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#### VOLUME 42February 2019

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**Front Cover Photo:** *Diuris pardina* in Kosciuszko National Park.

Courtesy of Amy Rowles

#### ECA Office Bearers 2018-2019

President: Belinda Pellow president@ecansw.org.au

1st Vice-President: Stephen Ambrose

2nd Vice-President: Danny Wotherspoon

Secretary: Adam Greenhalgh secretary@ecansw.org.au

Treasurer: Andrew Lothian treasurer@ecansw.org.au

Councillors: Martin Denny Alison Hunt Veronica Silver Daniel McDonald Narawan Williams Jason Berrigan Ashleigh McTackett John Travers

Administration Assistant: Membership Officer: Amy Rowles admin@ecansw.org.au

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#### ECA COUNCIL MEETINGS

The ECA Council meet every three months to discuss and deal with any current business of the association. Any member who wishes to view the minutes from any of the ECA council meetings may do so by contacting the Administration Assistant Amy Rowles admin@ecansw.org.au

#### Message from the Acting President

Dear Members,

I am writing this message on a cold winter's day (strange, considering that this issue of Consulting Ecology is February 2019!). It is equally unusual that I am the one writing it! However, I am keeping the seat warm for Belinda Pellow, who has temporarily stepped down from Presidential duties while she recovers from hospital surgery and receives post-operative treatment. On behalf of all ECA members, I wish Belinda a speedy and complete return to good health so that she can once again be at the head of the ECA Council table.

#### **Annual Conference and Workshops**

While you may not have heard from the ECA Council for a while, we have been really busy organising events and liaising with the NSW Government on your behalf.

Make sure that you register quickly for the ECA Annual Conference, annual general meeting and workshop that will be held at the Hunter Valley Retreat in Quorrobolong on 25-26 July 2019. The theme of the conference is BAM Assessment and Stewardship Site Management (25 July). We have a great line-up of speakers from the Office of Environment and Heritage (OEH) and Biodiversity Conservation Trust (BCT). Other OEH and BCT officers will also be present to mingle with the crowd on the day, so it will be a perfect opportunity for ECA members to corner a public servant and ask those difficult questions over lunch or a tea break!

But wait, there's more! (No, I'm not about to offer you a free set of steak knives). Stick around after the conference because on the following day we have a Nest Box Workshop. I'm honestly really excited about this one because the workshop presents latest research on nest box design and placement presented by university researchers, government scientists and ecological consultants.

Many thanks to ECA councillors, Daniel MacDonald, Jason Berrigan and Narawan Williams, in particular, for organising the topics and speakers for both of these events.

There are other workshops at various stages of planning, including one on Camera Trapping, Orchid Identification and Application of Environmental DNA in ecological consultancy. You will hear more about these over the coming months.

#### Liaison with OEH

The ECA Council has spent a considerable amount of time and resources over the last 12 months liaising with OEH and the BCT about the Biodiversity Offsets Scheme and the administration of BAM. This has been mostly in response to numerous concerns that ECA members have expressed to the Council about the BAM process. Belinda Pellow took up the challenge of liaising with OEH from the start of her ECA presidency. This has resulted in numerous email conversations and a few *ad hoc* meetings between representatives of the ECA and OEH. The clear message that the ECA Council is receiving from members is one of frustration, anger and confusion about aspects of the BAM process. The ECA now has an agreement to meet with OEH to discuss these problems on a regular basis (quarterly in the immediate term, probably biannually in the longer-term). The first of these regular meetings was held on 30 May when Alison Hunt, Martin Denny and I met with OEH Officers to discuss grievances made known to the ECA Council by members who are also BAM Accredited Persons. It was a positive meeting, but there is still a long way to go to smooth out the scheme's teething problems. As an ECA member you should have received an ECA Information Email in early June with a summary of the outcomes of that meeting. The next meeting with OEH (or its new incarnation) will be in late September/early October, so please send us topics for us to raise on your behalf.

#### **University Student Grants**

The ECA received 13 applications for student grants this year, all of which were a high standard. But at the end of the day there could only be three winners. These were:

**Conservation Grant** (\$2,000): Chantelle Doyle, University of NSW. Project: Ecology of the critically-endangered *Hibbertia spanantha*.

**Ray Williams Mammal Research Grant** (\$2,000): Amy Rowles, Western Sydney University. Project: Seasonal importance of high-elevation habitat for Australian bats.

**Terrestrial Ecology** (\$1,000): Grant Webster, Macquarie University. Project: The ecology of the newly described and endangered frog, *Uperoleia mahonyi*.

Thanks to John Travers, Alison Hunt and Jason Berrigan in joining me to select the successful projects this year. Thanks, also, to John for his generous donation of the Terrestrial Ecology Grant. We definitely wish all the applicants the very best with their projects!

#### ECA Facebook Page

The ECA's official Facebook has been up-and-running since December 2018. It's your opportunity to share information and techniques, seek assistance from peers and discuss the application of planning and biodiversity legislation. Check it out at the following link: <u>https://www.facebook.com/NSW-Ecological-Consultants-Association-2195001584086048/?ref=py\_c</u>. Many thanks to Jason Berrigan for setting up the Facebook page and acting in the role of page administrator.

#### **Consulting Ecology**

#### We need more articles!

I frequently (and I really do mean "frequently") hear ECA members say they really look forward to receiving their regular copy of *Consulting Ecology*. It is a valuable source of information, as well as containing really great pictures and a few articles of light and collegiate entertainment. It keeps us all in touch with one another and, more importantly, highlights some of the professional challenges and solutions that you may have thought were relevant just to you.

However, Consulting Ecology can only be compiled and despatched if we have material to put in it. You will have noticed that this edition is slimmer and later than usual. That is because Amy Rowles (ECA Admin Assistant) has not been receiving material from you (yes - YOU!) for inclusion in the journal. I realise that we are all extremely busy, but please send your interesting observations, comments about the ecological industry, photos of flora and fauna (camera trap photos are really interesting!), research articles, funny stories, in fact anything that is relevant to ecological consultancy. If Amy doesn't receive enough material for each issue, then that will definitely mean slimmer issues and delayed deliveries to your mail box.

See you all at the annual conference and workshop in July. In the meantime, happy consulting!

Oh, and don't forget to volunteer to stand for election to the ECA Council at this year's annual general meeting. It's a really rewarding experience. Everyone has a lot to offer, no matter how experienced they are, or how confident they feel about being involved. I'm sure that a few existing councillors would like a well-earned rest, and it is always great to have a few people on board with a fresh outlook and a range of new skills.

Dr Stephen Ambrose Acting President.



#### ECA RESEARCH GRANT WINNERS 2019

Terrestrial Ecology Grant: (\$1000)

Grant Webster, Macquarie University - The ecology of the newly-described and endangered frog, *Uperoleia mahonyi* 

Ray Williams Mammal Research Grant: (\$2000)

Amy Rowles, Western Sydney University – Seasonal importance of high-elevation habitat for Australian bats

ECA Conservation Grant (\$2000):

Chantelle Doyle, University of NSW - Ecology of the critically-endangered *Hibbertia spanantha* 



If you have 2nd hand ecological equipment that you would like to sell or would like to purchase you can place an ad in this newsletter. Free for members or \$40 for non -members. Contact admin@ecansw.org.au.

## EUROKY

#### Euroky: ability of an organism to adapt to changes in the environment

If you have any interesting observations or useful hints and information that you would like to share in the euroky column, please forward them to the newsletter editor or administration assistant to be included in the next edition.

#### HUNT FOR THE COCONUT CRAB

#### Martin Denny

The Coconut Crab (*Birgus latro*) is considered to be the largest land-living arthropod in the world, growing up to 4.1 kg and 1 metre in size. Luckily most are smaller, which is a relief as their pinchers are large and can inflict considerable pain if they latch onto an unsuspecting hand. Coconut Crabs are found throughout the Indian Ocean and the central Pacific Ocean, and are closely associated with coconut palms. They were once found in Australia but have long disappeared. The crabs range as far west as Zanzibar and east to the Cook Islands. Not all islands have these critters, but the Cook Islands, along with Christmas Island, are known to support large populations.



