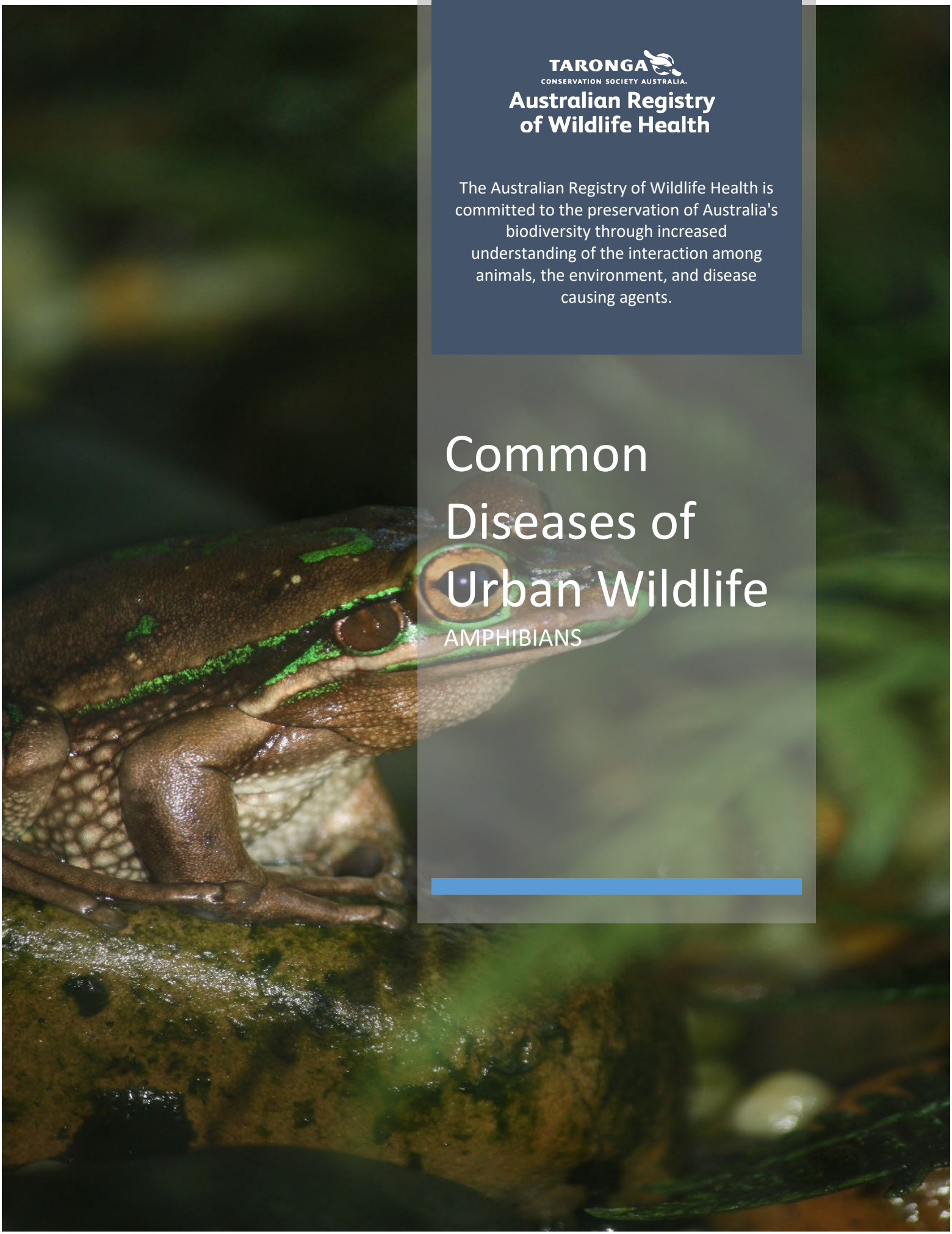

TARONGA
CONSERVATION SOCIETY AUSTRALIA.

Australian Registry of Wildlife Health

The Australian Registry of Wildlife Health is committed to the preservation of Australia's biodiversity through increased understanding of the interaction among animals, the environment, and disease causing agents.

Common Diseases of Urban Wildlife

AMPHIBIANS

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Contents

1 List of images

2 Introduction

Many diseases have been recognised and reported in free ranging Australian amphibians and those in rehabilitation or captive situations. The purpose of this document is to review the diseases that occur, in some cases frequently across numerous species and those that occur less often, affecting local populations, small groups or individuals.

Some diseases, although uncommon, have potential significance from a One Health perspective. We hope that this information assists with the timely recognition of common parasites, microbial infections, nutritional diseases, intoxicants and injuries to assist the appropriate care and welfare of wild animals. Throughout the text we offer advice from various authors towards achieving a diagnosis. As best-practice wildlife treatments change with advancing knowledge over time, treatment of amphibians in rehabilitation or captivity should be made in consultation with a veterinary professional.

A notifiable disease is one that must be reported to agricultural authorities. If you suspect or can confirm that an animal is showing symptoms of one of the diseases listed as reportable, you must report it to:

- your local vet or
- Wildlife Health Australia State Coordinator, www.wildlifehealthaustralia.com.au/AboutUs/ContactDetails.aspx
- your state or territory's department of primary industries or agriculture by phoning the Emergency Animal Disease Watch Hotline on 1800 675 888.

3 Parasitic Disease

Zoonotic: Unknown
Species records: All
Similar presentation to: Bacterial dermatitis, neoplasia

3.1 Ectoparasites

3.1.1 Flies (Diptera; *Batrachomyia* sp.)

Fly larvae migrate to the region of the dorsal lymph sac, burrowing through the skin. One to five maggots reside and mature in the lymph sac with their posterior spiracles in or near the frog's skin. Mature maggots exit via a hole in the skin, dropping off to pupate. Skin lesions may present as a typical deep ulcer with the maggot's spiracle visible next to a lump on the dorsal body, rump or base of a limb (Berger & Green, 2012). Some frogs survive infestation with little tissue damage, while others die at the time of larval emergence. Migrations of larvae to deeper tissues including the coelomic cavity are occasionally seen.

A useful summary of anuran myiasis, including various Australian cases, is documented by da Silva, et al. (2019). Several species of *Batrachomyia* flies occasionally parasitise Australian frogs. Elkan (1965) reports infestations in 11 amphibian species. Vogelnest (1994) described *B. mertensi* myiasis in a green tree frog (*Litoria caerulea*) and Lemkert (2000) reported *Batrachomyia* spp. infestations in the common eastern froglet (*Crinia signifera*), northern corroboree frog (*Pseudophryne pengilleyi*) and smooth toadlet (*Uperoleia laevigata*). *Batrachomyia strigapes* was detected in 5.1% of smooth toadlets (*U. laevigata*) examined in Sydney and infestation was associated with reduced frog weights (Schell & Burgin, 2001).

