg laying and live bearing.

Which way the lizard is evolving is impossible to say and may still be undetermined.

"Evolution is a random process rather than being directed," Whittington said. "With environmental changes, it could change the direction of selection and push it back the other way."

The idea that the skink could be moving away from live bearing and back to egg laying is a relatively new development in the field.

"Twenty years ago we thought it was difficult or impossible for egg laying to re-evolve," Whittington said. But a growing body of research since then has shown that it may be quite common. Recent analyses of genetic relationships between species revealed that certain egg layers are deeply nested within an evolutionary tree of live-bearing neighbours.

## A tiny scientific marvel: Olaf the IVF toad brings hope to at-risk species

By Katharine Gammon, *The Guardian*, 22 April 2020



*Above: Olaf the toad is the first of his species born via IVF using sperm that was frozen and thawed. Image: Fort Worth Zoo.*

Olaf grasps Diane Barber's gloved hands with his sticky, four-fingered legs. His skin is bumpy and moist, the colour of pebbles at the bottom of a river when dappled sun hits them. Olaf's eyes are deep amber. His body lifts and falls with each breath. "The males get really pretty," says Barber, ectotherms curator at Fort Worth zoo in Texas. "Sometimes they'll turn a solid yellow when they're in breeding form."

In some ways, this toad shouldn't exist at all. He is the progeny of an egg from a captive mother and sperm from a wild father – a hybrid from parents who were both dead. Olaf is not the first amphibian to be born via IVF – that has been happening for years – but he is the first of his species to be born from sperm that was frozen and thawed.

"We were able to recover a genetic lineage that had disappeared, so we were able to produce an offspring from dead parents," says Andy Kouba, an ecologist at Mississippi State University, who assisted with the project. "So that was an exciting first, to reintroduce genetic lines back into the population."

Scientists have a lot of tools to conserve species, says Kouba, but they still need to hedge against extinction in the wild. One way could be to bank the genetic lineages of species by freezing sperm and egg deposits and then later thawing and combining them in a dish to create offspring.

Amphibians are at the forefront of an uphill battle against extinction, losing a greater proportion of species than any other vertebrate group. The IUCN estimates that at least 41% of amphibians are at imminent risk of extinction. Habitat loss, climate crisis and a fungal disease all play a role in their demise.

But amphibians are not the only species that can benefit from new technologies. In vitro fertilisation, hormone therapy and cryopreservation are increasingly being used as tools for conservation – for amphibians and beyond. In a fast-changing world, frozen zoos may be the places where tissues are kept, in the hope of boosting numbers or resurrecting species in the future.

### Frozen zoos

How did Olaf come to exist at all? A year ago in Puerto Rico, researchers captured six male toads, injected them with hormones and then collected the sperm they ejected when they urinated. The toads usually pee when picked up by humans, but the researchers also barked at them, Kouba explains, as toads urinate when frightened, and dogs barking are a sure-fire way to scare them. The males were then released back into the wild.

The team of international scientists preserved the semen in liquid nitrogen and transported it to Fort Worth zoo, where female toads were injected with hormones to release eggs. Olaf and his roommates were created in a dish, eliciting cheers from the scientists when they hatched from their eggs.

The idea of creating a frozen zoo first emerged in the 1970s, when a medical pathologist named Kurt Benirschke started banking animal sperm and eggs at San Diego zoo in the same way that human gametes were starting to be stored: in giant vials of liquid nitrogen that dropped the temperature of the materials to -196 F. When Benirschke started banking genetic material, no technology existed to make use of it, but he believed it was important to carry on anyway.



*Above: It is estimated that at least 41% of amphibians are at imminent risk of extinction. Image: Fort Worth Zoo.*

Now, biobanks for animal species – from fish to reptiles to birds and even snails and molluscs – are popping up around the globe. The Frozen Zoo repository at San Diego zoo holds more than 10,000 cell cultures from nearly 1,000 different species. They are mostly from mammals, but also from birds, reptiles and amphibians.