A Coconut Crab shelter in the makatea showing the coconut husk lining

My experience of Coconut Crabs is mainly from one of the southern Cook Islands, Mauke Island. Here, they are known to occur, but not in large numbers. Locals will search for them at night when the crabs exit their day-time shelter within the rocky foreshore composed of fossilised coral (makatea). On other islands they burrow within the sandy soils near coconut palms. On Mauke Island they line a hollow in the makatea with coconut husk fibre.

Although they will eat coconuts they also forage for many other food items and will take them back to their rock crevices, hence the alternative name 'robber crab'. They have been known to kill and eat ship rats.

Coconut Crabs are related to terrestrial hermit crabs and the young utilize empty shells for protection, but the adults develop a tough exoskeleton on their abdomens.



Young Coconut Crab with protective shell

I was eager to see if I could photograph a coconut crab on Mauke Island and set out some open coconuts within the makatea and sat and waited. Before long, one emerged from its rock crevice and carefully started to eat the coconut, this gave me as good a picture as I was going to get. No use trying to catch it as it quickly scuttled away when I moved (and I like my fingers as they are).



Success, a Coconut Crab feeding on opened coconuts

The crabs are more common on some of the other Cook Islands, particularly the northern group. I was fortunate to travel to three such islands and, on Manahiki, I was given a meal that included Coconut Crabs. The large pinchers and legs contain much meat and the internal body organs are considered as 'crab caviar'. For the locals such a treat is boring and tinned spaghetti is better fare. The black piece of 'bread' is fried cassava and coconut cream.



The capture and eating of Coconut Crabs has been common throughout Polynesia, particularly for tourists. However, this has been over-exploited and is now banned on many of the islands.

On some of the remote northern Cook Islands there are restrictions on the taking of crabs and other food items as the islands are only serviced by freighter every 2 to 3 months. Consequently, the residents are dependant on what resources occur on their island. Conservation is part of their lives - a lesson for us well supplied Australians.



#### **ROCKY CREEK DAM TUNNEL**

#### Veronica Silver

Assessment of a 360 m long constructed tunnel at Rocky Creek Dam (Dorroughby, northern NSW) in June 2016 revealed occupation by a population of Eastern Horseshoe Bats (Rhinolophus megaphyllus). Eastern Horseshoe Bats are cave roosting specialists and prefer large cave and tunnel systems. Roosts of the Eastern Horseshoe Bat often have a restricted entrance with a narrow vertical drop (Churchill 2009). The bats were utilising a concrete ventilation shaft (gas vent) to enter and leave one end of the tunnel while access out of the other end was prevented by a gate with fine mesh which was designed to prevent human access (refer Plate 1). This gate was most likely installed in the 1980s therefore access via this end of the tunnel had been unavailable to the bats for at least 30 years. Works were proposed in the vicinity of the gas vent therefore GeoLINK recommended that two entry/ exit points be available to the bats. The gate therefore needed to be modified so that the bats had an alternative access to the tunnel during proposed works.

Following a literature review and discussion with Greg Ford, it was suggested that the existing gate could be modified by creating a horizontal 'slot' of minimum 200 mm high to allow bat egress. In the Pilliga region Murphy (2014) observed horseshoe bats leaving and entering a cave via a 200 mm high x 600 mm wide gap between an installed gate and the cave roof. Studies by Slade and Law (2008) found numbers of horseshoe bats declined significantly (with a significant increase in the number of aborted exit and entry flights) when gates to caves were installed with 125 mm spacings; with bat numbers and behaviour largely unaffected by gate spacings of 300 mm and 450 mm. 5

Due to project safety requirements, the proposed design of 200 mm width was not accepted, with a maximum slot height of 160 mm permitted. As the gas vent utilised by bats had an exit width of approximately 150 mm, a 160 mm slot was deemed acceptable as this was the maximum size conducive to safety requirements.

The new gate was installed in September 2016 with the following dimensions (refer Plate 2):

- Slot height: 160 mm
- Slot length: ~1600 mm
- Surrounding mesh dimensions: 190 mm x 190 mm

A site inspection in December 2018 recorded 102 Eastern Horseshoe Bats exiting the tunnel through the new gate, with bats seemingly passing through the slot with ease and utilising the full length of the slot to leave the tunnel. Two bats were also observed flying through the mesh to exit the tunnel. On this basis, the altered gate dimensions are clearly acceptable for horseshoe bats and could be adopted for similar situations where security or safety gating is required. A reduction to any width of less than 150 mm would be advised against on a precautionary basis.

#### **References:**

Churchill S.K. (2009). *Australian bats- Second Edition*. Sydney: Allen & Unwin.

Murphy, M.J., (2014). Roost caves of the Eastern Horseshoe Bat Rhinolophus megaphyllus Gray, 1834 (Chiroptera: Rhinolophidae) in the Pilliga forest in northern inland New South Wales, Australia. *Australian Zoologist* vol. 37(1).

Slade, P.S. & Law, B.S. (2008). An experimental test of gating derelict mines to conserve bat roost habitat in south eastern Australia. *Acta Chiropterologica* 10(2):367-376.



Plate 1 The bat 'unfriendly' former gate which restricted entry and egress



Plate 2 Bat-friendly redesigned gate with 160 mm high entry slot and large mesh (190 mm x 190 mm)

#### **UPCOMING ECA EVENTS**

#### ECA ANNUAL CONFERENCE

Date: 25-26 July 2019
Thursday conference: BAM Assessment and
Stewardship Site Management
Friday workshop : Nest-box workshop
Location: Hunter Valley Retreat, Quorrobolong NSW
Register: http://www.ecansw.org.au

ECA Camera Trap Workshop Date: 6 September 2019 Presenter: Paul Meek, DPI Location: Asquith Rugby Leagues Club, Waitara. Register: http://www.ecansw.org.au

#### PROPOSED FUTURE ECA WORKSHOPS

Orchid Workshop

Date: August 2019 Location: TBA Register your interest: admin@ecansw.org.au

eDNA Workshop
 Date: 2019
 Location: TBA
 Register your interest: admin@ecansw.org.au

## Vegetation Community Workshop—allocating PCT's Date: 2019 Location: TBA Register your interest: admin@ecansw.org.au

#### NON ECA EVENTS

#### • ESA19 Conference

Date: 24-29 November 2019 Location: Launceston, Tasmania Theme: Ecology: science for practical solutions Details: www.esa2019.org.au

## • EIANZ: Special forum on using eDNA for wildlife detection

Date: 13 August 2019 Location: Maiden Theatre, Mrs Macquarie Rd, Sydney. Details: www.eianz.org  RZS Forum: The Dingo Dilemma Date: 7 September 2019
 Location: Australian Museum, Sydney
 Details: www.rzsnsw.org.au

#### **ECA Membership Report**

In total we have 189 members, comprised of 141 Practising Ecological Consultants, 16 Associate (Consultants), 19 Associate (Government Ecological/ Environment Officer), 7 Associate (Non-practising), 1 Associate (Subscriber) and 5 Students. We currently have 11 applicants and have 11 new members and they are introduced below:

- · Shawn Ryan (Associate Ecological Consultant)
- Sarah Stevens (Practising Ecological Consultant)
- Kylie Bridges (Associate Ecological Consultant)
- Mathew Doherty Practising Ecological Consultant)
- Greg Chapman (Associate Non-practising)
- Emma Gray (Associate Ecological Consultant)
- · Addy Watson (Associate Ecological Consultant)
- Angela Carpenter (Associate Ecological Consultant)
- Yogesh Nair (Associate Ecological Consultant)
- · Vanessa Gorecki (Student)
- · Roxanne Zybenko Keane (Student)

#### RECENT LITERATURE AND NEW PUBLICATIONS

#### Journal Articles

**The responsibilities of ecological consultants in disseminating outcomes from threatened species surveys: a call to arms.** By Stephen Bell (*Australasian Plant Conservation* 27(2): 3-6).