3.2 Arachnids

3.2.1 Trombiculid mites

Larval trombiculid mites may infect the skin of frogs and toads causing small vesicles in the skin (Flynn, 1973). Chiggers occur in the terrestrial stages of amphibians. Unlike chiggers of other vertebrates, amphibian chiggers burrow into the skin, encyst and remain more than six months. Mortality is not reported. In Australia, larvae of two mite species (*Vercammenia gloriosa* and *V. zweifelorum*) have been identified in several frog species from Queensland. Pathology in the skin of an eastern stony creek frog, *Ranoidea wilcoxii* (formerly *Litoria wilcoxii*), infected with both mite species was described by Mendez, et al. (2010).

Pathology consists of raised orange or reddish-orange nodules (~ 0.5 - 4 mm) present on the skin of the body, limbs or toe webs. Lancing of the cysts reveals orange mites. Microscopically, cysts occur as cavities in the dermis and elicit minimal inflammatory cell reaction with lymphocytes, macrophages and fibrosis (Berger & Green, 2012).

3.2.2 Ticks

Tick infestations have not been reported in Australian amphibians, however *Amblyomma* sp. ticks have been reported infesting cane toads (*Rhinella marina*) in Central and South America. This is a widespread introduced amphibian pest species in tropical and sub-tropical parts of Australia. Australian *Amblyomma* ticks are well known parasites of native reptiles.

3.3 Endoparasites

Commenting on the prevalence of helminth infections generally, Barton (1995) found that of 424 native frogs collected, 300 (75.75%) were infected with at least one helminth species, although numbers of helminths in each individual were usually low, not more than four or five per animal.

3.3.1 Lungworm (*Rhabdias* sp.)

Rhabdias hylae is the most widespread species in Australia (Barton 1994). Larvae burrow through the skin migrating to the lungs directly or via the bloodstream. Some larvae fail to reach the lungs, encysting in other organs such as heart muscle, liver and eye, causing granuloma formation in these tissues (Williams 1960). Heavy infections are uncommon in wild amphibians (Tinsley, 1995). Experimentally, a culture of *R. sphaerocephala* larvae was shown to infect cane toads (*Rhinella marina*, formerly *Bufo marinus*) with large numbers of migrating larvae causing deaths of affected toads within 24 hours (Williams, 1960).

3.3.2 Sparganosis (*Spirometra erinacei*)

Spirometra erinacei is a common introduced tapeworm of feral cats in eastern Australia (Dickman, 1996) and also infects domestic dogs, native dingoes, foxes, and feral pigs (Gordon & Forsyth, 1954; Bowman, et al, 2002). Spargana of *Spirometra erinacei* infect many native vertebrate species including amphibians, dasyurids, monotremes and reptiles (Beveridge, 1978; Whittington, et al., 1992; Oakwood & Spratt, 2000).

A survey of free-ranging amphibians in eastern Australia between 1993 and 2000 by Berger, et al. (2009) found that infection with spargana (plerocercoids) of *S. erinacei* occurred in 12 out of 243 (4.9%) of sick frogs. Infections occurred in skeletal muscle and subcutis, especially the thighs, of adult *Litoria caerulea*, *L. aurea*, *L. gracilenta*, and *L. peronii*. Three frogs were also infected in the coelomic cavity. Heavy burdens in seven frogs were associated with poor body condition and debilitating

lesions, whereas lighter infections in five sick frogs were considered likely to be incidental to other diseases. In severe infections, a large proportion of thigh muscle was replaced with spargana and various amounts of fibrosis, and some frogs also had myonecrosis, granulomatous inflammation, haemorrhage, and skin ulceration. Concurrent infections were common. The authors concluded that sparganosis is one of a few currently recognised serious diseases affecting free-ranging frogs in Australia.

3.3.3 Trematodes

Metacercariae of various trematode species occur in tadpoles and frogs. The definitive host may be snakes, frogs, birds or mammals (Reichenbach-Klinke & Elkan, 1965). Usually, encysted larvae are not pathogenic although infections have been found in vital organs such as eyes, heart, liver, lung and central nervous system (CNS) where they may cause disease. Metacercaria of *Fibricola* sp. occur over the skin of tadpoles of various frog species in Australia and can occur at high prevalence.

Gross pathology includes many smooth, round raised orange lumps < 1 mm occurring over the tail, and dorsal and lateral body. Histopathology includes cysts in the myxomatous tissue in the subcutis over the body and tail and muscles adjacent to the notochord. Cysts surrounding the larvae have rings of fibroblasts and fibrocytes with almost no inflammatory cells. There are occasional foci of mixed inflammation in the myxomatous tissue assumed to be the remains of degenerate cysts (Berger & Green, 2012).

Barton (1994) reports 10 families of digenea (trematodes) identified in Australian amphibians, including the introduced cane toad (*Rhinella marina*). Surveys of Australian frogs by Barton (1995) found six species in native frogs that also occurred in *R. marina* which also had two species not found in native frogs. The acquisition of parasitic species from native amphibians and reptiles by *R. marina* is postulated. Evidence of introduction of exotic parasites with *R. marina* has not been found.

3.3.4 Oligochaeta (*Dero* [*Allodera*] *hylae*)

Parasitic (or symbiotic) oligochaetes are darkly pigmented, elongate, segmented worm-like organisms within the lumina of the Wolffian ducts ("ureters") of treefrogs (*Hyla* sp., *Litoria* sp. and *Osteopilus* sp.) and rarely toads (*Bufo* sp.) recorded from southeastern USA and Australia. Morbidity or mortality is generally not reported, even in heavy infections. Multiple para-renal oligochaetes may cause marked dilation of the Wolffian ducts. In heavy infections organisms may be present in the urinary bladder (Harman & Lawler, 1975; Hill, et al., 1997; Berger & Green, 2012). In cases of heavy parasite burden in captive anurans, high numbers of oligochaetes may result in severe localised and systemic disease, such as bacterial septicaemia due to rupture of the urinary tract (Frenckel & Harman, 1985).

