## <u>Lorikeet paralysis syndrome project – a year in review</u>

### **Summary**

- Current hypotheses
  - LPS is caused by a toxin that interferes with the function of the neuromuscular junction.
  - This toxin is of plant origin and that this plant only blossoms or fruits during the late spring, summer and early autumn.
- Recent hypothesis expansion
  - LPS might be also be caused by a toxin produced by fungi growing on in plants, their flowers, or in or on their fruit or ingestion of a venomous insect or spider living on or in fruit could cause LPS
- Citizen Science Project (iNaturalist) update
  - o 118 members
  - o 250 observations
  - o 805 records of plants that rainbow lorikeets have been feeding on
    - 130 species of plants identified 30 potentially toxic
- New areas of investigation update
  - Next generation sequencing (Sequencing plant DNA found in lorikeet droppings to determine what lorikeets with LPS have been eating)
    - Preliminary findings show that this tool does work with samples from rainbow lorikeets and suggests that lorikeets with LPS feed on figs (*Ficus* spp.) and possibly other fruits prior to the onset of LPS.
    - Next step examine more samples from lorikeets with LPS and control lorikeets that do not have LPS.
    - Expand our testing to determine what bacteria, fungi and even insects and spiders, that the lorikeets may have ingested prior to developing LPS.
  - Geospatial analysis
    - Collated data will be used to conduct a geospatial analysis to look at any relationships and patterns between sites, conditions and environments.

### **Investigative team**

- David Phalen, Professor, Sydney School of Veterinary Science
- Lauren Bassett, Research Associate, Sydney School of Veterinary Science
- Maya Yaffe, Volunteer, Second Year Veterinary Student, Sydney School of Veterinary Science
- Claude Lacasse, Wildlife Veterinarian, Wacol Animal Care Centre, RSPCA Oueensland
- Mainity Batista Linhares, Diplomate American College of Veterinary Pathologists, Lecturer, School of Veterinary Science, University of Queensland and
- Rachele Wilson, Research Assistant, PhD Candidate, Griffith University and University of Queensland.
- Holly Somerset Bowden, Third Year Bachelor of Science and Bachelor of Advanced Studies (Taronga Wildlife Conservation), Faculty of Science
- Simon Cropper, Ecologist
- Dr. Karrie Rose, Veterinary Pathologist Registrar, Australian Registry of Wildlife Health, Taronga Conservation Society Australi

While not part of the investigative team, we are grateful for the support of Holly Parsons from Birdlife Australia who provided critical advice on setting up our iNaturalist citizen scientist platform.

### **Background**

Lorikeet paralysis syndrome (LPS) is a seasonal disease, causing thousands of rainbow lorikeets to present to wildlife rehabilitators and wildlife veterinarians every year in southeast Queensland and northern NSW, stretching their resources. This year, 2022, was no exception and despite the unusual weather, thousands of lorikeets with LPS presented to carers and wildlife veterinarians

Our team, with the financial support of WIRES, is the leading research group investigating the cause of LPS. We refer you to a recent publication of ours to see what the most current information is on the signs of this disease, what the prognosis is for lorikeets with LPS is and what the current thinking is regarding the possible causes are for LPS.

C Lacasse, K Rose, M Allen, MP Ward, LA Pulscher, A Giles, J Hall, DN Phalen. 2021. Investigation into clinicopathological and pathological findings, prognosis, and aetiology of lorikeet paralysis syndrome in rainbow lorikeets (*Trichoglossus haematodus*). Australian Veterinary Journal 99(10): 432-444. (https://onlinelibrary.wiley.com/doi/pdf/10.1111/avj.13107).

This year, it also became clear that other diseases, in addition to LPS, are impacting wild lorikeets and our investigations have expanded to determine the cause of lorikeet deaths on the south coast and northeast regions of New South Wales.

### Investigation into the aetiology of LPS

Based on the findings reported in the Australian Veterinary Journal, it is our current hypothesis that LPS is caused by a toxin that interferes with the function of the neuromuscular junction. We also hypothesize that this toxin is of plant origin and that this plant only blossoms or fruits during the late spring, summer and early autumn. We have recently expanded our hypothesis to include the possibility that LPS might be caused by a toxin produced by fungi growing on in plants, their flowers, or in or on their fruit. We are even considering the possibility that ingestion of a venomous insect or spider living on or in fruit could cause LPS.