Abstract: Ecological consultants are often in the enviable position of being paid to botanically explore and seek out threatened plants. Yes, there are attractive jobs in remote or pristine locations where few botanists have trod before, but there are also less desirable projects in weed-infested remnants across highly fragmented landscapes or in heavily urbanised environments. Both offer the potential to uncover important information on threatened plants. But are we, as consultants, fulfilling our responsibilities for the cause of conservation by disseminating the outcomes of threatened species surveys and monitoring? Habitat suitability, live abundance and their link to road mortality of Tasmanian wildlife. Hanh K. Nguyen , Matthew W. Fielding , Jessie C. Buettel and Barry W. Brook (2019). Wildlife Research 46(3) 236-246 https://doi.org/10.1071/WR18128

Attenuated post-fire fauna succession: the effects of surrounding landscape context on post-fire colonisation of fauna.. Angela Simms , Meaghan Scott, Simon Watson and Steve Leonard (2019). Wildlife Research 46(3) 247-255 https:// doi.org/10.1071/WR18131

**Confirmation of little eagle (Hieraaetus morphnoides) migration by satellite telemetry.** Renée Brawata, Stuart Rae, Bernd Gruber , Sam Reid and David Roberts (2019) Australian Journal of Zoology 66(4) 247-250 https://doi.org/10.1071/ ZO18060

**Time budget of the squirrel glider (Petaurus norfolcensis) in subtropical Australia.** David J. Sharpe and Ross L. Goldingay (2019). Australian Journal of Zoology 66(4) 251-260 https://doi.org/10.1071/ZO18049

Gut content and stable isotope analysis of tadpoles in floodplain wetlands. J. F. Ocock, K. J. Brandis, J. Wolfenden, K. M. Jenkins and S. Wassens (2019) Australian Journal of Zoology 66(4) 261-271 https:// doi.org/10.1071/ZO18043

Are koalas detected more effectively by systematic spotlighting or diurnal searches? Lachlan Wilmott, Dympna Cullen , George Madani , Martin Krogh and Kylie Madden (2018). Australian Mammalogy 41(1) 157-160 https://doi.org/10.1071/AM18006 Submitted: 26 June 2017 Accepted: 4 May 2018 Published: 7 June 2018

Abstract: Koalas (*Phascolarctos cinereus*) are difficult to detect due to their cryptic nature, occurrence at low densities and broad distribution. We compare the relative effectiveness of two common, direct survey techniques used to detect koalas: spotlighting and diurnal searches. Seventy-six 2-ha sites were surveyed using both spotlighting and diurnal searching. Each site was surveyed for 0.5 person-hours, such that search area and effort were equal. In this study, spotlighting was found to be 3.25 times more effective at detecting koalas than day searches. Therefore, where access and terrain allows, spotlighting surveys offer a significant advantage over diurnal searches in the detection of koalas. The role of Lantana camara in areas of bell miner (Manorina melanophrys) associated dieback and its implications for terrestrial mammal and insectivorous bat populations. Penny E. Wright, Kathryn T. A. Lambert and Paul G. McDonald (2018) Australian Mammalogy 41(1) 49-56 https:// doi.org/10.1071/AM17003

Movement of small mammals through a roadunderpass is facilitated by a wildlife railing. Ross L. Goldingay, Brendan D. Taylor and Jonathan L. Parkyn (2018). Australian Mammalogy 41(1) 142-146 https://doi.org/10.1071/AM17056

Quantitative interpretation of images of long-nosed potoroos at baited camera-traps: defining a 'visit'. Peter Jarman and Michael Driessen (2018). Australian Mammalogy 41(1) 147-149 https://doi.org/10.1071/ AM17057

**Can a common snake provide conservation insights?** Ross L. Goldingay (2019). Australian Journal of Zoology 66(4) 279-285 https://doi.org/10.1071/ ZO18079

(Cryptophis Abstract: The small-eyed snake nigrescens) is a common non-threatened species in eastern Australia. It coexists with the threatened broadheaded snake (Hoplocephalus bungaroides), a species adversely affected by habitat disturbance and subject to poaching. The small-eyed snake is a habitat generalist and not subject to poaching. It may prey on other snakes, including the broad-headed snake, and, like the broad-headed snake, may shelter under thermally favourable loose rocks during the cooler months of the year. This may lead to interactions between these species due to the limited availability of such rocks, and possibly exacerbate other threats to the broad-headed snake, such as poaching and the loss of thermally favourable rocks. I conducted repeat surveys for snakes at 64 rock outcrops in Royal National Park over a 16year period. I predicted that site use by the small-eyed snake would not be influenced by a disturbance variable previously documented to influence site use by the broad-headed snake. Observations were consistent prediction, confirming with this the unique vulnerability of the broad-headed snake. I used my long -term data to analyse the co-occurrence of the two species. The broad-headed snake was detected as frequently at sites with and without the small-eyed snake, suggesting that these species occupy outcrops independently of each other. Therefore, interactions with the small-eyed snake will not reduce the effectiveness of habitat restoration for the broad-headed snake in Royal National Park.

#### **Recent Book Releases**

Information Source: CSIRO Publishing Website http://www.publish.csiro.au

#### Title: Cats in Australia: Companion and Killer

Author: John Woinarski, Sarah Legge, Chris Dickman RRP: \$59.99

No. Pages: 344 Publisher: CSIRO Publishing Date: June 2019



Across the world, cats are loved as pets or are kept or tolerated for their role in controlling some animal pests. But cats,

both pets and feral, also kill many native animals and this toll can be enormous. Cats have been remarkably successful in Australia, spreading pervasively across the continent and many islands, occurring in all environments, and proving to be adept and adaptable hunters. A large proportion of Australia's distinctive fauna is threatened and recent research highlights the significant role that cats play in the decline and extinction of native species.

#### Title: Community-based control of invasive species.

Author: Paul Martin , Theodore Alter, Don Hine, Tanya Howard.

RRP: \$99.99

No. Pages: 288

#### Publisher: CSIRO Publishing

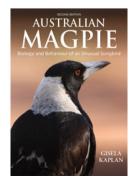
#### Date: June 2019

Invasive species are among the greatest challenges to environmental sustainability and agricultural productivity in the world. One of the most promising approaches to managing invasive species is voluntary citizen stewardship. However, in order for control measures to be effective, private citizens often need to make sustained and sometimes burdensome commitments.



#### Title: Australian Magpie: Biology and Behaviour of an

<u>Unusual Songbird</u> Author: Gisela Kaplan RRP: \$45.00 No. Pages: 280 Publisher: CSIRO Publishing Date: May 2019



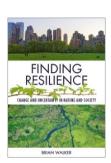
Title: <u>Wildlife of the Otways and</u> <u>Shipwreck Coast</u> Author: Grant Palmer RRP: \$49.99 No. Pages: 304 Publisher: CSIRO Publishing Date: April 2019





Title: Finding Resilience: Change and uncertainty in

<u>Nature and Society</u> Author: Brian Walker RRP: \$59.99 No. Pages: 168 Publisher: CSIRO Publishing Date: March 2019



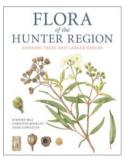
An analysis of how ecosystems, societies and people cope with disturbance and adversity.

Floods, fires, famines, epidemics and disasters of all kinds are on the increase, and as their frequency rises so does the call for greater resilience. But what does that mean? The word is used differently in psychology, ecology, economics and engineering and runs the risk of becoming meaningless jargon. This would be most unfortunate because, if we are to successfully navigate very real and dangerous global trends, it is resilience that needs to be understood and fostered.