3.4 Protozoal diseases

3.4.1 Trypanosomiasis

Over 60 species have been reported in anurans but the taxonomy is confused. Most infections are non-pathogenic. Trypanosomes are common in frogs from Queensland, but none has been associated with disease. Transmission is via leeches (Delvinquier & Freeland, 1989). Microscopically, typical trypanosomes are seen on stained blood smear slides or tissue squash-smears. Live organisms are readily detected in a fresh whole-mount unstained drop of blood (Berger & Green, 2018).

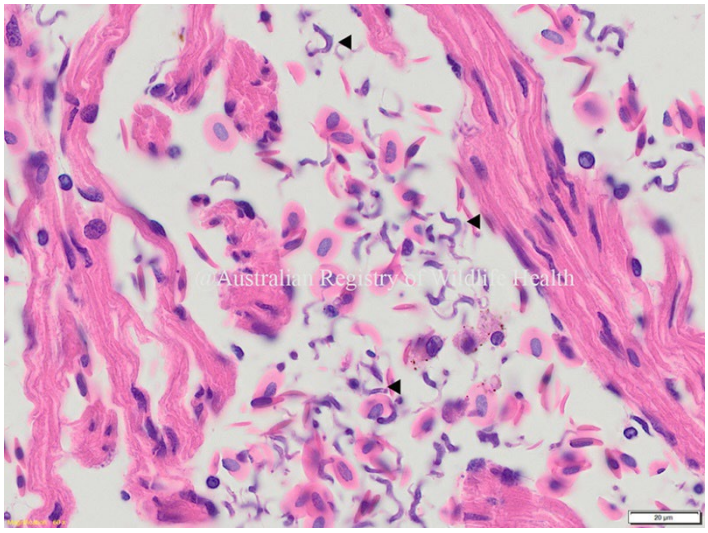

Figure 1 H&E section of a Green and Golden Bell Frog heart with severe trypanosomiasis

3.4.2 Coccidiosis

Coccidial infection is fairly common but usually innocuous. In heavy infections, coccidiosis in tadpoles can cause reduced growth and thickening of the intestinal wall (Berger & Green, 2018). Microscopically, slight to massive numbers of oocysts are present in enteric epithelium with variable degrees of inflammation. Infections in tadpoles expire at metamorphosis. Australian parasites are likely to be *Goussia* spp. (Berger, 2001).

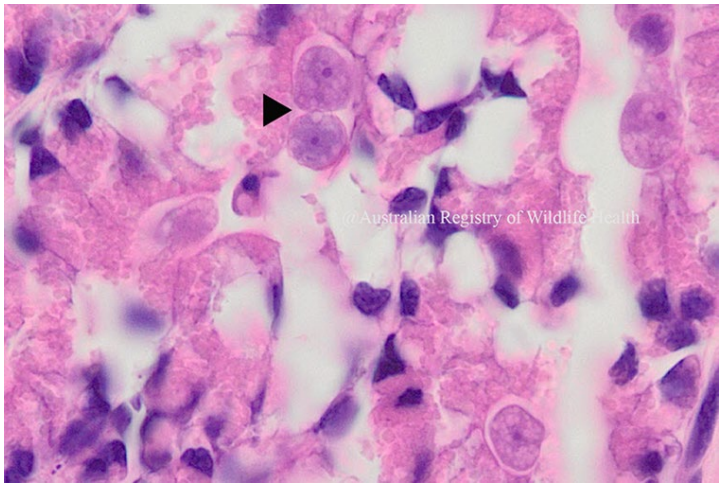

Figure 2 H&E stained lacrimal gland with coccidia from a Green and Golden Bell Frog

3.4.3 Amoebiasis

Amoebae, most often *Entamoeba* sp., are known to occur most often in the gastrointestinal tract, liver, or kidney of stressed amphibians (Wright, 2006). Clinical entero-invasive infections of amphibians in zoos and low elevation neotropical locations are reported infrequently. Subclinical non-invasive infections may be more common (Berger & Green, 2018).

Gastrointestinal presentations are most common clinically and symptoms include anorexia, weight loss, diarrhoea, blood in the faeces, and dehydration. Dissemination to the liver or kidney may also occur (Poynton & Whitaker, 2001). It is possible to make a presumptive diagnosis of amoebiasis through positive identification of amoeba in very fresh faecal samples or cloacal wash specimens from diseased animals. Definitive diagnosis generally requires histological confirmation of invasive amoeba within tissues (Poynton & Whitaker, 2001). Treatment involves supportive therapy to counter

dehydration and gastrointestinal compromise as well as oral or bath administration of anti-amoeboid drugs such as metronidazole (Wright, 2006).

3.5 Myxozoan and Microsporidial diseases

3.5.1 *Myxidium* spp.

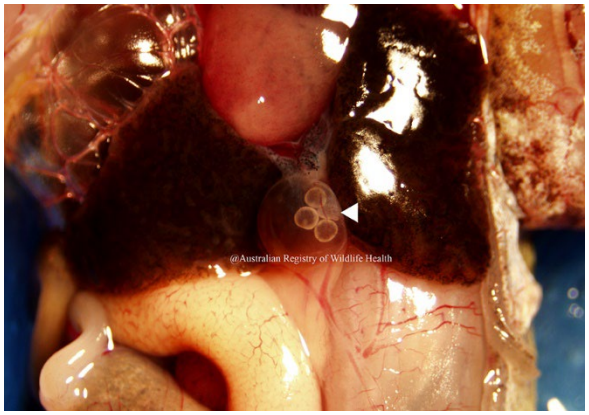

Figure 3 Myxidium trophozoites in the gall bladder of a Booroolong frog

Two species of *Myxidium* have been identified in Australian frogs as emerging pathogens. Brain and liver genotypes occur in tadpoles and adults of native frogs including green tree frogs (*Litoria caerulea*) (Hill, et al., 1997), green and golden bell frogs (*L. aurea*), southern bell frog (*L. raniformis*) and cane toads (*Rhinella marina*) (Delvinquier & Freeland, 1988; Hartigan, et al., 2011). The lifecycles are unknown but may involve invertebrate hosts. Infection may result in lethargy and emaciation. The brain genotype can cause circling and paralysis.

Within the gall bladder large trophozoites (1-5 mm) may present as white flocculent material (Berger & Green, 2018). Stages may be found in the liver adjacent to or within bile ducts. In Australia, moderate to severe biliary duct proliferation, lymphoplasmacytic hepatitis and periportal fibrosis occurs. The brain genotype also infects CNS and root ganglia with extrasporogonic developmental stages occurring within myelinated axons. CNS stages are 5-25 µm diameter and consist of numerous small cells within a primary cell wall. Infection may cause multifocal non-suppurative meningoencephalitis in the CNS. Light infections not associated with lesions have also been observed (Hartigan, et al., 2011).

