Cockatoo Hindlimb Paralysis Syndrome (CHiPS) the past, present and future

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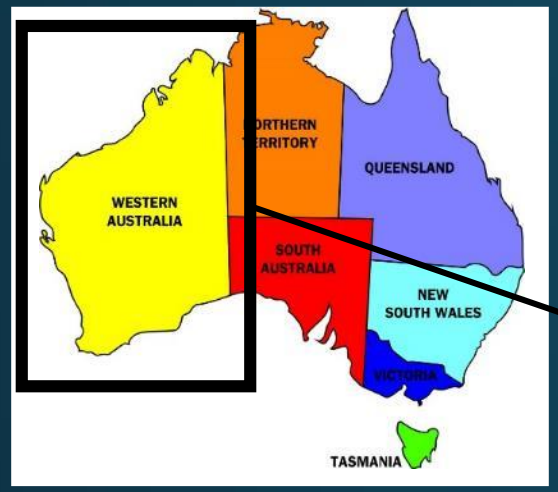
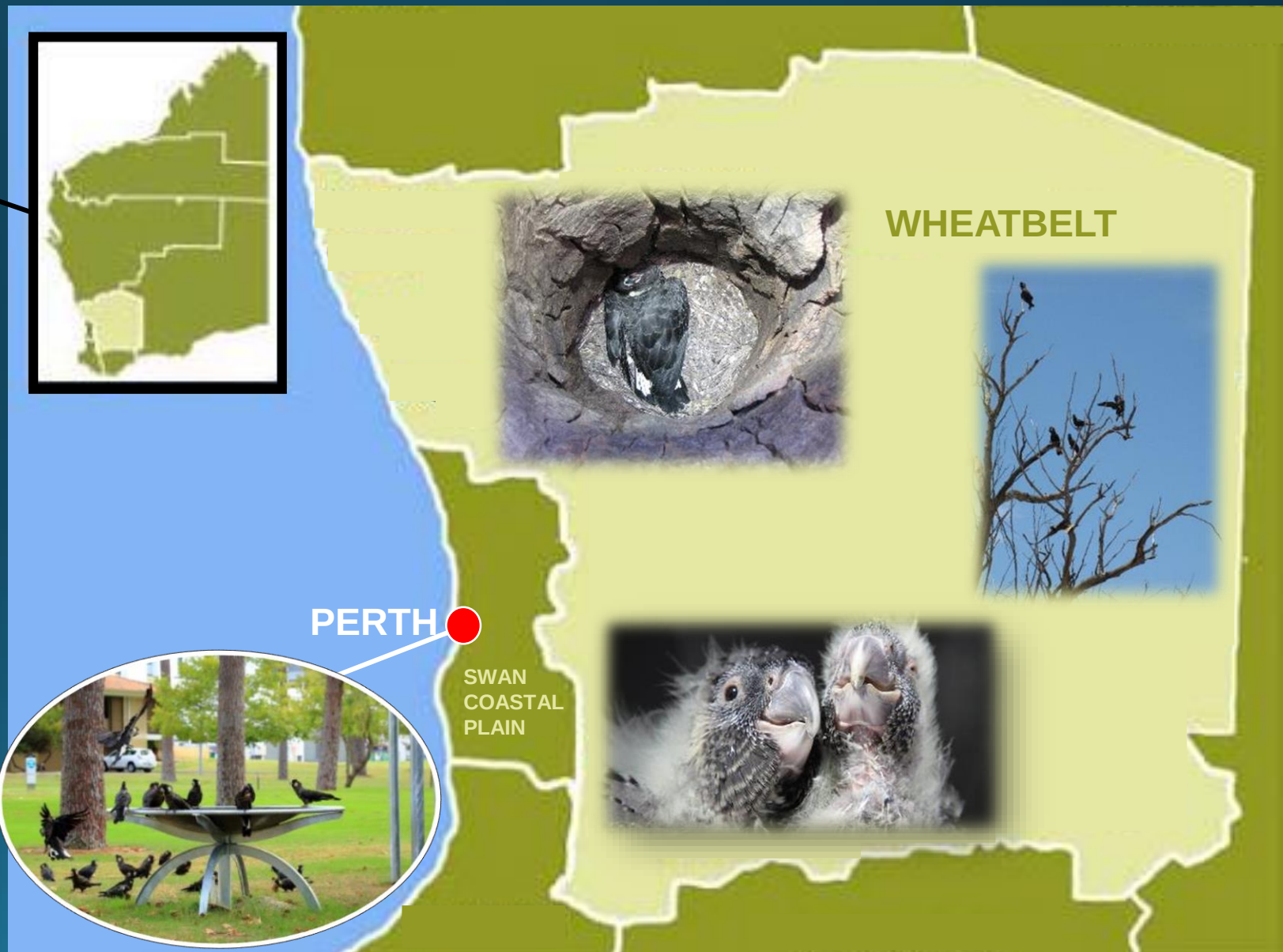
Carnaby's Cockatoos in Perth



- One of 3 Black Cockatoo species endemic to WA
- 'Threatened' at state, national & international level
- Main breeding area in the wheatbelt; populations move to the Swan Coastal Plain in summer