Finding Resilience is international in scope and unravels how ecosystems, societies and people cope with disturbance and adversity. An authoritative but plain English account which is based on the experiences of researchers, the fascinating stories from around the world reveal what resilience is, how it works in different kinds of systems, how it is expressed, and how it can be gained and lost.

#### Title: Flora of the Hunter Region

Author: Stephen Bell, Christine Rockley and Anne Llewellyn RRP: \$79.99 No. Pages: 136 Publisher: CSIRO Publishing Date: March 2019



A botanical identification guide which combines art and science to describe the 54 endemic trees and large shrubs of the Hunter region.

The Hunter Region, between the Hawkesbury and Manning rivers in eastern New South Wales, hosts a rich diversity of vegetation, with many species found nowhere else. Spanning an area from the coast to the tablelands and slopes, its rainforests, wet and dry sclerophyll forests, woodlands, heathlands, grasslands and swamps are known for their beauty and ecological significance.

#### SCAT AND HAIR ANALYSIS: DO'S, DON'TS AND POSSIBILITIES

Georgeanna Story, Scats About Ecological scatsabout@gmail.com



Amongst the many fauna monitoring tools available for biodiversity surveys, scat and hair analysis is frequently considered a valuable technique. Most practitioners include one or both in surveys for a range of reasons, such as to detect species, to assess species abundance or to determine predation impact (eg. Glen et al, 2006; Norton et al 2006). Survey protocols depend on the purpose but often involve systematic or incidental collection, and identification can either occur on site or be sent to specialised consultants. As one of those consultants it seemed appropriate to give a quick overview of best practice for collection and storage to ensure that samples are in the best possible condition and those surveying stay as safe as possible.

When dealing with scats it's best to treat all samples as a potential source of tapeworm eggs from the hydatid, *Echinococcus granulosus*. Hydatid infection in carnivores is wide spread across Australia and eggs persist for long periods in the environment. Infection of humans is caused through ingestion or inhalation of eggs, so use gloves and avoid breathing in dust from and around the scat. It is best to secure samples in separate paper bags and allow the scat to dry out, reducing the likelihood of fungal growth. Fungal growth damages the scat and its contents, reducing the probability of a confident identification. It also allows the sample to be directly heat treated before handling. Cooking scats at 100°C for several hours kills the eggs and allows for safe handling. We are all familiar with the odour of predator scats and how useful it can be in scat identification but please make sure that the scat has been heat treated before having a sniff! If you are sending off scats for analysis ensure all samples are clearly labelled and they are safely packaged to minimise damage during transport. Also, be kind to the postman and contain any potent smells.

The collection and storage of hair samples on wafers, tapes or as tufts (Figure 1) is a little less fraught with safety concerns but there are still a couple of points to consider. Often there are very few hairs collected on wafers and tapes, so for identification, every hair counts. If wafers and tapes get overly hot the glue will melt slightly and the hairs sink in. When the glue resolidifies, the hair is immersed in, rather than sitting on top of the glue. In such situations it is very difficult to extract hairs, especially without damage and this effects the probability of identification. Such treatment is especially problematic for short fine hairs from species such as Pseudomys, Antechinus and Petaurus. So if possible, don't leave the samples in hot cars or in tubs in the sun. In situations where hair is being collected from an animal, a tuft should be collected on the back between the shoulder blades. There are a number of different types of hairs across the body and they can differ in their diagnostic properties. Between the shoulder blades generally gives the greatest density of diagnostic hairs.







Figure 1. Example of funnel, tube and wafers in use.

Every survey technique comes with certain limitations and scat and hair analysis is no exception. Scat detectability varies with species or sites and different hair traps target different animals (Lindenmayer et al 1999). Identification ability and confidence can also vary. For scats and pellets, the more diagnostic features the sample contains the better the chance of a confident identification (Figure 2). In addition to the typical characteristics of size, shape and smell we can also use features of the sample content and the presence of grooming hairs. The fresher and more intact the sample the better but there are times when a single hair in a crumbled mass of scat can give you a positive identification. There are also some species that are more likely to have grooming hairs in their scats. Brushtail possums, quolls, swamp wallabies and water rats are often very generous.







Figure 2. Example of the size and shape differences in Australian mammalian scats

When it comes to identifying hair (Figure 3), we can also run into limitations when the hairs come from parts of the body that possess fewer diagnostic features, such as the muzzle, legs and tail. There are also closely related species that have hair with very similar characteristics. In these situations, the diagnostic features vary in a tendency of a particular characteristic rather than a definite difference. To differentiate such species the more diagnostic hair the better but there are times when you just can't pick the difference, or feel a probable identification is more suitable. I imagine most of you would have received results that have *Antechinus* sp. or *Rattus* sp. Unfortunately, this is a limitation of the technique (Lobert et al. 2001).



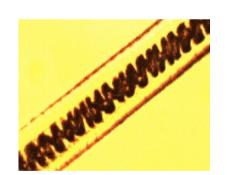




Figure 3. Example of the size and shape differences in Australian mammalian hair

Advancements in molecular techniques are proving useful in scat and hair identification (eg. Berry et al. 2007). Epithelial cells in the scat coating can provide identification of the predator scat identity and screening of content can identify prey. If using hair, extraction of genetic material from the follicle can identify the species. These genetic methods also come with limitations. There are times when the analysis is inconclusive due to the quality of DNA or issues with contamination. Cost can also be a limiting factor for many projects, especially if analysis is outside the typical suite of species. Continuing development will help to make this method more affordable in the future.

With this combination of traditional methods and new technologies available, scat and hair analysis will continue to be an attractive tool for biodiversity monitoring.

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#### THE GREENSPACE BIRD CALCULATOR: A TOOL TO HELP MONITOR AVIAN BIODIVERSITY IN URBAN GREENSPACES

Corey T. Callaghan

#### Background

Urban greenspaces (e.g., cemeteries, parks, habitat corridors, urban wetlands) are constantly subjected to development. Hence, the question often needs to be asked: how many species are in that local urban greenspace? This seems simple. But is it? If you had to provide an estimate – with lower and upper bounds – of the number of birds in your local park, would you be able to do so? This question is also becoming increasingly important as researchers and society are realizing the importance of conserving urban biodiversity. So, if we don't know how many species are in a given park then it is difficult to prioritize these greenspaces and make cases for preservation of their existence to the various policy-makers and stakeholders. Hence this was the motivation for creating the Greenspace Bird Calculator: a web-app aimed at enhancing our collective knowledge of bird diversity in urban greenspaces.

The Greenspace Bird Calculator relies on eBird data. eBird is a global citizen science project with > 600 million observations and > 400 thousand participants. It works by enlisting birdwatchers to submit observations of birds in the form of 'checklists', and they make all their data freely accessible to researchers and practitioners. Often, these data have a bias towards urban areas – the areas where people live. This is problematic for some ecological questions, but this can be advantageous if the focus is on ecological questions within urban areas. The web-app depends on two key inputs: eBird data and delineated urban greenspaces (Figure 1). It also relies on previously published research which investigates the validity and utility of eBird data in urban greenspaces.