3.5.2 *Myxosporeans* (*Myxobolus* spp.)

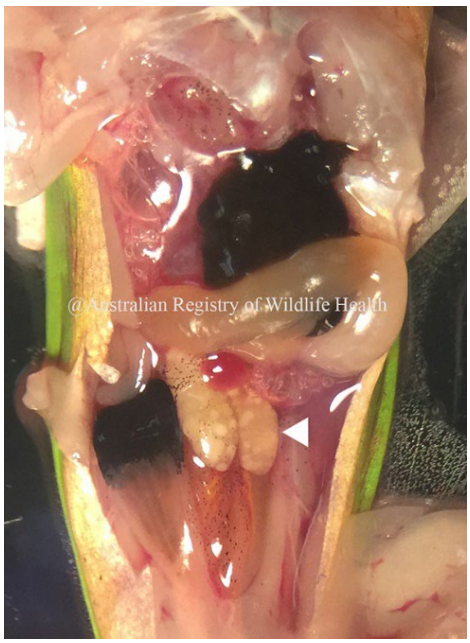

Figure 4 Myxobolus hylae cysts in the testes of a green and golden bell frog

Two *Myxobolus* species have been described in the urogenital system of Australian frogs: *M. hylae*, and *M. fallax*. Frogs infected with *M. hylae* are reported to be lethargic, thin and, in cases of high parasite burden, gonads may be swollen and covered with white cysts. Microscopically, infection results in partial replacement of the gonads by large, encapsulated cysts and degenerate cysts are infiltrated with granulomatous inflammation (Johnston & Bancroft, 1918).

The two species were distinguished from each other based on myxospore morphology of museum material of *M. hylae* and specimens of *M. fallax* (Browne, et al., 2002). As yet, no other *Myxobolus* species has been identified in Australian frogs. *Myxobolus* spp. have been reported in the common green tree frog (*Litoria caerulea*), eastern dwarf tree frog (*L. fallax*), stony creek frog (*L. lesueuri*), Peron's tree frog (*L. peronii*) and endangered green and golden bell frog (*L. aurea*) and southern bell frog (*L. raniformis*) species, though only as incidental findings (Berger, 2001, Mann, et al., 2010).

Given the distribution of these frog species it is likely that *Myxobolus* spp. can be found across the entire east coast of Australia (Hartigan, et al., 2013).

3.5.3 *Cystodiscus* spp.

Cystodiscus australis and *C. axonis* (originally thought to be a single species *Cystodiscus immersus*) were introduced to Australia with the cane toad (*Rhinella marina*) in 1935 (Hartigan, et al., 2010; 2011; 2012a; 2012b). Based on case reports and wildlife disease screening, *Cystodiscus* species has not been identified in Western Australia or the Northern Territory (Hartigan, et al., unpublished). The absence of *Cystodiscus* parasites in the Northern Territory, despite presence of known susceptible hosts *R. marina* and *Litoria caerulea* suggests the absence of a putative invertebrate host in this area. The movement of *Cystodiscus* species into new areas has been linked to frogs accidentally translocated with fresh produce (Hartigan, et al., 2012c). The southward spread and increased prevalence in southern distributed frog populations is of concern as some of Australia's most endangered frog species are in southern states and species with no previous exposure to these parasites may be more susceptible to *Cystodiscus* infection and disease. Moreover, *Litoria* species in particular the green tree frog (*L. caerulea*) are popular species within the international pet trade which could, potentially, spread *Cystodiscus* spp. around the world.

Both *Cystodiscus* species can infect and cause disease in tadpoles, although not all host species are affected in the same way (Hartigan, et al., 2012a). Frogs with severe disease exhibit neurological dysfunction, lose the ability to right themselves, and in some cases lose hindlimb movement. Lesions caused by either *Cystodiscus* species include inflammation and hyperplasia of the liver, and morphologic appearance of presporogenic stages of both species are similar. Brain lesions attributed to *C. axonis* are more severe in some species (booroolong frog, *L. booroolongensis*, yellow-spotted bell frog, *L. castanea* and southern bell frog, *L. raniformis*) and include haemorrhage, gliosis and necrosis (Hartigan, et al., 2012a). It is speculated that infection with *Cystodiscus* species may cause delayed metamorphosis leading to tadpole overwintering (Hartigan, et al., 2012a). Both Australian *Cystodiscus* species appear to be emerging parasites.

3.5.4 Microsporidian myositis *(Pleistosphora* spp.*)*

Sporadic infection of tadpoles and adult anurans is recorded. Intense muscular infections may cause death. Hepatic infections are described from captive juveniles in Australia. Clinical signs of infection include lethargy, atrophy and emaciation. Inapparent or mild to intense white streaking of skeletal muscles may be seen at post-mortem. Microscopically, intracytoplasmic organisms occur within indistinct parasitophorous vacuoles. Inflammation may be absent or mildly lymphohistiocytic. Muscle regeneration may occur with long chains of sarcoblasts adjacent to damaged muscle (Berger, 2001).
Intramuscular injections with chloramphenicol sodium succinate for 18 days and topical administration of oxytetracycline HCl with polymyxin B sulphate for 21 days, have been used for successful treatment overseas (Graczyk, et al., 1996).

4 Bacterial diseases

4.1 Bacterial dermatosepticaemia (Red leg syndrome, Red leg disease)

Zoonotic: No
Species records: All
Similar presentation to: Bacterial septicaemia, flavobacteriosis, fungal dermatopathies (e.g. chytridiomycosis), viral aetiologies, environmental toxins, general irritants

Red leg is probably the most over-diagnosed and misdiagnosed disease of all amphibians. The disease is described as a generalised systemic bacterial infection associated with cutaneous erythema occurring most often on the ventrum or extremities. Historically, *Aeromonas hydrophila* was frequently implicated as the bacterial aetiological agent, however many other Gram-negative bacilli including aeromonads, pseudomonads, *Flavobacterium indologenes* and *F. meningosepticum* (Anver & Pond, 1984; Olson, et al., 1992; Taylor, et al., 1993) and enterobacteria (*Citrobacter*, *Proteus*, *Salmonella*) may be involved. Some Gram-positive bacteria (*Streptococcus* and *Staphylococcus*) have also been linked to this syndrome (Crawshaw, 1992; Mauel, et al., 2002). Consequently, red leg syndrome is now a more general term associated with peracute to acute bacterial septicaemia, and no longer considered synonymous with *A. hydrophila* associated disease (Densmore & Green, 2007).