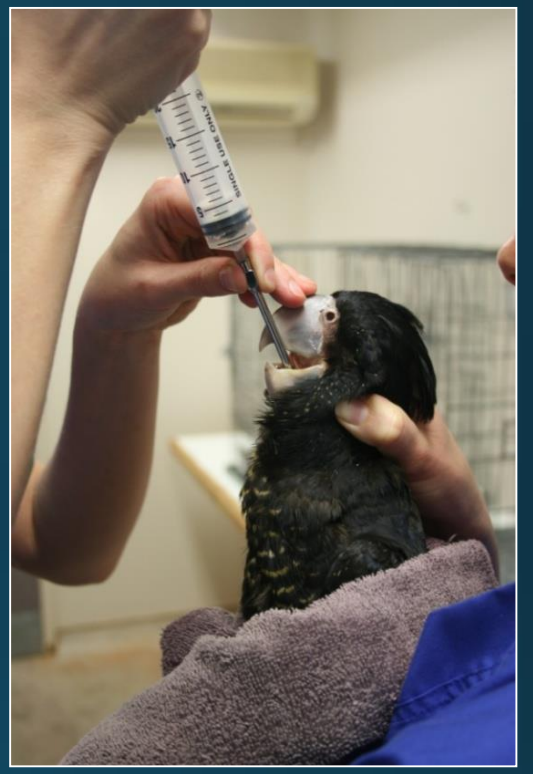
Perth Zoo and Carnaby's Cockatoo

- Cooperative program

- Dept Parks & Wildlife (DPaW) to treat sick and injured Black Cockatoos since 2000

- 200 birds admitted per year; approximately half Carnaby's cockatoos

- Triage, treatment & supportive care

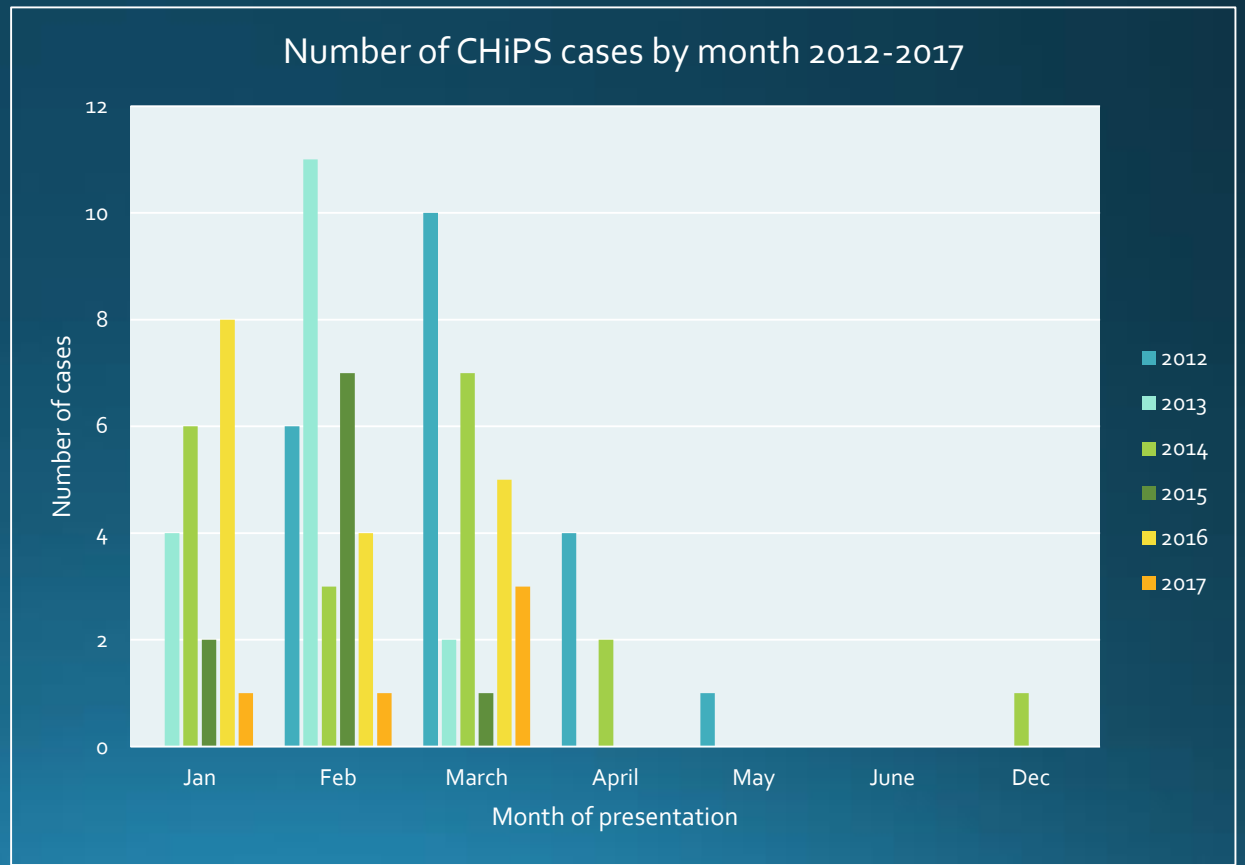


The Emergence of “CHiPS”

Cockatoo Hindlimb Paralysis Syndrome (“CHiPS”) was first defined as a disease entity in 2012, when a cluster of 21 Carnaby’s Cockatoos presented with varying degrees of hindlimb paresis or paralysis from January to June.