#### Providing basic information on bird communities

Currently, the Greenspace Bird Calculator provides (Figure 2): (1) the total species richness, shown as a speciesaccumulation curve, (2) a subsetted bird community species richness, where any species on < 5% of all eBird checklists were removed from the analysis, (3) the number of eBird checklists, shown through time, and (4) a list of species and their reporting rate – the percentage of eBird checklists that they are reported on. We statistically assess the available data in eBird and provide accessible interpretation for inclusion into urban greenspace management and planning <u>https://coreytcallaghan.github.io/urban\_greenspaces/#/</u> Here is an example, looking at Randwick Environment Park <u>https://coreytcallaghan.github.io/urban\_greenspaces/#/</u>greenspace/aus-nswrandwick environment park and here is a local oval <u>https://coreytcallaghan.github.io/urban\_greenspaces/#/</u>greenspace/aus-nswrandwick environment park and here is a local oval <u>https://coreytcallaghan.github.io/urban\_greenspaces/#/</u>greenspace/aus-nswrandwick environment park and here is a local oval <u>https://coreytcallaghan.github.io/urban\_greenspaces/#/</u>greenspace/#/

#### Relevance for the consulting world

I foresee this tool being used by a number of end-users, including the public, local councils, and ecological consultants. All of which might at some point be interested in the bird diversity in their local parks. For example, these data can be used to (1) understand the differences in bird diversity among a handful of local parks and greenspaces, and (2) better understand which species are likely to occur in the park for more prioritized and targeted surveys. The information presented is also provided as 'raw data' so that the figures can be downloaded and used in various reports.

#### Next versions

The Greenspace Bird Calculator is an iterative tool, and this is the first version. You may notice that not many

greenspaces have been fully delineated yet. This is why we have provided an adaptable framework so anyone can delineate a greenspace and send it to us for inclusion when we re-run the data algorithms (quarterly). At the moment, this is just the first release of the web-app, and we aim to look at trends over time in a more rigorous manner in the future. In the first iteration of the GBC we disregard inter and intra-annual changes in species richness. In highly migratory systems (e.g., northern hemisphere) there are distinct seasonal differences in urban greenspace usage by birds. In Australia, these differences are relatively minor for terrestrial species (i.e., excluding shorebirds). However, we will continue to develop the web-app, allowing end-users to investigate temporal differences if interested, but this relies on sufficient eBird data from all seasons to investigate with confidence. eBird data are relatively new to Australia, and thus many sites are lacking long-term datasets, limiting our ability to track species richness through time. However, given the current uptake of the eBird project in Australia, and globally, we anticipate that inter and intra-annual changes in species richness will be feasible in the near future. It is our hope that people will contribute to development of the Greenspace Bird Calculator by (1) delineating more greenspaces, and (2) contributing data from their local greenspaces to eBird. These efforts, combined with the natural growth in eBird data can provide tangible data to better understand how birds are using urban greenspaces.

#### Conclusions

I believe that this project demonstrates the evolution of citizen science data – taking lots of data and transforming it into tractable results easily conveyed with researchers and the general public alike. It is my hope that the data continues to increase, providing opportunities to further enhance the statistical studies of these citizen science data. For more information on the Greenspace Bird Calculator, please see a recent publication in Australian Zoologist.

Callaghan et al. 2019. <u>The Greenspace Bird Calculator: a citizen-driven tool for monitoring avian biodiversity in</u> <u>urban greenspaces</u>. Australian Zoologist. DOI: https://doi.org/10.7882/AZ.2019.009.

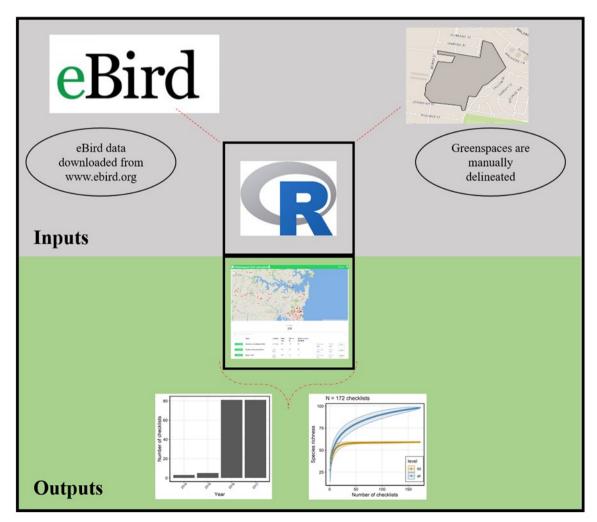
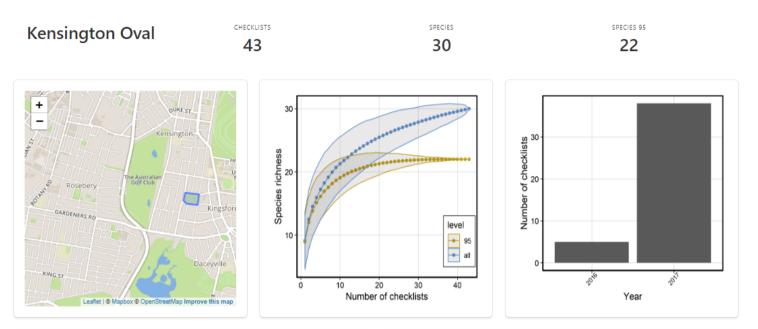


Figure 1. The workflow of the Greenspace Bird Calculator, showing the inputs and outputs, analyzed using R statistical software. Reproduced from Callaghan et al. 2019.

#### 🞾 Greenspace bird calculator🌲



#### Background

This GBC web-app provides summarized data for urban greenspaces throughout the world, working to summarize eBird data. eBird is a large citizen science project with > 500 million observations globally. The purpose of this web-app is to provide council managers, local land management groups, and any other interested parties with detailed information about the species richness of their local urban greenspaces. We have been working on understanding the value of eBird data at an urban greenspace -- see here --- as well as understanding how eBird data can be used to estimate biodiversity metrics within urban greenspaces --- see here. For each greenspace with more than 40 eBird checklists, we present the following:

1.) Bootstrapped species richness accumulation curves

2.) The number of eBird checklists submitted through time

Rank	Species	Reporting rate (%)
1	Noisy Miner	100
2	Rainbow Lorikeet	95.3
3	Pied Currawong	86
4	Rock Pigeon	83.7
5	Australian Magpie	72.1
6	Australian Raven	62.8
7	Welcome Swallow	60.5
В	Australian Ibis	58.1
9	Australasian Figbird	51.2
10	Crested Diseon	A 8 V

### Figure 2. An example of a given greenspace's homepage – showing the various outputs for that urban greenspace. Reproduced from Callaghan et al. 2019.



Figure 3. A Pied Currawong is a common inhabitant of urban greenspaces in Sydney. But is it in your local urban greenspace?

#### ENVIRONMENTAL DNA: A TOOL FOR THE FUTURE IN ECOLOGICAL CONSULTANCY

Dr Stephen Ambrose, Principal Ecologist, Ambrose Ecological Services Pty Ltd stephen@ambecol.com.au

#### INTRODUCTION

Ecological consultancy is on the verge of a new revolution. Some will argue that the revolution is already underway.

A key part of our work is documenting the biodiversity of a site or region under investigation, and important first process before we can even contemplate predicting the impacts of a proposed development or activity. The way in which we define biodiversity has become much more refined, and we rely heavily on DNA analyses to define biodiversity units such as populations, sub-populations, species and sub-species.

But DNA analysis can also help us in another way. There are many flora and fauna taxa that are cryptic and are hard to detect. Flora may only be detectable and identified easily when they are in flower, fauna may be camouflaged, secretive in behaviour, or present in an area from time-to-time (e.g. migratory or nomadic species, or those with large home ranges). Yet, when present in an area, they can leave traces of genomic DNA in the external environment. Environmental DNA (eDNA) is a complex mixture of genomic DNA from many different organisms found in an environmental sample (e.g. air, soil, sediment, aquatic and faecal samples). This DNA can be extracted from an environmental sample through filtering the air or water, from sifting sediments, or from bulk samples. The aim of this DNA extraction is to obtain the most comprehensive DNA-based taxonomic or functional information as possible for the ecosystem under investigation.

Total eDNA contains both intracellular and extracellular DNA. (Taberlet et al. 2018).