Scientists had been working on the process of bringing together sperm and egg outside the bodies of animals like rabbits and guinea pigs since the 1870s. But making babies in a test tube is no simple task because every species is different. IVF success rates for mammals are much higher than with reptiles, and with some species, it's incredibly low. Even within species, some cells are more difficult than others. When it comes to amphibians, Kouba says, their sperm is pretty simple to freeze – but eggs are not, because they are so much larger than the sperm. "Inside that cell, you have water content, and when you start to freeze anything with water, they form ice crystals that rupture the cell."

Because fish such as salmon and cod are widely available commercially, they have been studied much more than amphibians, and amphibian researchers like Barber and Kouba are waiting for breakthroughs that they can adopt. "There is a huge interest in freezing sperm to move genetics around the world for commercially viable species," Kouba says.

One reason that conservationists want to boost the numbers of amphibian populations is because diseases are devastating them in the wild. Kouba says cryopreservation has a place as a last-ditch effort to bank the remaining genetic material from a dying species, but that it is really better to take action before a species' numbers have crashed. "We can produce tens of thousands for reintroduction, which may allow a species to return to a healthy population," he says.

That's because amphibians have a naturally high mutation rate, so sending thousands of amphibians into the wild to mate and repopulate could mean they create a disease-resistant strain on their own. "You release animals with the hope that disease resistant lines will develop, and those animals will reproduce," says Kouba.

## **Healthy herds**

In Colorado, Jennifer Barfield is using IVF to create bison with healthy genes to introduce into existing wild herds. Left to their own devices, bison breed well but what they can't do is move around the globe – so to transport new genes to different areas requires the use of IVF. Using assisted reproductive technologies, she has helped produce healthy Yellowstone bison from ones that had a bacterial disease called brucellosis and established a new conservation herd in Colorado called the Laramie Foothills Bison Conservation Herd.

There is another advantage to using IVF in bison says Barfield. "Bio-banking the genetic preservation is just a good idea, like a form of genetic insurance. You never know how science will advance to use that material in the future."

She says that one of the challenges in endangered species is that often researchers don't know enough about the reproductive physiology of rare animals to apply solutions before the animals become extinct. "You can't experiment on endangered species," says Barfield.

In some species, assisted reproduction is the last hope. There are only two remaining northern white rhinos left in the world, both female, both past reproductive age. Last year, researchers were able to harvest eggs from one of the females and have created three embryos with frozen sperm from dead males. The embryos are now frozen, with the hope of implanting them into surrogate southern white rhino females later this year. The eventual goal is to create five animals that could live in the wild in Africa – a plan that could take decades to come to fruition.

Back at Fort Worth zoo, Barber carefully puts Olaf back in his cage in the isolation room. In the wild, Puerto Rican crested toads like Olaf spend 95% of their time in small holes between crushed rocks, coming out to feed at night and when it rains. In his home at the zoo, the staff have constructed small pyramids made from sections of white PVC pipes for Olaf and the other toads to hang out in. Of the toads that used to live in this room, 193 were recently shipped via FedEx back to Puerto Rico for reintroduction; now more are being raised here. Other rooms house frogs raised from other



parts of North America, all in need of a population boost.

Barber says that in the 1980s zoos thought of themselves as arks. "That was kind of the buzz word for zoos: we're becoming arks instead of just having animals for exhibit. We need to start doing more about thinking about conservation and maintaining species long term."

But those arks are full, and more species need assistance – there's no space to keep animals just as an insurance policy. "We're not arks, we're life rafts," Barber says. "And we have very limited seating."

As she speaks about her passion for overlooked species, Barber's eyes fill with emotion. "Especially with the stuff that I work with – the creepy crawlies – most people wonder: why should we care? And it's like, well, we should care about everything because we're all connected. Sometimes it's just really hard to communicate why we should care and why it is our responsibility to try to mitigate things that we humans have caused."

So far, IVF conservation work is only being done with about 10 species – but Kouba predicts it will become much more widespread in the next decade. "I think it's going to become mainstream," he says, adding that it could become a primary resource for conservation. "We left those species in the world and we were able to collect their material, bring it back and put new founder lines back in the population, so we don't need to remove animals from the wild any more.

"That will revolutionise how we manage wildlife."