Epidemiology 2012-2017

- Only Carnaby's Cockatoos affected (n=90)
- No gender or age specificity
- Most cases found within 15km of Perth city
- 88% of cases presented at PZVD from January to March



DIFFERENTIAL DIAGNOSES FOR HINDLIMB PARESIS/PARALYSIS IN BIRDS

- Anticholinesterase pesticide poisoning (organophosphates,carbamates)
- Organochlorine pesticide poisoning
- Other pesticides (e.g. pyrethrins, glyphosate, imidacloprid)
- Heavy metal poisoning
- Other toxin (e.g. rodenticides, mycotoxins)
- Spinal trauma
- Nutritional deficiency/resource competition
- Infectious disease: virus/bacterial/parasitic
- Botulism

ACTIONS FOR REFINING DIFFERENTIAL LIST

1. Clinical and post mortem examination; index case definition
2. Toxin source investigation
3. Tissue residue examinations: pesticides, heavy metals
4. Cholinesterase (ChE) levels in blood and brain

Clinical and post mortem examination

Examination	Findings
General examination	• No evidence of primary trauma (bruising, fractures) • Ulceration over keel associated with prolonged recumbency • No regurgitation (common in black cockatoos with heavy metal poisoning)
Neurological examination & systematic evaluation of paresis/paralysis	• No significant changes other than presenting paresis/paralysis • Neurological deficit ranging from reduced foot clench and reduced voluntary leg movement, through to full paralysis. Deep pain generally present. Loss of cloacal tone commonly seen.
Radiography	• No significant changes
Clinical pathology – CBC/biochemistry	• No significant changes
Gross pathology	• Often poor body condition and secondary ulceration over keel • No evidence of primary spinal trauma
Histopathology	• No evidence of infectious process, nutritional deficiency or primary trauma

CHiPS Index Case

- Carnaby's Cockatoos only
- Birds of any age and gender
- Bilateral paresis or paralysis not associated with primary trauma
- No other neurological signs (eg cranial nerve involvement, tremors, seizures)

- Good appetite but variable body condition
- No signs of infection or systemic illness

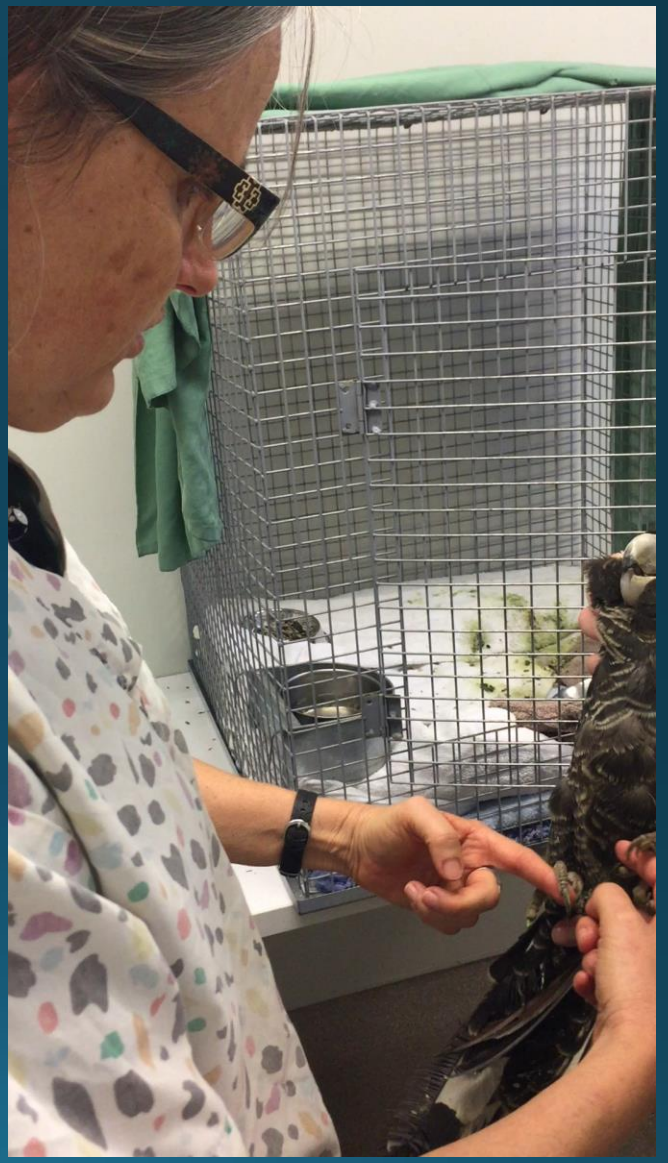
- Cases can recover with supportive nursing alone

Clinical evaluation

- Initial exam under manual restraint - triage
- GA, radiographs, clinical examination within 24-48hrs of admit, serum store for future AChE analysis & NTE assays