Erythema is due to vasodilation, congestion or ecchymotic haemorrhages. Other clinical signs attributed to red leg syndrome include anorexia, swelling, oedema (generalised or localised to the extremities or the lymphatic sacs), coelomic effusions, and epidermal erosions, ulcers, sloughing, or necrosis (Densmore & Green, 2007) and may include haemorrhages in internal organs, ascites and pale livers (Berger, 2001). Alternatively, the disease may present as sudden death, with few or no overt signs.

Post-mortem bacterial invasion of organs is a common phenomenon among aquatic vertebrates, including amphibians, and it occurs much more rapidly than in terrestrial, endothermic vertebrates. Therefore, diagnosis of dermatosepticaemia is considered unreliable when it is based solely on bacterial isolation from tissues of an amphibian that died between one and three hours before the necropsy. A full diagnostic workup including gross pathology, histopathology, and microbial culture are recommended to achieve a definitive diagnosis and avoid misdiagnosis. Differential diagnosis must include other nonbacterial pathogens that may present with similar clinical signs including ranaviruses (Cunningham, et al., 1996a) and chytrid infection by *Batrachochytrium dendrobatidis* (Berger, 2001).

Histologically, there may be degenerative myopathy and multiple foci of coagulative necrosis with clumps of bacteria (Berger, 2001). Many bacteria can be isolated from healthy animals and from the environment (Carr, et al., 1976; Hird, et al., 1981) suggesting that disease occurs secondary to stresses caused by poor husbandry such as overcrowding, dirty conditions, trauma, temperature changes, and after transport (Hubbard, 1981; Glorioso, et al., 1974).

Treatment of dermatosepticaemia may or may not be effective depending upon the chronicity of the infection, causative agent, and antibiotic efficacy. Culture and sensitivity testing is recommended before treatment begins. Parenteral treatment with broad spectrum antimicrobials may be the best option but should be administered together with antifungal therapy to avoid overgrowth of opportunistic fungi and intercurrent mycotic infection. Correcting poor husbandry and alleviating stress may help prevent the development.

4.2 Chlamydia sp.

Outbreaks of chlamydiosis have been reported in captive amphibians, usually involving fulminant, multisystemic infections. Frogs may be found behaving abnormally, sitting unprotected during the day, lethargic and in poor nutritional condition. Clinically, non-regenerative anaemia with low PCV and low total white cell count, low total protein, and elevated aspartate amino transferase may be seen. Thickened, non-collapsing lungs, and a small spleen may be seen grossly. Histopathology shows spleen and bone marrow haemopoietic cell depletion, severe chronic mononuclear pneumonia with inflammation and thickening of the septa, alveolar spaces containing masses of free monocytes, lymphocytes, plasma cells and erythrocytes, and renal tubular epithelial cells containing round, intracytoplasmic deposits of brown pigment that did not stain for iron with Perl's stain. Electron

microscopy may be used to identify chlamydial particles in membrane bound inclusions. *Chlamydia pneumoniae* has been confirmed by PCR and culture of lung (Berger, et al., 1999).

4.3 Flavobacteriosis

Flavobacteria are Gram-negative yellow pigment-producing bacteria that are commonly recognised pathogens of lower vertebrates, and are widely present in aquatic environments, with numerous reports appearing in the literature in both wild and captive amphibians (Densmore & Green, 2007). Berger and Green (2012) suggest that bacterial septicaemias caused by flavobacteria are a threat to captive amphibian colonies but are less common in free-living amphibians.

Clinical signs are non-specific and often closely resembles the presentation of bacterial dermatosepticaemia (see 4.1). Clinical signs may include: marked lethargy, anorexia and weight loss, anasarca, dyspnoea, congestion of toe-webs, dermal petechiae, panophthalmitis, corneal oedema, neurological signs (including anisocoria, head tilt, loss of righting reflex, abnormal posture, meningitis), otitis interna, marked haemorrhage in major muscle groups, purulent pericarditis, hepatosplenomegaly and a distended gastrointestinal tract with viscous haemorrhagic contents (Olson, et al. 1992; Green, 1999; Taylor, et al. 2001; Keller & Shilton, 2002; Berger & Green, 2012). Flavobacterial meningitis has been linked with immunosuppression related to pesticide exposure (Hayes, et al., 2006).

Although reports of histological changes are sketchy, there may be infiltrates of macrophages, neutrophils and lymphocytes in the liver, spleen, mesonephroi, serosal surfaces and epicardium. Macrophages may contain Gram-negative bacilli. Neutrophils may be found in the anterior chamber of the eye with retinal detachment and severe conjunctival and corneal oedema. The pericardium may have a thick layer of fibrin, neutrophils and bacteria and the liver may show hepatocellular degeneration and coagulative necrosis (Berger & Green, 2012).

Diagnoses of flavobacteriosis may be achieved through bacterial culture and molecular analysis including PCR (Green, et al., 1999; Mauel, et al., 2002; Olson, et al., 1992). Crawshaw (1992) and Hadfield and Whitaker (2005) have reported achieving the best treatment with antibiotic regimes based on antimicrobial sensitivity testing using premortem bacterial culture.

4.4 Mycobacteriosis

Zoonotic: Yes
Species records: All
Similar presentation to: Other bacteria, mycosis, viral aetiologies, endo- and ecto-parasites

Berger and Green (2012) note that mycobacteriosis is rare in free-living amphibians, however certain mycobacteria can cause chronic problems in captive amphibian colonies. Early signs of disease may be subtle or unapparent, and fulminant clinical signs often do not develop until the disease has become widely systemic (Densmore & Green, 2007). Clinical signs include lethargy, poor body condition, and wasting as well as a mucopurulent nasal or oral discharge. *M. liflandii* infection causes skin ulcers, hydrocoelom and inability to dive (Berger & Green, 2012). Affected skin frequently involves the digits and mouth and may present as miliary lesions.

The gross pathology of mycobacteriosis includes solitary, miliary or prominent and coalescing granulomas that may be irregular and poorly demarcated, or large and encapsulated. Liver, spleen, intestine and dermis are most often affected, but granulomas may be present in nearly any organ or tissue. Mycobacteriosis should be included in the differential diagnosis when granulomatous, histiocytic, lymphocytic, caseous, or pyogranulomatous nodules are found in any organ, including the

skin (Densmore & Green, 2007). As various species of mycobacteria are zoonotic, where infection is suspected appropriate precautions should be taken when handling animals or related biological specimens.