Intracellular DNA originates from living cells or living multicellular organisms that are present in the environmental sample. Extracellular DNA results from cell death and subsequent destruction of cell structures, and can be degraded through physical, chemical or biological processes. For example, DNA molecules can be cut into smaller fragments by nucleases. After its release, extracellular eDNA may be adsorbed by inorganic or organic surface-reactive particles such as clay, sand, silt and humic substances. When present in an environmental sample, it can come from unicellular organisms such as bacteria and other micro-organisms that are either in an active state (cells) or in a dormant stage (spores). It may also come from multicellular individuals such as meiofauna (e.g. nematodes, rotifers) or dissociated fragments of larger organisms (e.g. root fragments) and can be in active or dormant stages (e.g. seeds, pupae, pollen). Free DNA molecules can occur in aquatic environments or adsorbed on the surface of different types of organic or mineral particles.

Extracellular DNA is released in the environment, mostly from decaying cells or sloughed material (e.g. tissues, faeces and chemical secretions).

Although eDNA sampling and analysis has been underway for just over 10 years, its uptake for practical purposes has, up to now, been surprisingly slow. Its past application has been restricted largely to academic studies of ecology, paleoecology, archaeology and forensics, and has only more recently become involved in biodiversity management. Some of the reasons for its slow uptake for ecological consultancy work have been the costs involved and, despite recent technological advances, extracting relevant information from eDNA is not straightforward and not exempt from biases. It also involves a team of people working together, including those with ecological knowledge and skills, eDNA sampling skills in the field, molecular biologists in the laboratory who are skilled at producing the DNA sequence results, and those who are skilled in bioinformatics for dealing with massive amounts of sequence data. Nevertheless, universities around the world, and especially in

Australia, are currently training lots of postgraduate students in these techniques, many of which will no doubt enter the ecological consultancy industry, either as specialist ecological consultants, or as sub-contractors offering their specialist services to ecological consultants. This has already begun to happen in parts of Europe, the United Kingdom and the United States. It's beginning to happen in Australia with several commercial molecular biological companies actively advertising their services to the environmental consultancy industry. Therefore, eDNA analysis is likely to become an increasingly important tool in ecological consultancy.

As the older (already established) generation of ecological consultants, we should strive to understand how it works and, if applicable, embrace this new technology. So let us delve deeper into what is involved. In doing so, I will focus my discussion on the relevance of eDNA sampling to ecological consultancy, discuss what is involved in collecting field samples, provide examples of how this approach has been applied, and present some of the pitfalls and biases of eDNA sampling and analysis. I have not delved into the discussion of laboratory analyses of samples (DNA extraction, sequencing, analysis and interpretation of data) other than to give a very brief overview of the methodology; that is something I have left for others who are qualified and experienced in this area to do another time.

#### **OVERVIEW OF AN eDNA INVESTIGATION**

If the aim is to identify the taxa present in an eDNA sample, there are two main approaches, each based on PCR (polymerase chain reaction) of the sample in the laboratory. One can either:

- 1. determine the presence or absence of a single species, using a species-specific approach based on quantitative PCR (single-species identification); or
- 2. engage in shotgun sequencing, which has the potential of revealing the DNA of multiple taxa within a sample (DNA metabarcoding).

DNA metabarcoding involves examining metabarcode sequences amplified from eDNA. A metabarcode consists of a short and a taxonomically informative DNA segment flanked by two conserved segments on either side that serve as primer anchors for the PCR. Shotgun sequencing involves the sequencing of random DNA fragments from a DNA extract, generally using next-generation sequencers. However, taxa identification based on shotgun sequencing is difficult to achieve because it requires high sequencing depths and extensive reference databases for taxonomic assignment (Glenn 2011).

It is likely that the species-specific approach would be the most valuable approach for ecological consultants engaged in development or activity applications, especially when attempting to confirm the presence of a threatened species on the site under investigation. The metabarcoding approach may be of some value in documenting the biodiversity of a site or area, but it is probable that taxonomic reference databases would not be comprehensive enough to allow precise identification of all the DNA recorded in samples.

A third approach, which is less relevant to our main line of work is the metagenomics approach. This is based on shotgun sequencing of eDNA without any targeted PCR, and is used to study the functional characteristics of genomes, mostly of micro-organisms (Simon & Daniel 2011).

The main steps of an eDNA study, showing the three possible approaches are shown in Figure 1 and how they have been applied to three real-life studies are shown in Figure 2. Taberlet *et al.* (2018) warn that each approach can produce possible errors and artifacts, and identify the following parameters that need to be considered:

- 1. the different controls to be included at various steps of the process (extraction negative controls, PCR negative controls, PCR positive controls of known composition, replicated samples, etc.);
- 2. the sampling strategy (how many samples, how many sample replicates, how to spatially distribute the samples, at which time of the year, etc.);
- 3. the sample preservation method and the DNA extraction protocol (should the samples be preserved before DNA extraction, or do they have to be extracted immediately in the field to avoid degradation and/or micro

-organism development; choice of extraction protocol according to the scientific questions; logistics, financial constraints, etc.);

- 4. the protocol of DNA amplification in DNA metabarcoding [which metabarcode(s) to analyse, which multiplexing strategy to adopt according to the number of samples and sequencing platform, etc.];
- 5. the sequencing strategy (should the sequencing be done in-house or outsourced); and
- 6. the strategy for data analysis.

#### ENVIRONMENTAL DNA SAMPLING

#### Where to Sample?

Herder *et al.* (2014) indicate that eDNA studies can be applied to aquatic habitats (ponds, lakes, creeks, rivers, streams, pooled water in depressions), marine habitats, soils and sediments, animal traces (e.g. terrestrial twigs, leaf litter, hair, faeces, shed skin) and environmental samplers (e.g. pollen and honey from bees, blood from leeches and mosquitoes, owl pellets, faeces from predators). Bohmann *et al.* (2014) summarise a range of studies in which eDNA sampling has been used (Table 1).

#### Soil Samples

When soil samples are used as a source of eDNA, several soil cores are collected separately (e.g. Yoccoz *et al.* 2012), or pooled together to obtain a maximum representation of the local biodiver-sity (e.g. Taberlet *et al.* 2012). The samples are usually then frozen before DNA extraction, or are processed directly after sampling (e.g. Taberlet *et al.* 2012).

#### **Faecal Samples**

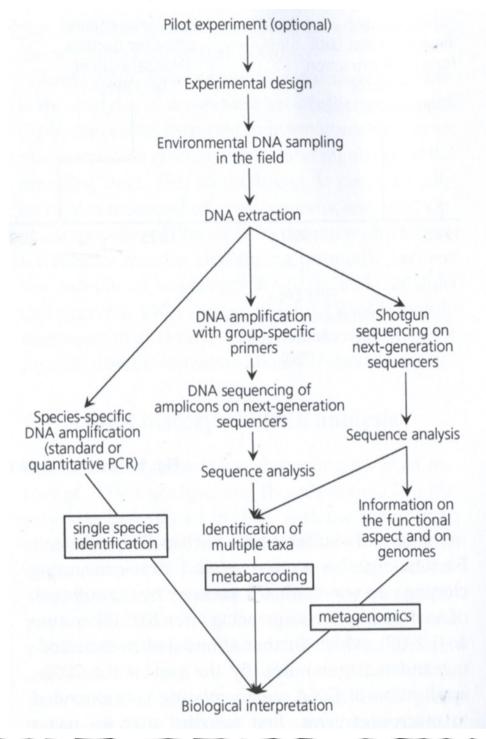
When using faecal samples, the most common strategy consists of collecting the samples individually, and dehydrating them quickly by placing the material into alcohol or into a tube filled with silica gel or a combination of both methods.

#### Water Samples

The strategy for sampling water samples is much more complex. Two main sampling approaches are proposed in the literature, both based on DNA concentration from different volumes of wa-ter.