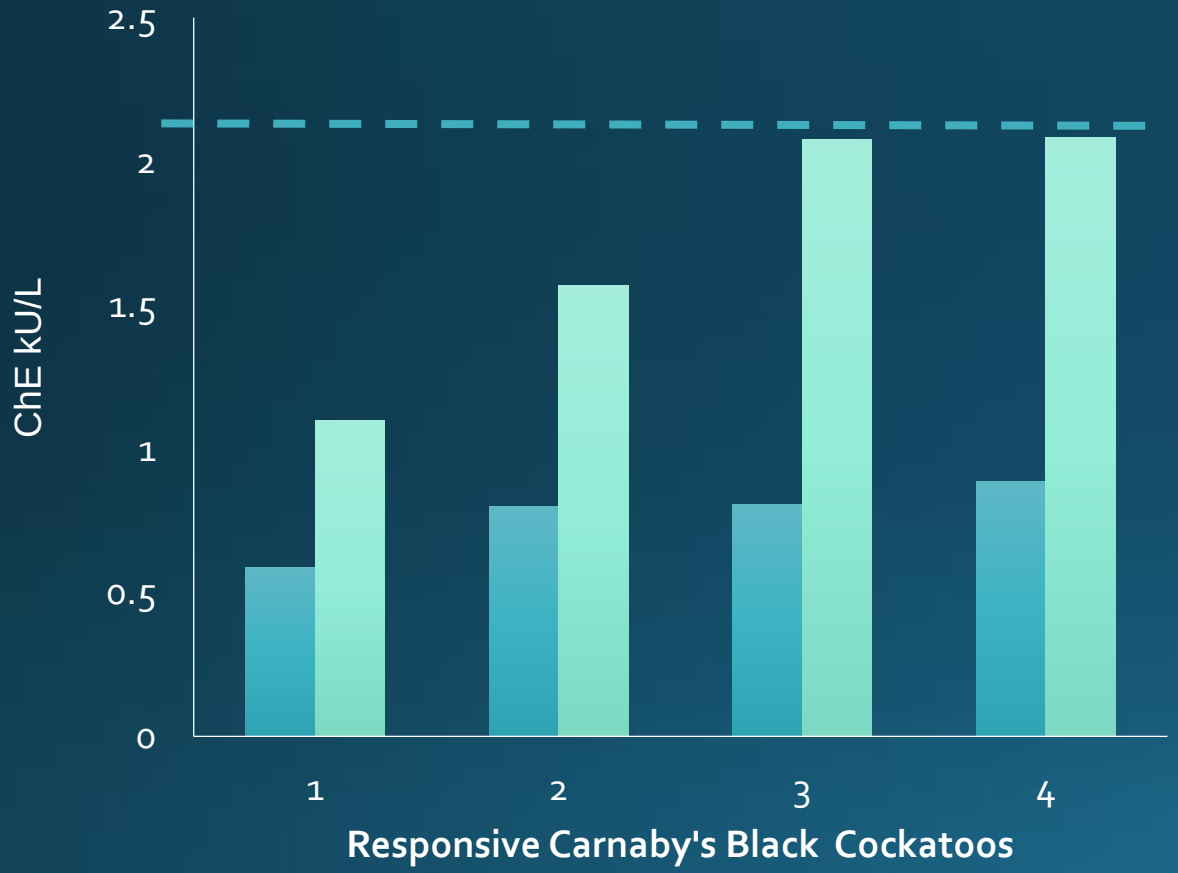
- Supportive care & housing modification
- Monitoring of CHiPs signs q 3 days to reassess & evaluate prognosis

- If severe, not compatible with future release eg. severe respiratory distress, severe keel trauma, poor body condition euthanased & necropsied collecting brain, crop, liver & GIT for future toxicologic examination & serum & plasma store.



ChE Levels as an Indicator of anti-ChE Pesticide Exposure

i) Serum ChE in Recovering CHiPS cases



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DEVELOPMENT OF REFERENCE RANGES FOR PLASMA TOTAL CHOLINESTERASE AND BRAIN ACETYLCHOLINESTERASE ACTIVITY IN FREE-RANGING CARNABY'S BLACK-COCKATOOS (*CALYPTORHYNCHUS LATIROSTRIS*)