### Citizen science project

To identify the plants that lorikeets feed on during the times of the year when LPS does not occur and compare them to the plants that they feed on when LPS occurs, we have started a citizen science project on iNaturalist. Our team of citizen scientists, at the time of this writing, includes 118 members and more are joining each week. Our citizen scientists have contributed 250 observations of individual plants that lorikeets have fed on to the project.

Of equal importance, iNaturalist has a large, ever-growing database of rainbow lorikeet images that have been recorded for other projects. Combined, we now have approximately 805 records of the plants that rainbow lorikeets have been feeding on and have identified more than 130 species of plants on which they feed. Of these 130 species, 30 are potentially toxic and 11 meet the criteria of blooming or fruiting during the same time that LPS cases occur. While the ultimate goal of this study is to identify the plant or plants that are most likely to cause LPS, the data that we are generating is also demonstrating how lorikeets use native and introduced vegetation in an urban environment to meet their nutritional needs.

### Promoting the LPS studies and engaging with the public

Promoting the LPS studies to the public has been crucial for recruiting project members to undertake research in the field and raise awareness of LPS in the community. A comprehensive media campaign has been rolled out to inform the public about this disease and the efforts underway to determine its cause. The study has been promoted through various channels, including direct email, radio, print media, social media and online. Information about this project can be found on the University of Sydney website and has been included in multiple short radio interviews, multiple in print and online media reports, in the newsletters of councils in areas where LPS occurs and plant societies and bird watching organisations. The University of Sydney has also hosted two webinars for citizen scientists to introduce them to the research program. These events have benefited from the involvement of WIRES through the provision of speakers and event promotion support. LPS project members continue to be updated on the project on a weekly basis on the iNaturalist site.

# Using next generation sequencing and lorikeet droppings to determine what lorikeets with LPS have been eating

Phase 1: Our team has established a collaboration with Rachele Wilson at Griffith University. Rachele has developed a screening tool to identify the plants that an animal has eaten by the plant DNA present in their faeces. She has used this tool extensively to study the diet of urban flying-foxes. Using this technology, we have done a preliminary 'proof-of concept' study to determine if we can use it to identify the plants rainbow lorikeets have been feeding on just prior to the onset of LPS.

Our preliminary findings show that this tool does work with samples from rainbow lorikeets and suggests that lorikeets with LPS feed on figs (*Ficus* spp.) and possibly other fruits prior to the onset of LPS. This has made us consider the possibly that a bacterium or a fungus may grow on or in these fruits and may produce the toxin causing LPS, as figs are not thought to be toxic themselves. Another possibility is that a venomous insect around or on the fruit (possibly a wasp or ant) might be ingested by or sting or bite the lorikeet feeding from a plant, causing LPS. Our preliminary findings have also opened our eyes to the diversity of species of plants that rainbow lorikeets feed on and we have found that they feed various plant species than have not been detected by our citizen scientists.

Phase 2: The next step in this project is to examine more samples from lorikeets with LPS and control lorikeets that present at the same time, but do not have LPS. Our objective is to collect samples from 40 lorikeets with LPS and 40 control lorikeets and repeat the analysis. In this phase of the project, we will expand our testing to determine what bacteria, fungi and even insects and spiders, that the lorikeets may have ingested prior to developing LPS.

### Geospatial analysis

Our team has established a collaboration with Simon Cropper, an experienced ecologist, to do some geospatial analysis of our collated data, comparing sick bird locations with tree locations. Simon brings with him experience and expertise in conservation, geographical information systems and environmental data management. Our combined citizen science and iNaturalist data (gathered by Holly and Maya), paper data on sick lorikeet locations, and council GIS asset data will be used to conduct a geospatial analysis to look at any relationships and patterns between sites, conditions and environments.

### Could flying-foxes get a form of LPS?

For the past two years in the mid to late summer paralysed flying-foxes have been presenting to carers and wildlife veterinarians in the Brisbane area and in northern New South Wales. Our team has joined up with Wildlife Health Australia and eminent bat ecologists and pathologists to use the same tools that we are using in our LPS investigation to determine if the cause of LPS may also be the cause of the paralysis in flying-foxes.