The first method is based on DNA precipitation using ethanol and sodium acetate and/or cell remains centrifugation (Ficetola *et al.* 2008). Ethanol acts as preservative of DNA, therefore samples can be stored for a few days, or weeks, at room temperature, which can be advantageous when sampling locations are distant from laboratory facilities. The main limitation of this technique is that the sampled volume of water is relatively low. If a species is present at high density, the amount of DNA released in the environment is high and this method would probably allow detection even with a low volume of water (e.g. three times 15 mL, Ficetola *et al.* 2008). However, if the organisms are present at low densities, or if they have limited mobility in the environment, the area where eDNA is present is very restricted. If the sampling points are too far from the eDNA source, the species might not be detected. Therefore, the sampling strategy should be revised, and the number of samples per site should be increased. An alternative sampling strategy consists of taking dif-ferent sub-samples of water from the study area. Those sub-samples are then mixed together for homogenization and a few sub-samples are taken from this large volume of water to be transferred to tubes filled with ethanol and sodium acetate (e.g. Biggs *et al.* 2014; Herder *et al.* 2013a, 2013b; Piag-gio *et al.* 2014). Because the eDNA is not distributed homogeneously in a water, sampling at different points within the study area increases the chance of collecting eDNA released from the target species.

Figure 1 The main steps of an eDNA study, showing the three possible approaches: single-species identification, metabarcoding and metagenomics (from Taberlet *et al.* 2018).



#### For further reading on eDNA:

• ABC News online story has useful information about artificial bat roosts and discusses how eDNA in bat faeces collected from caves is used to identify bat species and estimate the sizes of local colonies.

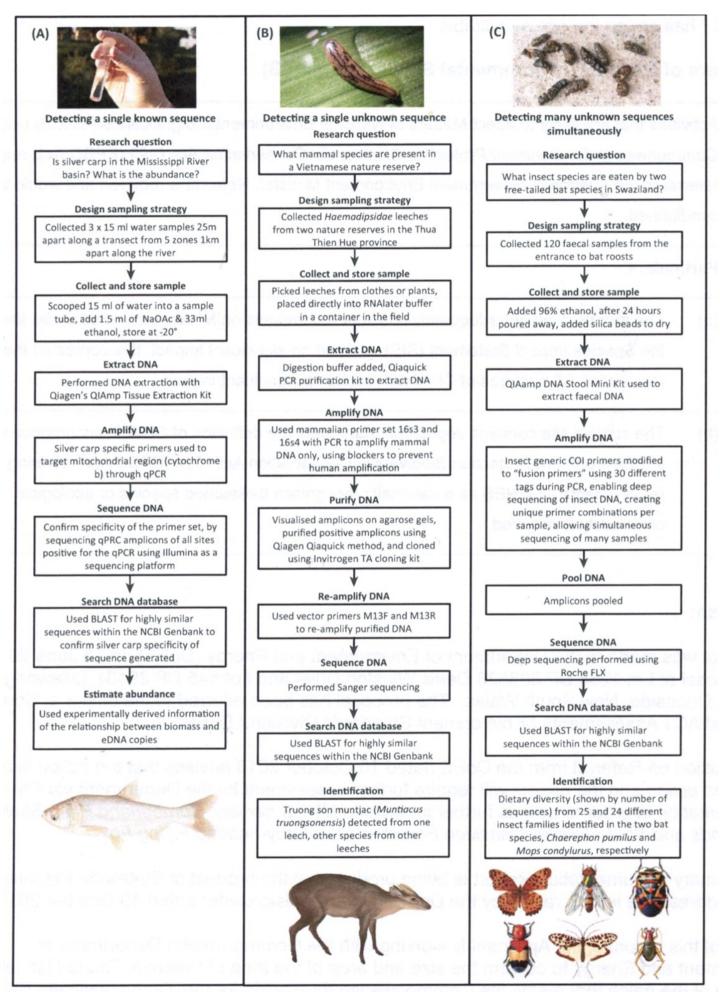
https://www.abc.net.au/news/2018-11-19/ghost-bat-motels-built-near-pilbara-mines/10481728

• eDNA sampling is being used to research Platypus.

https://pursuit.unimelb.edu.au/articles/on-the-dna-trail-of-the-platypus

https://www.abc.net.au/news/2018-11-10/platypus-vulnerable-to-extinction-researchers-say/10477902

### Figure 2 Case studies that illustrate the research questions and the eDNA methods used (from Bohmann *et al.* 2014)



#### Table 1 Examples of the application of eDNA sampling (from Bohmann et al. 2014)

Sample	Application	Studies of importance	Refs
	ential for conservation biology	and policy-making decisions	
Blood meal	Species detection	DNA of rare mammals such as the elusive Truong Son muntjac (Muntiacus truongsonensis) identified in leeches collected in Vietnam	[58]
Faeces	Population genetics	Highly fragmented and isolated populations of giant panda ( <i>Ailuropoda melanoleuca</i> ) were analysed and landscape genetic patterns, divergence time, and population structure identified	[68]
Honey	Species detection	Plant and insect DNA identified in just 1 ml of honey	[69]
Seawater	Species detection	Harbour porpoise ( <i>Phocoena phocoena</i> ) and long-finned pilot whale ( <i>Globicephala melas</i> ) detected in the western Baltic	[30]
Snow	Species detection	Wolf ( <i>Canis lupus</i> ) DNA isolated from blood spots in the Italian Alps and Arctic fox ( <i>Alopex lagopus</i> ) DNA isolated from footprints	[70,71]
Soil	Species detection	Vertebrate mitochondrial DNA (mtDNA) identified in soil samples collected in a zoological garden and a safari park matched to the elephant and tiger inhabitants, respectively	[29]
Applications with pot	tential for ecology (including p	alaeo- and macroecology)	
Cave sediments	Reconstructing past flora and fauna	Extinct biota identified from cave sediment in New Zealand, revealing two species of ratite moa and 29 species of plants from the prehuman era	[42]
Fresh water	Species detection and biomass estimation	Diversity of rare and threatened freshwater fish, amphibians, mammals, insects, and crustaceans was quantified in eDNA from small water samples collected in lakes, ponds, and streams	[28]
Ice cores	Reconstructing past flora and fauna	Plant and insect diversity from the past million years was catalogued from deep ice cores in Greenland	[72]
Nunatak sediments	Reconstructing past flora and fauna	Reconstruction of vegetation from the end of the Holocene Thermal Maximum $[5528 \pm 75 \text{ calibrated years before present (BP)}]$ from bedrock protruding through ice sheets (nunatak sediments)	[43]
Permafrost	Reconstructing past flora and fauna, habitat conservation	Fungal, bryophyte, enchytraeid, beetle, and bird DNA identified in frozen sediment of late-Pleistocene age (circa 16 000–50 000 years BP)	[73, reviewe in 74]
Saliva/twigs	Species detection	DNA in saliva on browsed twigs identified browsing moose ( <i>Alces alces</i> ), red deer ( <i>Cervus elaphus</i> ), and roe deer ( <i>Capreolus capreolus</i> ), amplifying in some samples up to 24 weeks after the browsing event	[75]
Applications with pot	ential for the understanding of		
Air	Invasive-species detection	The presence of genetically modified organisms was detected from samples of air containing low levels of pollen	[76]
Fresh water	Wildlife-disease detection	Detecting the chytrid fungus <i>Batrachochytrium dendrobatidis</i> , which is likely to be a primary cause of amphibian population declines, in water samples	[77]
Fresh water	Invasive-species detection	The American Bullfrog ( <i>Lithobates catesbeianus</i> ) was successfully identified, showing that early detection of invasive species at low densities is possible and has implications for management	[44]