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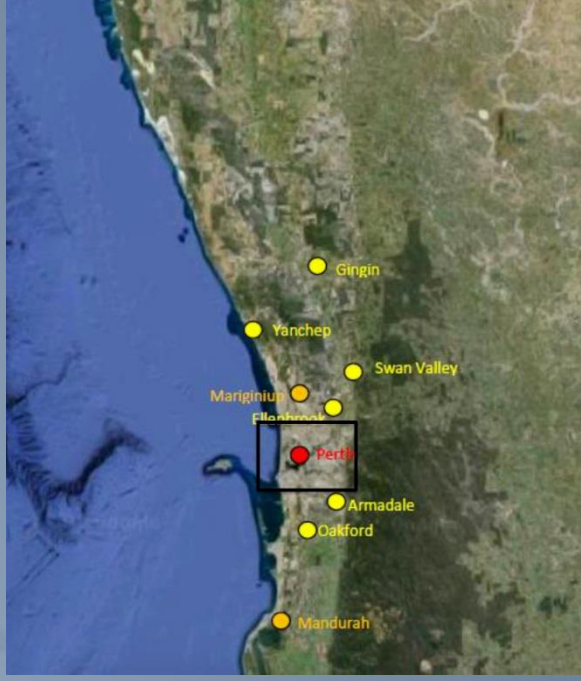
ABSTRACT: Published avian reference ranges for plasma cholinesterase (ChE) and brain acetylcholinesterase (AChE) are numerous. However, a consistently reported recommendation is the need for species- and laboratory-specific reference ranges because of variables, including assay methods, sample storage conditions, season, and bird sex, age, and physiologic status. We developed normal reference ranges for brain AChE and plasma total ChE (tChE) activity for Carnaby's Black-Cockatoos (*Calyptorhynchus latirostris*) using a standardized protocol (substrate acetylthiocholine at 25 C). We report reference ranges for brain AChE (19–41 µmol/min per g, mean 21±6.38) and plasma tChE (0.41–0.53 µmol/min per mL, mean 0.47±0.11) (n=15). This information will be of use in the ongoing field investigation of a paresis-paralysis syndrome in the endangered Carnaby's Black-Cockatoos, suspected to be associated with exposure to anticholinesterase compounds and add to the paucity of reference ranges for plasma tChE and brain AChE in Australian psittacine birds.
Key words: Anticholinesterase compounds, black cockatoos, psittacine, reference ranges.

Toxin source investigation



- Investigation of pesticide use in high caseload areas
  - No changes in practices for many years
  - The only pesticides in use known to be toxic to birds

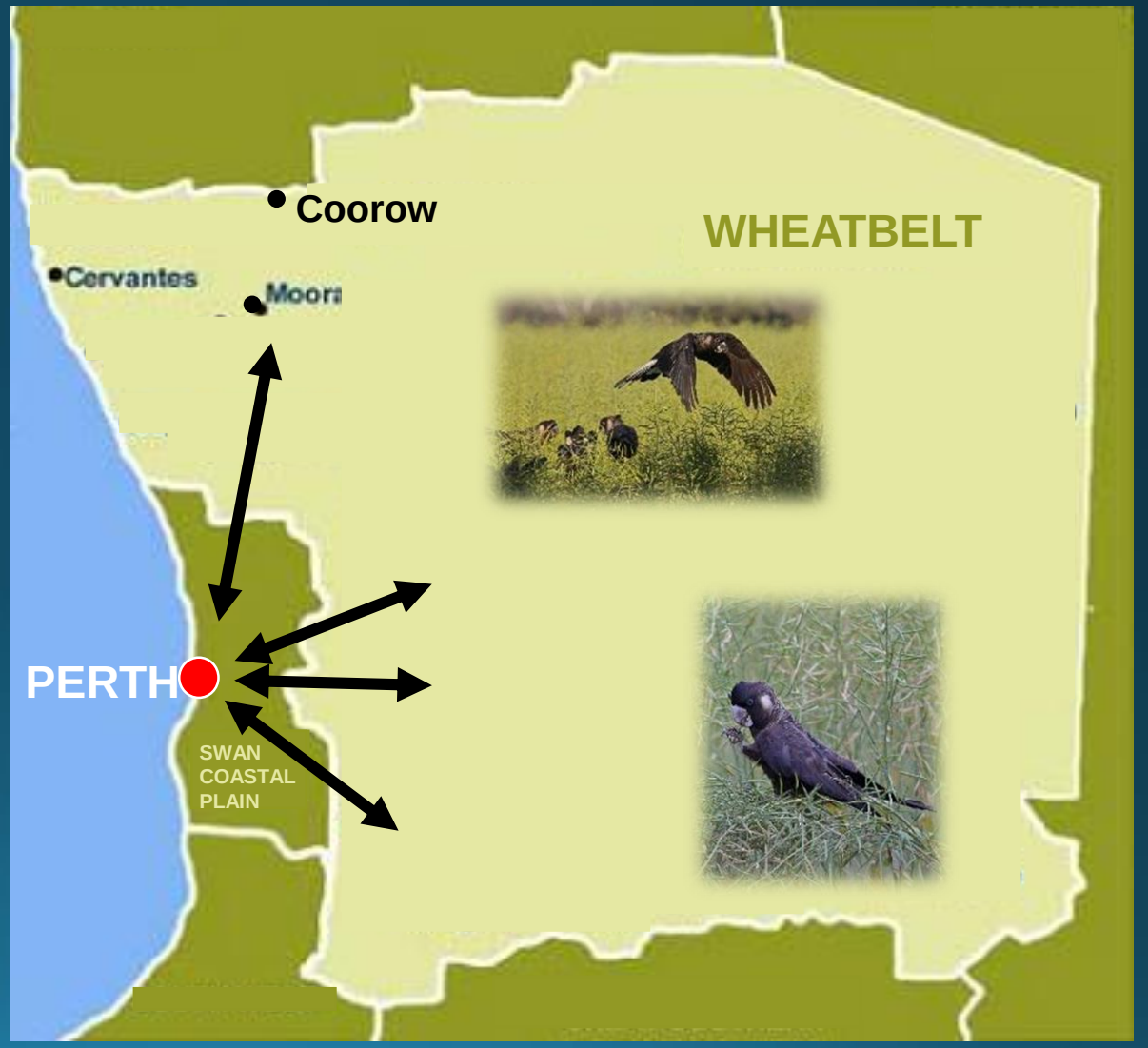
  - pyrethrins; no Organophosphates (OP's) or carbamates reported
  - No reports of other affected species

- Exposure in inland breeding areas – delayed or variable effect
  - CHiPS seasonal pattern coincides with influx of Carnaby's Cockatoos from the wheatbelt

Wheatbelt Exposure to Pesticides

Wheatbelt Mortality Events – OP poisoning?