Histopathological changes include granulomatous inflammation which in amphibians consists of epithelioid macrophages, small lymphocyte-like macrophages and true lymphocytes. Early granulomas are solidly cellular, poorly demarcated, and have been confused with lymphosarcoma (Green, 2001). Acid fast staining (Ziehl-Neelsen) is recommended for all caseous and non-caseous granulomata of amphibians, especially those in long-term captive situations (Berger & Green, 2012). If Gram-positive bacilli are evident in inflammatory nodules histologically and ZN acid fast stain is inconclusive, then alternative acid fast stains such as Fite-Faraco, or PCR may prove useful. The lack of any reported effective treatment of mycobacteriosis for amphibians warrants the culling of affected animals and the thorough disinfection of holding facilities (Pessier, 2002; Taylor, et al., 2001).

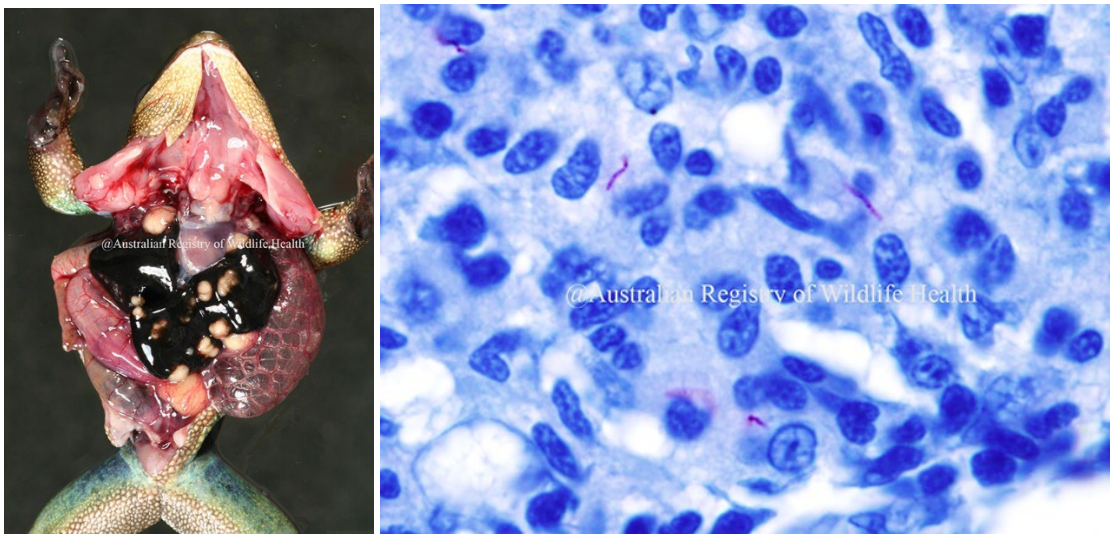

Figure 5 Mycobacterial granulomas in the liver of a green and golden bell frog, and ZN stain showing acid fast organisms of Mycobacterium marinum from the same individual

4.5 Salmonellosis

Zoonotic: Yes
Species records: All
Similar presentation to: No clinical signs

In Australia, Salmonellae were isolated from 12.7% (19 out of 150) of *Rhinella marina* collected from the wild and nine serotypes were identified, all of which had previously been isolated here from humans and livestock (O'Shea, et al., 1990). Salmonellae are zoonotic bacteria which can cause significant gastroenteritis and more serious diseases. Amphibians may carry pathogenic *Salmonella* species, but frogs showing signs of disease are rarely reported (Reichenbach-Klinke & Elkan, 1965; Anver & Pond, 1984). Salmonellae are generally not of significance to the amphibian host, but may infect humans and other animals. An outbreak of gastroenteritis in humans near Rockhampton was thought to have possibly arisen from green tree frogs (*Litoria caerulea*) contaminating drinking water in rainwater tanks (Taylor, et al., 2000).

4.6 Spinal arthropathy associated with Ochrobactrum anthropi

Cane toads (*Rhinella marina*, formerly *Chaunus [Bufo] marinus*) in Australia commonly have nodular proliferations at intervertebral joints often causing ankylosis. Pathology involves bone and cartilage

proliferation, often with associated pyogranulomatous inflammation. An interaction between degenerative and bacterial aetiologies is suggested (Shilton, et al., 2008).

5 Viral diseases

5.1 Ranaviruses

Zoonotic: No
Species records: All
Similar presentation to: May be no clinical signs
Reportable OIE List Disease: Yes

The ranaviruses are the best described pathogenic amphibian virus group composed of viruses from the genus *Ranavirus* family *Iridoviridae* (Densmore & Green, 2007). Ranaviruses are associated with disease outbreaks causing significant mortality and morbidity in wild amphibians, reptiles, and cultivated and wild fish (Gray & Chinchar, 2015). They are reported from most continents, including Australia (Hyatt, et al., 2002; Weir, et al., 2012). Ranaviral disease of amphibians was included as a notifiable list disease by the World Organisation for Animal Health in 2008 (OIE, 2016). Amphibian ranaviral disease is considered an emerging infectious disease, having been detected over an increasing species and geographic range (Hemingway, et al., 2009).

Bohle iridovirus (BIV), a ranavirus, was isolated from metamorphs of captive ornate burrowing frogs (*Limnodynastes ornatus*), from Bohle in northern Queensland, where it caused mortality during and after metamorphosis (Speare & Smith, 1992). Laboratory studies have shown that both introduced cane toads (*Rhinella marina*) and native frogs are vulnerable to BIV (Speare, 2000; Cullen & Owens, 2002). Tadpoles appear the most susceptible, while juvenile frogs were more susceptible than adults.

Mahaffey Road virus, an irido-like virus was associated with disease and mortalities in captive magnificent tree frogs (*Litoria splendida*) and green tree frogs (*L. caerulea*) in the Northern Territory. Viral isolates cultured from both species were determined to belong to the genus ranavirus and were indistinguishable from BIV (Weir, et al., 2012). While disease from ranavirus is infrequently observed in wild amphibians in Australia, antibodies to ranaviruses have been detected widely in cane toads (*R. marina*) in New South Wales, Queensland and Northern Territory with an overall prevalence of 2.7% (range 0-18%). (Zupanovic, et al., 1998). Wild, moribund adult *L. caerulea* from Townsville and captive juvenile red-backed toadlets (*P. coriacea*) from Sydney have tested positive for BIV with PCR (Speare, 2000; Cullen & Owens, 2002).