- *This article was amended on 13 May 2020. An earlier version incorrectly stated that Olaf the toad was the first amphibian to be born using frozen sperm. He was in fact the first of his species to be born via that method.*

## **Baby crocodiles at large in Broome after slipping out of Malcolm Douglas Crocodile Park**

*By Erin Parke, ABC Kimberley, 26 April 2020*

Authorities have ordered upgrades to fencing at a crocodile park in Broome after a mass escape of hatchlings, most of which are still on the run.



*Above: One of the crocodile hatchlings found at Cable Beach. Both were given a health check by the staff at Native Anima, Rescue Broome.*

*Image: supplied by Native Animal Rescue Broome.*

The baby crocodiles are thought to have slipped through a fence, with two discovered by locals at nearby Cable Beach in mid-April.

Nine other hatchlings from the same nest remain unaccounted for.

In a separate incident, a slightly older crocodile – around 20 centimetres long – escaped its pen and slipped out a gate, and was found in a roadside drain.



*Above: One of the escaped hatchlings was found hiding in rocks at nearby Cable Beach.*

*Image: Abby Murray*

Park owner Valerie Douglas said she was installing new fencing along the park perimeter. "Everything's under control and we are making changes so it doesn't happen again," she said. "The 11 hatchlings escaped from a nest that was missed by staff when they were collecting eggs in January, and some of them got away.

"So we are paying for new fencing to make sure it doesn't happen again."

She said the reptiles that escaped were not big enough to pose a risk to people or pets.

The two crocodiles at Cable Beach were found last week by local women going for walks with their children.

Matilda, whose parents have asked for her surname not to be used, says she spotted the small reptile hiding next to a rock at sunset.

"When I first saw it I thought it was a lizard, but then I realised it was a crocodile," she said.

"We stayed with it, because it was getting dark and we didn't want to lose it."

Both of the baby crocodiles found at the beach have been given a health check and returned to the Malcolm Douglas Crocodile Park, which spans two large properties.

#### **Croc parks covered by farming licence**

The animals had escaped from the 'old' Malcolm Douglas Crocodile Park, which sits on 2 hectares of prime real estate opposite Cable Beach.

The park was opened by the legendary filmmaker in 1983, as a place for tourists to gather and watch the enormous 'salties' feed on raw chickens and where Mr Douglas oversaw farming for crocodile skins.

It has been closed to the public since 2012, after Mr Douglas relocated the tourism side of the business to a new site 16km east of Broome and was killed in a freak car accident on the property.



*Above: The old Broome Crocodile Park is located near Cable Beach in Broome.*

*Image: Andrew Seabourne.*

There are now about 2,500 crocodiles across the two sites, which are covered by a crocodile farming licence that requires the animals to be safely secured.

The Department of Biodiversity and Cultural Attractions said in a statement that the properties were inspected annually.

"Parks and Wildlife Service is working closely with park owners ... to ensure the park meets the required standards," the statement said.

"An assessment of the park was undertaken on Friday ... and recommendations were made to the park, which included repairs to sections of the mesh fence."

#### **Colourful history**

It's not the first time crocodiles have escaped or been stolen from the park.

In 2016, at least six small crocodiles disappeared.

One was found killed by a car in a nearby carpark, and another was found swimming in the backyard pool of a neighbouring property.

At the time, owner Valerie Douglas said the animals were stolen during a break-in and then set free.

In October 2019 authorities were called in when a crocodile named Cinnamon went missing.

The 15-month-old animal had been regularly handled by tourists as part of daily tours, and police were unable to locate him.

Broome residents are asked to call the local office of the Parks and Wildlife Service if they see a crocodile, so staff can determine if it is an escapee or one that lives in the creek network along the coast.

#### **Green sea turtle released more than a year after being found with horrific injuries on Great Keppel Island following boat strike**

*By Erin Semmler, ABC Capricornia, 7 June 2020*

A vulnerable green sea turtle that washed up with life-threatening injuries on a remote beach has returned to the sea — 18 months, and thousands of dollars worth of food later.

In November 2018, a Great Keppel Island resident raised the alarm after finding Denise with a sliced throat, cut eyelid and half a flipper missing.

In rough seas, rescuers loaded the injured turtle onto a body-board and carefully towed her to an accessible beach.