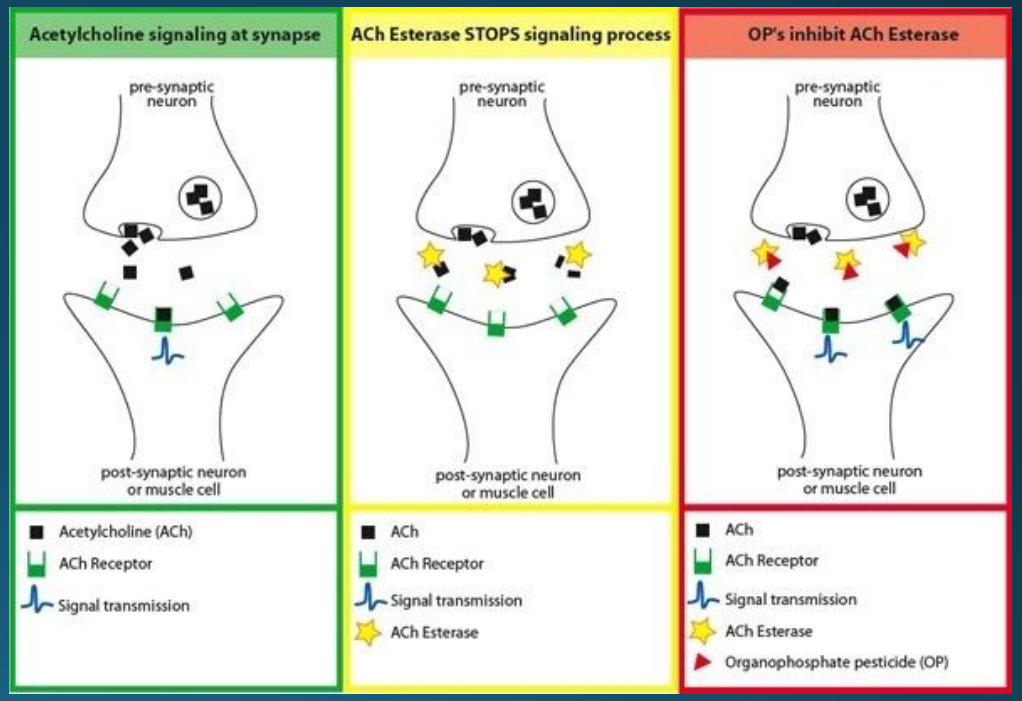
- Live birds with CHiPS signs and dead hens in nest hollows found at Coorow (2009, n=16) and Coorow (2012, n=7).
- At the time of year the mortalities occurred (September-October), growers are routinely spraying canola crops with organophosphate (OP) pesticides.
- Carnaby's Cockatoos feed extensively on canola during breeding; other black cockatoo species do not feed on canola.



Anti-cholinesterase compounds and their toxicity in non-target species

- Include organophosphates (OP's) carbamates, neonicitinoids
- Acetylcholine (ACh) is a neurotransmitter

- Acute exposure can result in death by respiratory or cardiac arrest
- Sublethal exposures can lead to a range of systemic biochemical, physiological and behavioural effects



Anti-cholinesterase compounds

- Accidental or deliberate OP poisoning reported in many wild Australian native birds including sulphur-crested cockatoos, corellas and galahs.
- In humans, can cause acute, intermediate or 'delayed neuropathy syndrome' OPIDN

AOP	OPIDN
•Acute onset •Varying degrees of muscle paralysis •Death due to cardiac or respiratory arrest **•Birds more sensitive when compared to mammals (Walker 1982, Goldstein et al 1999)** •Birds of prey feeding on baited animals (Goldstein et al 1999, Elliot et al 1997) •Other birds feeding on crops (Benson et al 1971, White et al 1983, Flickinger et al 1984)	•Onset up to 2-3 weeks following exposure to OPs (Emerick et al 2012) •Flaccid paralysis of hindlimbs •Paralysis of forelimbs in severe cases

Diagnosis acute OP toxicity cf. delayed neuropathy

AOP	OPIDN
Necropsy: No significant findings	No significant findings
Histopathology: No significant findings	Sensory-motor axonopathy (Emerick et al 2012) •CNS and PNS •Wallerian degeneration
•Cholinesterase quantification •Plasma (BChE) •Brain (AChE) •>20% inhibition diagnostic for exposure •>50% inhibition diagnostic for toxicity •Toxicology	•NTE quantification & Reactivation •70-80% inhibition diagnostic (Emerick et al, 2012) •Inability to reactivate •Toxicology

Mode of action

AOP	OPIDN
Inhibition of AChE •Binding and phosphorylation by OPs •Within hours of exposure •Disturbances in synaptic transmission in CNS and PNS •Reversible •All OP's, some carbamates	Inhibition **and** ageing of Neuropathy target esterase (NTE) •Binding and phosphorylation by OPs •Within hours of exposure (Correll et al 1990, Sogorb et al 2010) •Irreversible ageing of NTE •Time dependent process •Only oxon forms of OPs •Animal must survive acute immediate cholinergic syndrome (Correll et al 1990)

Testing hypothesis: “CHiPS is caused by anticholinesterase toxin”

Not able to conduct experimental studies.