Two syndromes in frogs are associated with ranavirus infection: ulcerative syndrome and haemorrhagic syndrome. The most common presentation is sudden death due to peracute systemic haemorrhagic disease. Clinical signs in tadpoles and metamorphs can include decreased activity, ascites, focal haemorrhages and death. Adults may exhibit decreased activity, erratic swimming, lethargy, loss of equilibrium, buoyancy problems, skin ulceration, focal and systemic haemorrhages and death (Berger & Green, 2012; Jerrett, et al., 2015). Mortality and morbidity vary from species to species (0–100%), and may be variable depending on virus type, and age and health status of the host. Typical histopathology includes renal, pulmonary, hepatic, splenic and haemopoietic necroses and haemorrhages (Berger & Green, 2012).

Ranavirus infection and disease may be diagnosed through primary isolation in cell culture, molecular identification (PCR and sequencing of the major capsid protein), light and electron microscopy, or a combination of these techniques (Gould, et al., 1995; Green, 2001; Kattenbelt, et al., 2000; Mao, et al., 1996; Weir, et al., 2012; Wolf, et al., 1968).

Figure 6 Magnificent tree frog with oedema and haemorrhage of the legs due to a ranaviral infection, and a fixed specimen of a magnificent tree frog with haemorrhage adjacent to the spinal column from the same infection (Images courtesy of Berrimah Veterinary Laboratory, Northern Territory)

Horizontal transmission occurs via contaminated water, animal-to-animal contact and cannibalism. The virus can be spread between widely separated river systems and impoundments, which suggests viral persistence and the existence of transmission mechanisms other than direct horizontal transmission. Possible vectors include nets, boats and other equipment, or amphibians used for bait by recreational fishers. Birds are potential mechanical vectors. Temperature is considered a likely factor influencing disease outbreaks, with the prevalence or severity of outbreaks greater during warmer months (DAFF, 2012).

6 Fungal diseases

6.1 Chytridiomycosis (Batrachochytrium dendrobatidis; Bd)

Zoonotic: No
Species records: All
Similar presentation to: Bacterial infections (including 'red leg'), ranavirus, intoxication, other fungal infections
Reportable OIE List Disease: Yes

The impact of chytridiomycosis (Bd) on frogs may be the most spectacular loss of vertebrate biodiversity due to disease in recorded history and is responsible for numerous amphibian extinctions (Skeratt, et al., 2007). *Bd* in free-living amphibian populations was first described in Australia and in Central America during the mid-1990s (Berger, et al., 1998; Pessier, et al., 1999). *Bd* remains common in surviving populations, with seasonal outbreaks. While predominantly known as a disease of wild amphibians, *Bd* also affects captive populations (Mazzoni, et al., 2003; Parker, et al., 2002; Pessier, et al., 1999).

Chytrids are ubiquitous, keratinophilic or chitinophilic, sporozooic fungi located in moist and aquatic environments. *Bd* is a **notifiable OIE List A Disease**, which has a very wide amphibian host range although susceptibility varies among species. Mortality rates range from 0-100% and incubation times from about 10 to 70 days, varying with temperature, host species and age, and fungal dose and strain. *Bd* infects superficial keratinizing cells of the adult skin and tadpole mouthparts (Berger & Green, 2012).

In tadpoles, infection of the mouth parts with *Bd* is characterised by multifocal to diffuse loss of black colour on jaw sheaths ("beaks") and tooth rows. While tadpoles may survive infection, they may have

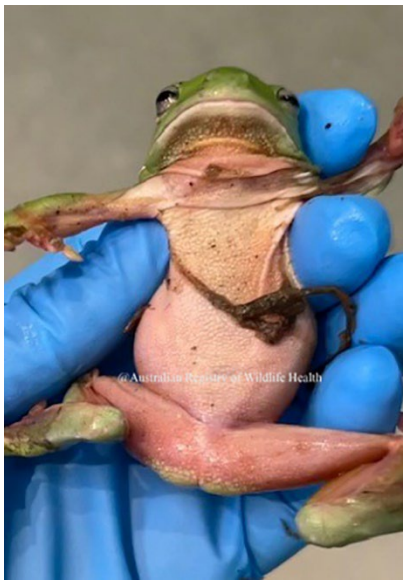

Figure 7 Green tree frog with accumulation of shed skin and reddened ventral skin due to chytrid fungus infection

reduced growth rates and smaller size at metamorphosis. In captivity it is typical to see high mortality rates two to three weeks after metamorphosis, when the skin over the body becomes keratinized and infection spreads. Gross pathology and clinical signs in frogs include discoloured and reddened skin, inappetence, lethargy, loss of righting reflex and seizures. Sick frogs usually die within days. There may be accumulations of shed skin, thickened translucent-to-opaque moults, and occasionally paint-brush haemorrhages in ventral skin. Infections are most intense in ventral abdominal and thigh skin, toe tips and toe webs. In terminal stages electrolyte concentrations are greatly decreased, but other biochemical parameters are normal (Berger & Green, 2012).

Microscopically, Bd produces spherical or oval sporangia, not hyphae. Sporangia divide internally to form the infective, waterborne flagellated zoospores that are released via a discharge tube through the skin. Bd is an intracellular parasite, infecting cells of the stratum granulosum and stratum corneum. In histological sections, Bd is spherical or oval with occasional discharge papillae seen projecting from the skin surface. The contents of zoosporangia vary, and four developmental stages can be identified: (1) Dark basophilic, rather homogenous mass; (2) Sporangia become multinucleate; (3) Cytoplasm divides to form distinct zoospores; (4) Once the zoospores are released via the discharge papilla, the empty zoosporangia remain. In some empty colonial stages, thin septa are visible dividing the sporangium into two, four or more internal compartments. The observation of internal septa within sporangia increases confidence in the diagnosis. Empty sporangia may collapse into an irregular shape. During this terminal stage the empty shell sometimes becomes colonised by bacteria. Empty sporangia are the most common stage present, particularly in the sloughing surface layer. Immunohistochemistry, PAS or silver stains are useful for confirming suspect cases. Focal hyperkeratosis, erosions and disorganised cell layers are common in infected areas. The epidermis may be thicker or thinner than normal. Ulcers occur in some species. Inflammation is minimal, but may be marked if secondary bacterial and, or opportunistic fungal infections develop.