With the help of a forklift, she was ferried 15 kilometres to the mainland and driven another 50km to Rockhampton veterinarian Greg Muir.

During her 18-month recovery at the Quoin Island Turtle Rehabilitation Centre, Denise put on more than 30kg and ate more than \$15,000 worth of squid, sardines and pilchards.



*Above: Denise's flipper was amputated after a suspected boat strike.*

*Image: Alma Street Veterinary Hospital.*

Manager Kim van Oudheusden said Denise would not have survived without the help from so many people.

"The vet kept her overnight and stitched that back up, then she came here in a car and then we took her on a boat — it was quite an operation.

"We are so grateful to all those people that help us out and do these amazing things because without them, we would not be able to rescue these turtles."

She was named after one of her rescuers, Denise Weisse, president of the Keppel Turtle Fund Inc.

Carers at Quoin Island have rescued and rehabilitated hundreds of turtles since 2011 but owner Bob McCosker developed a strong bond with Denise during her lengthy stay.

"I laid down beside her and while I was pulling her stitches out of her eyes, she did not move,



*Above: Rescuers spent four hours moving the severely injured turtle from the remote beach.*

*Image: Lyndie Malan.*

didn't flinch — she just watched me and she knew what we were doing.

### **Breeding the next generation**

Marine biologist Liam Turner and his partner Hayley Coen have been caretakers at the Centre for six months.

"It's a really good feeling to release a breeding female out into the wild," Mr Turner said.

They hoped Denise might bump into Yoda, another mature green sea turtle that was released from the centre in March.

"They have a shared experience so hopefully they remember each other, maybe give a scratch to each other," Ms Coen said.

It takes females of the vulnerable species' 30 to 40 years to reach reproductive maturity.

"Once she's in the water she's fine, but obviously they don't navigate the land too well with only the one [front] flipper," Mr McCosker said.

### **Fishers urged to be careful**

Mr McCosker says because turtles live underwater and out of sight, humans often forget about them.

"We have to be careful and be mindful; what we do on the mainland affects these gorgeous animals out in the sea," he said.

He urged boaters to slow down and keep an eye out for marine life.

"A propeller had got caught between her head and her flipper, which obviously did massive damage to her," Mr McCosker said.

"Just really watch the water, not just for where you're going but for animal life."





*Above: Mr McCosker is happy Denise is healthy and free, but sad to know of the struggles she will face without her left flipper.  
Image: Erin Semmler.*

Whether it be fishing line, hooks or crab pots, he said the centre saw constant injuries and deaths caused by recreational fishing.

"[Fishing line is] to be there for the next 1,000 years, not the next couple of weeks, it's there forever," Mr McCosker said.

"What we need is a massive movement to get into a biodegradable fishing line. Any form of plastic or nylon, it doesn't belong in the ocean."

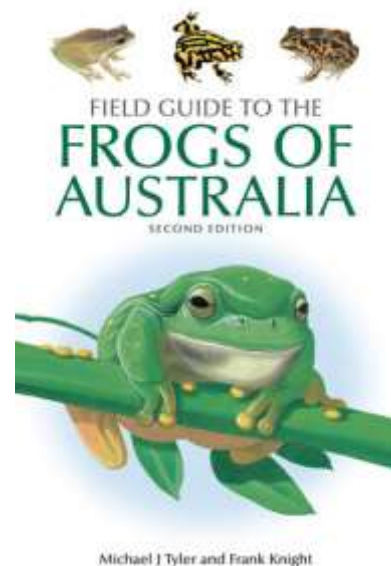
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### **Field Guide to the Frogs Of Australia,** Michael Tyler and Frank Knight

CSIRO, 208 pages. This review by Ian Fraser, *Talking Naturally*' blog, <http://ianfrasertalkingnaturally.blogspot.com.au/>

It is now a decade since this formidable author-artist team produced the first edition of this work, in which they filled the last major gap in 21st century Australian terrestrial vertebrate field guides. Sadly Tyler, who became something of a legend, died just a few weeks ago (26 March). He started his professional life as a laboratory technician at Adelaide University in the school of Human Physiology and Pharmacology, nearly died at one stage from lead poisoning from handling old museum specimen frog labels, studied frogs for his Master of Science in his 30s and spent the rest of his working life in the Zoology Department. He was also an Honorary Associate at the SA Museum for decades, and then Curator Emeritus. He was widely regarded as the doyen of Australian frog specialists.