However:

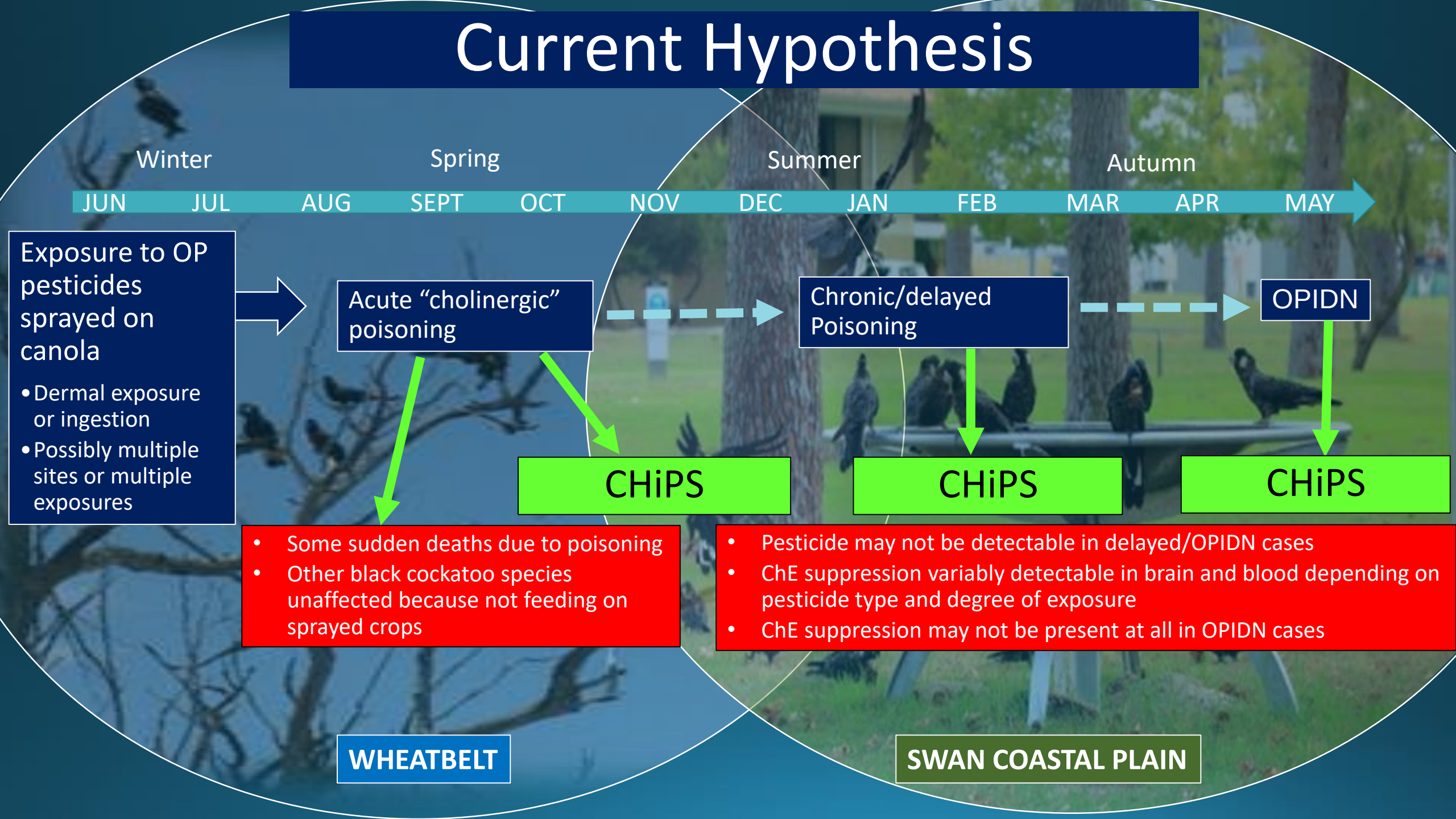
- Some birds responded to atropine (antidote to OPs)
- Some birds have been found to recover if provided with supportive care for at least 1 week
- NTE assays - significant difference between the CHiPS and control cases for NTE inhibition.