In summary our work into the cause of LPS is proceeding at a rapid pace. Our ability to do this work has been largely due to our great team of citizen scientists, the support of other scientists with various skills, and the financial support of WIRES. Given all these resources we are looking forward to a very productive year and will keep you informed about our progress.

### List of Plant Genus/Species Rainbow Lorikeets Feed on (observational and faecal data)

Rusty gum (Angophora	Pink Silk Tree (Albizia	Shingle tree (Acrocarpus
leiocarpa)	julibrissin)	fraxinifolius)
Bloodwood tree (Corymbia	Golden Penda	Gymea spear (Doryanthes
gummifera)	(Xanthostemon chrysanthus)	palmeri)
Rough barked apple	Broad-Leaved Paperbark	Scarlet pimpernel (Anagallis
(Angophora floribunda)	(Melaleuca quinquenervia)	arvensis)
Swamp Box (Lophostemon	Wallum bottlebrush	Acacia genus
suaveolens)	(Melaleuca pachyphylla)	
Poplar box (Eucalyptus	New Zealand Christmas	Cadagai ( <i>Corymbia</i>
populnea)	Tree (Metrosideros excelsa)	torelliana)
Forest Red Gum	Lemon-scented teatree	Callitris genus (Cypress
(Eucalyptus tereticornis)	(Leptospermum petersonii)	pine)
Tallowwood (Eucalyptus	Wax Mallow (Malvaviscus	Firewheel tree (Stenocarpus
microcorys)	arboreus Cav)	sinuatus)
Narrow-leaved ironbark	Cherry Blossom (Prunus	Forest grass tree
(Eucalyptus crebra)	serrulata)	(Xanthorrhoea johnsonii)
Red ironbark (Eucalyptus	Yellow elder (Tecoma stans)	White Clover (Trifolium
sideroxylon)		repens)
Yellow gum (Eucalyptus	Weeping lilly pilly (Syzgium	Pink cedar (Acrocarpus
leucoxylon)	floribundum)	fraxinifolius)
Northern grey ironbark	Scrub cherry (Syzygium	Wild tamarind (Leucaena
(Eucalyptus siderephloia)	australe)	leucocephala)
Bangalay (Eucalyptus	Powderpuff lilly pilly	Crows ash (Flindersia
botryoides)	(Syzygium wilsonii)	australis)
Marri (Corymbia	Candelabra aloe (Aloe	Leptospermum genus
calophylla)	arborescens)	
Plunkett mallee (Eucalyptus	Amyema Genus	Elephant's Ear (Macaranga
curtisii)		tanarius)
Swamp bloodwood	Peanut tree (Sterculia	Ice cream bean (Inga edulis)
(Corymbia ptychocarpa)	quadrifida)	
Brush box (Lophstemon	Pine cones (Conifers)	Peltophorum genus
confertus)		