In 2010, in reviewing the first edition, I wrote: "This is what I want of a modern field guide; three or four species to a page, so the illustrations are large and clear, with enough space for some useful text, including distribution notes (in addition to a map), behaviour (which however really refers only to breeding biology), conservation status, habitat, call and similar species. Where relevant, top or bottom views are also shown, and colour variations as appropriate. You need this book." All that still applies to this edition, though the 227 species covered there have now grown to 258 (including 10 non-natives). Moreover, in his Preface Tyler tells us that he had another ten species waiting to be described and expected more. Sadly he won't be describing them, though others inspired by him doubtless will, but this guide will remain a fitting testament to his massive contribution to Australian frogger. That alone is a good enough reason to buy it.



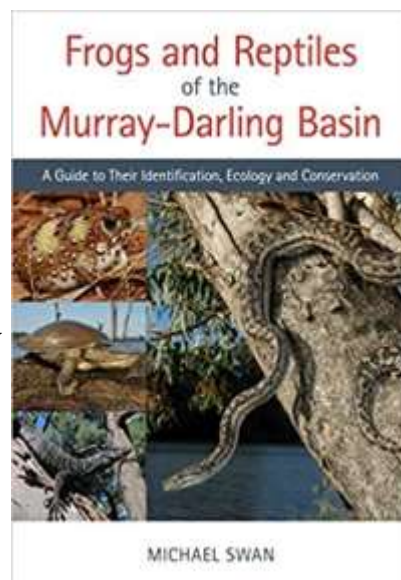
**Frogs and Reptiles of the Murray Darling Basin: A guide to their identification, ecology and conservation**, Michael Swan, CSIRO, 352 pages.  
 This review by Ian Fraser, 'Talking Naturally' blog,  
<http://ianfrasertalkingnaturally.blogspot.com.au/>

Australia seems to be abundantly blessed with good quality field guides to reptiles and frogs; this is good for both herpetologists and the rest of us, because we're pretty well off for reptiles and frogs too. In addition to national guides for both reptiles and frogs (e.g. see the previous review), there are state guides to the reptiles of NSW and Victoria and to the frogs of Queensland, and regional guides to both e.g. the Perth area and the Victorian mallee.

This title chooses to cover the mighty Murray-Darling Basin, a million square kilometres of inland south-eastern Australia. The author, Michael Swan, is former senior reptile and frog keeper for both Melbourne Zoo and Healesville Sanctuary and coordinates the Lilydale High School reptile collection, "the largest school collection of reptiles and amphibians in Australia"! Lucky Lilydale High students I say – how I'd have loved to attend such a school. (I do wonder how many other school collections it's actually competing with though.)

A note here; Michael is not to be confused with Gerry Swan, co-author of excellent guides to both national and NSW reptile field guides. This book completely overlaps a previous CSIRO title, Reptiles of the NSW Murray Catchment, though that one, with a smaller catchment area and no frogs to consider, was able to offer quite a lot more detail per species. This of course raises a perennial question as to how many field guides we need for a given group of animals, though even the burgeoning herpetology field is relatively modest compared with the five major Australian national bird field guides. I some time ago decided that only the market can meaningfully answer that conundrum.

So, this book? It's good, as I'd expect from CSIRO. It begins excellently, with an introduction to each of the basin's 22 sub-catchments, with useful but succinct information on their geography, towns, vegetation, significant wetlands and numbers and key species of 'herps'. Species maps are good, large enough to be clear, though this is not always true for the photos unfortunately. There is a brief introduction to each family and genus, and the expected species notes on size, description, habitats, notes and similar species (mostly all just single-sentence paragraphs) and sub-catchment occurrence. If your 'thing' is herpetology you'll probably want to add this to your library, and if you live in the catchment it's probably a worthwhile investment. As I said, it's mostly a market decision, but if you're interested in such a guide you wouldn't be disappointed in this purchase.



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