Responses consistent with anti-cholinesterase toxicity +/- a delayed onset OP induced neuropathy

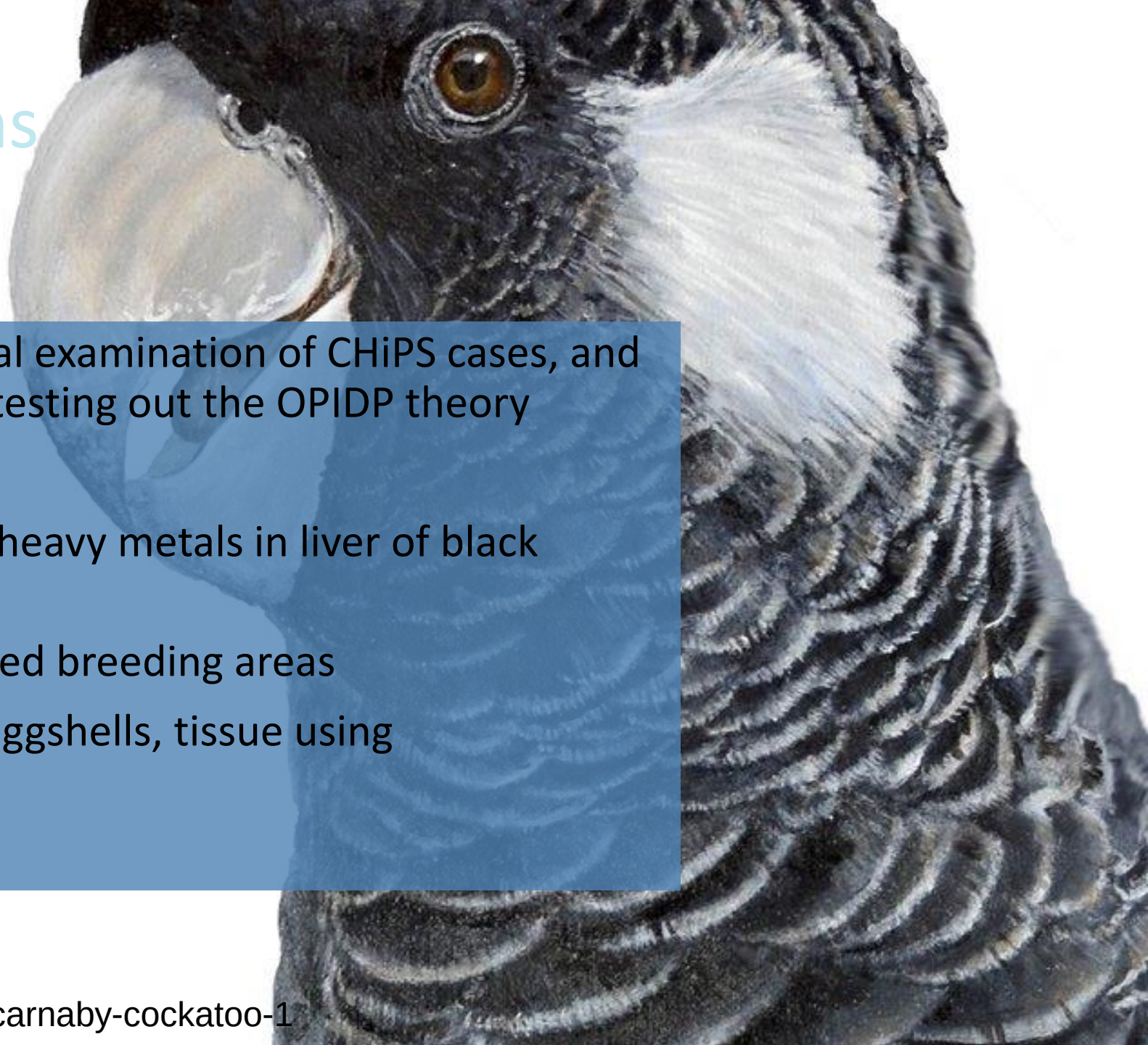
Further examinations undertaken

Examination	Findings
OP panel (13 pesticides commonly used in WA) in digestive tract contents	all samples negative
Carbamates (9 pesticides commonly used in WA) in digestive tract contents (n=14)	all samples negative
Imidacloprid in digestive tract contents (n=14) Imidacloprid & Clothianidin (n=2) -2017	all samples negative Low levels (n=1)
Fipronil in digestive tract contents (n=2)	all samples negative
Heavy metals in fat, muscle, liver and skin (n=10): • lead, mercury • cadmium, nickel and zinc • chromium • copper and zinc (Coorow birds; n=2)	 • not detected • detected in subclinical quantities • detected in subclinical quantities • elevated in liver samples (46-100mg/kg; cf 30-60mg/kg Macdonald et al 2010) – general ill health
Viral testing	Viral testing (Newcastle Disease Virus, Beak and Feather Disease Virus, Avian Influenza) – negative

Current Hypothesis



Ongoing Investigations



- Repeated & thorough histological examination of CHiPS cases, and development of new assays for testing out the OPIDP theory
- Establishing ‘normal ranges’ for heavy metals in liver of black cockatoos (*underway*)
- Ecological investigation in targeted breeding areas
- Further toxicology testing (e.g. eggshells, tissue using metabolomics)

Source: https://estore.parrots.org/products/carnaby-cockatoo-1

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Additional Images: R.Dawson, K.Lightbody, P.Brigg

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