The second strategy is based on filtration of different volumes of water and is mostly applied in large and/or flowing water bodies. Using filtration, larger volumes of water can be accommodated, which theoretically increases the detection probability (Wilcox *et al.* 2013). However, this does not mean that filtration always performs better than the precipitation method. This method is more time consuming and more expensive (e.g. filters, pump etc.). By filtering larger volumes of water more inhibitors are also concentrated in the sample. And more importantly, eDNA is often highly degraded into mostly small fragments (Deagle *et al.* 2006). Those fragments might not be retained by the filters as pore sizes are too large (see below), thereby lowering the detection probability. Four main types of filters are used: glass fibre filter (Jerde *et al.* 2011), cellulose nitrate filter (Goldberg *et al.* 2011), carbonate filter (Takahara *et al.* 2012) or nylon filters (Thomsen *et al.* 2012b)

Filtration can be performed in the field (e.g. Goldberg *et al.* 2011; Thomsen *et al.* 2012a). The filtered samples are then stored in alcohol, or on ice, and sent to the laboratory for analysis. This may be a limitation if the study is performed on a large-scale and when different persons sample different points, as numerous pumps will need to be purchased. Alternatively, filtration can be performed subsequently in the laboratory (Jerde *et al.* 2011). In this case, water is collected in a sterile container, stored on ice and sent immediately to the laboratory for analysis. The samples should be processed in the laboratory within 24 hours (Jerde *et al.* 2013) and, therefore, the eDNA laboratory should not be too distant from the sampling points. Alternatively, water samples should be frozen until filtration can be performed (e.g. Thomsen *et al.* 2012b). This can be a limitation when sampling locations are in a remote environment, or when transport to the laboratory takes more than 24 hours.

#### Sources of Error

If eDNA sampling and analysis is inadequate, it can lead to false positives (type I error: eDNA detected where target species is not present) and false negatives (type II error: eDNA not detected where target species is present). Bohmann *et al.* (2014) identify the most likely sources of these errors and general strategies for avoiding them (see Table 2). For those interested in a more detailed treatment of sources of these errors and their avoidance, then I highly recommend reading Chapters 2 (*DNA Metabarcode Choice and Design*), 4 (*Sampling*), 5 (*DNA Extraction*), 6 (*DNA Amplification and Multiplexing*), 7 (*DNA Sequencing*) and 8 (*DNA Metabarcoding Data Analysis*) in Taberlet *et al.* (2018).

Table 2	Sources of uncertainty from eDNA and how they may be overcome (from Bohmann <i>et al.</i> 2014)
	Sources of uncertainty from eDivit and now ency may be overcome (from Dominant et all 2011)

Potential Error	Solution	
False positives (type I error: eDNA detected where target species is not present) resulting from false detection of eDNA from other sources, e.g. tributaries into a major river, ballast water discharge, sewage and waste water, excrement of animals that prey on the target species, dead target species carried on boats, or unsterilized equipment.	To ensure false positives do not occur via contamination between samples when using the same equipment, equipment must be sterilised thoroughly or, preferably, not reused. Quality control to avoid false positives should be implemented in the sampling strategy; e.g. blank samples can be taken into the field to ensure con- tamination does not occur in the transport phase, and samples can be taken from adjacent areas where target species are known to occur. Sampling design should incorporate a risk assessment of target and non-target eDNA.	
False positives resulting from PCR primers and probes that do not have a high enough level of specificity, allowing the amplification of "lookalike" non-target species.	<i>In silico</i> testing of species-specific DNA-based probes and primers, e.g. comparing sequences with the Basic Local Alignment Search Tool (BLAST), or using ecoPCR software, as well as <i>in vitro</i> testing of probes and pri- mers against target tissue-derived DNA; genetic distanc- es should also be reported.	
False negatives (type II error: eDNA not detected where tar- get species is present) resulting from insufficient sensitiv- ity or failure of methods to perform as expected.	Rigorous testing of primers against target species' DNA must be undertaken to ensure successful amplification, as well as optimising protocols to be confident of spe- cies detection before sample collection begins.	
The inability of eDNA to distinguish between live or dead organisms, including digested or faecal remains of target organisms derived from their predators (e.g. foxes prey- ing on small mammals).	Repeated temporal sampling of the same area will alle- viate this problem to some extent. Because dead bod- ies, predators' faecal matter, or other introduced sources of DNA decompose and degrade over time, a species that is permanently present in an environment will still be detected after the introduced contaminants have been degraded beyond the point of DNA amplifica- tion. The study's risk assessment should include any visually observed dead organisms.	

#### WHERE TO FROM HERE?

A number of seminars or workshops on the application of eDNA studies have been or will be presented to practising ecological consultants in Australia. For instance, the *Ecological Consultants Association of Victoria* invited Helen Barclay, Managing Director of EnviroDNA to speak on this topic in June 2018. EnviroDNA will also be presenting a whole-day forum on eDNA in Melbourne in collaboration with Environment Institute of Australia and New Zealand on 3 April 2019.

The Council of the Ecological Consultants Association of NSW (ECA NSW) is considering running a day-long

workshop this year on the use of eDNA. But this workshop will proceed only if our membership shows Jerde, C.L., Mahon, A.R., Chadderton, W.L. and Lodge, sufficient interest in attending. I hope that this short D.M. (2011). "Sight-unseen" detection of rare aquatic introduction to eDNA studies has whetted your appetite for such a workshop!

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## **Contributions to the Newsletter, Volume 43**

Contributions to the next newsletter should be forwarded to the administration assistant Amy Rowles <u>admin@ecansw.org.au</u> by the **30th of July 2019**.

- Articles may be emailed in WORD, with photos included or referenced in an attached file as a jpg.
- Please keep file size to a minimum, however there is no limit on article size (within reason)
- Ensure all photos are owned by you, or you have permission from the owner
- Ensure that any data presented is yours and you have permission from your client to refer to a specific site (if not please generalise the location).
- All articles will be reviewed by the editorial committee, and we reserve the right to request amendments to submitted articles or not to publish.
- Please avoid inflammatory comments about specific persons or entity

The following contributions are welcome and encouraged:

- Relevant articles
- ◊ Anecdotal ecological observations
- Hints and information
- ♦ Upcoming events
- ◊ Recent literature
- New publications (including reviews)
- Photographs







**Above**: a ringtail possum caught within a tree clearance near to Willow Tree. Often I find possums are calmer and easier to check over for injury if they are first swaddled and held close to your body. This possum was uninjured and released within a nest box in adjacent vegetation . Photo courtesy of Ben Ellis



Above right: Eastern Horseshoe Bat. **Right:** Greater Broad-nosed Bat. **Below Right:** Eastern Bentwing Bat. Photos courtesy of Amy Rowles





Above: *Homalictis urbanus* bee. Photo courtesy of Rochelle Lawson.

**Below**: *Parsonsia dorrigoensis* (Milky Silkpod) located within an ecotone between mesic and dry woodland. This species is most easily identified by the milky sap that extrudes when broken and its shiny purplish lamina. Photo courtesy of Ben Ellis.







**Above**: Eastern Pygmy-possum in Kosciuszko National Park. Photo courtesy of Elliot Leach



Above: Coastal Petaltail Dragonfly freshly emerged from exuviae at Chaffey Creek. Photo courtesy of David Havilah.

**Below:** Diuris disposita (Willawarrin Doubletail) found within a disturbed low groundcover adjacent to dry woodland within a private site in Collombatti. A total of less than 50 plants are known across 3 populations. I believe this could be an underestimated population size based on a lack of systematic survey being conducted and the fact that the orchid is cryptic and produces a very non-conspicuous flower. Marking the individuals found could only be done by crawling along the ground on hands and knees. Photo courtesy of Ben Ellis



Left: A vast swathe of almost monoculture Kangaroo Apple, after wildfire in tall heath. Good for a feed soon. Taken 26th August 2018 at Cape St George Lighthouse, Jervis Bay. Photo courtesy of Danny Wotherspoon