Weeping Bottlebrush	Tuckeroo tree (Cupaniopsis	Mango (Mangifera genus)
(Melaleuca viminalis)	anacardioides)	ividige (ividigiteta genus)
Crimson Bottlebrush	Foxtail (Agave attenuata)	Common wild oat (Avena
(Melaleuca citrina)		fatua)
Silky oak ( <i>Grevillea</i>	Century plant (Agave	Indian cherry ( <i>Cordia</i>
robusta)	americana)	dichotoma)
Mundubbera grevillea	Rough century plant (Agave	Grey mangrove (Avicennia
(Grevillea whiteana)	asperrima)	marina)
Broad-Leaf Banksia	Custard Apple (Annona	White mulberry ( <i>Morus</i>
(Banksia robur)	reticulata)	alba)
Coastal Banksia (Banksia	Camphor laurels	East Indian crabgrass
integrifolia)	(Cinnamomum camphora)	(Digitaria setigera)
Cocos Palm (Syagrus	Dracaena genus	Watermelon (Citrullus lanatus)
romanzoffiana)	Dylah on two (Figure olegation)	,
Triangle palm (Dypsis	Rubber tree (Ficus elastica)	Asparagales sp.
decaryi)	Dlug is some de (I	Drygologists
Queen Palm (Syagrus	Blue jacaranda (Jacaranda	Dyschoriste sp.
romanzoffiana)	mimosifolia)	Daniela (Citari
Mexican Fan Palm	Poincianas (Delonix regia)	Pomelo (Citrus maxima)
(Washingtonia robusta)		
Australian fan palm	Murraya genus	Slender wild oat (Avena
(Livistona australis)		barbata)
Date palm ( <i>Phoenix genus</i> )	Neem Tree (Azadirachta indica)	Cyperus sp.
Alexandra palm	Sasanqua Camellia	Jackfruit (Artocarpus
(Archontophoenix	(Camellia saanqua)	heterophyllus)
alexandrae)		
Areca palm (Dypsis	Frangipani	Platanus sp.
lutescens)		-
Palm (Sabal genus)	Cycads	White fig (Ficus virens)
Colville's Glory (Colvillea	Duranta erecta	Chinese Banyan (Ficus
racemosa)		microcarpa)
Cockspur coraltree	Geranium genus	Moreton Bay fig (Ficus
(Erythrina crista-galli)		macrophylla)
Common Coral tree	Brazilian peppertree	Blue fig (Elaeocarpus
(Erythrina lysistemon)	(Schinus terebinthifolia)	grandis)
Flame bottletree	Broad-leaved peppertree	Tipuana ( <i>Tipuana</i> tipu)
(Brachychiton acerifolius)	(Schinus terebinthifolius)	1 ( T
Umbrella Tree	Blueberry Ash tree	Common guava (Psidium
(Heptapleurum	(Elaeocarpus reticulatus)	guajava)
actinophyllum)		[G
Xanthorrhoea genus	Brunsfelsia genus	Sensitive plant ( <i>Mimosa</i>
		pudica)
Weeping Boer bean (Schotia	Avocado (Persea	Sage (Salvia genus)
brachypetala)	Americana)	
o. achyperana)		
African Tulip (Spathodea	Blue Quandong	Hoop pine (Araucaria
campanulata)	(Elaeocarpus grandis)	cunninghamii)
campanana,	(Lincocai pus gi anais)	commissionin)

Pink Evodia (Melicope	Orange Trumpet vine	Box tree (Lophostemon
elleryana)	(Pyrostegia venusta)	confertus)
Black sheoak (Allocasuarina	Passionfruit (Passiflora	Mushroom (Fungi)
littoralis)	edulis)	
Brazil Raintree (Brunsfelsia	Morning glory ( <i>Ipomoea</i>	Mandarin tree (Citrus
paucilflora)	obscura)	reticulata)
Blackbean	Peacock flower (Caesalpinia	Curtain fig (Ficus
(Castanospermum australe)	pulcherrima)	macrocarpa)
Royal poinciana (Delonix	Sausage tree (Kigelia	
regia)	africana)	

# Plant Genus/Species known to be toxic (from previous list)

Cocos Palm (Syagrus romanzoffiana)	Mexican Fan Palm (Washingtonia robusta)	Foxtail (Agave attenuata)
Colville's Glory (Colvillea racemosa)	Brazil Raintree (Brunsfelsia paucilflora)	Century plant (Agave americana)
Cockspur coraltree (Erythrina crista-galli)	Blue jacaranda ( <i>Jacaranda</i> mimosifolia)	Rough century plant ( <i>Agave asperrima</i> )
Common Coral tree (Erythrina lysistemon)	Blackbean (Castanospermum australe)	Cycad
Flame bottletree (Brachychiton acerifolius)	Scarlet pimpernel ( <i>Lysimachia arvensis</i> )	Camphor laurels (Cinnamomum camphora)
Umbrella Tree (Heptapleurum actinophyllum)	Royal poinciana ( <i>Delonix</i> regia)	Dracaena genus
Xanthorrhoea genus	Morning glory ( <i>Ipomoea</i> obscura)	Rubber tree (Ficus elastica)
Weeping Boer bean (Schotia brachypetala)	Peacock flower (Caesalpinia pulcherrima)	Poincianas - Flamboyant tree (Delonix regia)
African Tulip (Spathodea campanulata)	Broad-leaved peppertree (Schinus terebinthifolius)	Frangipani
Duranta erecta	Brazilian peppertree (Schinus terebinthifolia)	Sausage tree (Kigelia africana)

# A Big Thank You

This important project examining Lorikeet Paralysis Syndrome would not be possible without the generous support of the New South Wales Wildlife Information, Rescue and Education Service (WIRES).