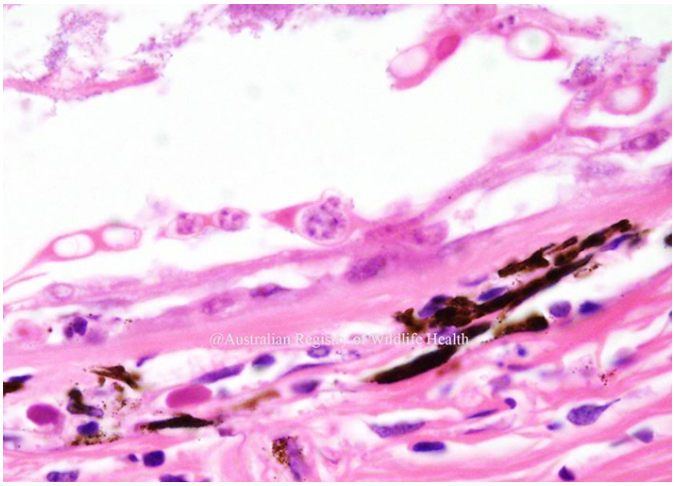

Figure 8 H&E stained section of skin from a brown-striped frog with chytrid sporangia present

Distinctive lesions are not seen in internal organs. The cause of death appears related to loss of electrolytes across the epidermis. Wet mounts can be made from shedding skin, whole skin pieces or tadpole mouths. The round to oval intracellular sporangia (5 - 13 µm) occur in clumps. Old, empty, septate sporangia are the most prevalent stage in shedding skin, although sporangia containing zoospores are commonly found. Discharge tubes usually point perpendicularly to the skin's surface and thus appear as small circles, which can be difficult to discern. Epidermal cell nuclei are of similar size to sporangia but can be differentiated by their

irregular, indistinct membranes and flat, granular, grey appearance. Congo red, cotton blue and Dip Quick stains can be used if desired (Berger & Green, 2012).

Figure 9 Sloughed skin heat fixed and stained with Dip Quick

Chytridiomycosis can only be diagnosed by laboratory tests. The clinical signs of chytridiomycosis are non-specific. In ill amphibians, all diagnostic tests (including histology and wet skin mounts) are accurate. However, for screening healthy animals, swabbing for standard or real-time PCR is much more sensitive. Caution in interpreting PCR results from skin swabs of wild and group-caged amphibians is advised however, since a positive result confirms the presence of the organism but does not necessarily confirm an infection in the individual (Densmore & Green, 2007). Culture requires special techniques and is insensitive for diagnosis. Sample from ventral surfaces of adults by collecting skin scrapings or smears, excising pieces of skin, or by swabbing. Toe-webbing, and/or toes can be excised from live or dead frogs, and strips of skin from the inguinal area (pelvic patch) can be collected from dead animals. Tadpoles need to be sacrificed if microscopy is to be done. Whole tadpoles can be collected and preserved and sectioned through the mouthparts to include the dark brown keratinized jaw sheaths or tooth rows. Tail stumps in metamorphs are a sensitive site to sample. In live tadpoles the mouthparts can be swabbed for PCR (Berger & Green, 2012).

Successful treatment of chytridiomycosis in captive anurans is reported, which included bath treatment with 0.01% itraconazole (Pessier, 2002). Because Bd is transmissible by movement of infective zoospores, isolating the infected animals and disinfecting equipment and surroundings are essential in managing the disease (Johnson & Speare, 2005, 2003; Johnson et al., 2003). Colony managers should establish a quarantine period for all recently captured amphibians and use effective antifungal agents to prevent potential dissemination of the pathogen (Johnson & Speare, 2003; Johnson et al., 2003).

6.2 Oomycosis

Water moulds (family Saprolegniaceae, phylum Oomycota) are ubiquitous in surface water. The term, saprolegniasis, may be used for infections by multiple genera including *Leptolegnia*, *Achlya*, *Aphanomyces*. Oomycetes are not true fungi and are principally saprophytes that invade dead or infertile eggs and larval skin wounds, but some species appear to be pathogenic. Secondary infections of aquatic amphibians often are associated with anchorworms (*Lernaea* sp.), leech bite wounds, other wounds, gangrene of extremities subsequent to gas bubble disease, and ranaviral skin ulcers. Saprolegniasis may have its greatest impact on amphibian eggs, resulting in highly variable mortality levels depending on environmental factors and overall condition (or fertility) of the eggs (Blaustein et al., 1994). However, water moulds also commonly invade eggs after death, so it may be difficult to determine whether these organisms represent a causative factor in an egg mortality event (Densmore & Green, 2007). Two epidemics of *Aphanomyce*s sp. in cane toad tadpoles (*Rhinella marina*) in Queensland have been described with no associated underlying disease. Tufts of hyphae were attached to the nostrils, mouthparts, other parts of the head, and occasionally to the hind legs and tail (Berger & Green, 2012).

The gross pathology of oomycosis presents as white, pale tan, or pale grey fuzzy cottony growths from the skin of infected animals or the capsules of infected eggs, but may vary in coloration depending on host, duration of infection, species of mould, and water quality, including suspended particulate matter in the water (Densmore & Green, 2007). Infection may be focal or generalised. Hyphae are best observed in the immersed specimen, since filaments collapse out of water and become very difficult to detect lying on the skin (Berger & Green, 2012). Erythematous or ulcerated skin may also be visible. Although infections generally affect the tail, hindlimbs, gills, and oral mucous membranes

without becoming systemic, lesions sometimes deeply penetrate and involve underlying tissues (Densmore & Green, 2007).

Microscopically, examination of fungal-infected tissues or lesions by wet mounts often reveals mats of aseptate, sparsely branching fungal filaments. Histologically, fungal filaments and zoospores are evident in lesions, although stain affinity (hematoxylin and eosin, periodic acid-Schiff) may be poor (Green, 2001). Inflammatory response is generally minimal, but lesions may show evidence of erosion or ulceration, necrosis, and oedema, depending on the severity (Densmore & Green, 2007). Deeper invasion into dermis and muscles is variable.

It is possible to diagnose oomycosis presumptively through observation alone, but a definitive diagnosis is dependent on histology, culture of the water mould, or molecular confirmation (Densmore & Green, 2007). Living material is needed for isolations.

Descriptions of various effective therapeutic regimes appear in the literature, including bath treatment with antifungal agents such as benzalkonium chloride, copper sulphate, or potassium permanganate (Taylor et al., 2001).

6.3 Zygomycoses

The literature includes reports of zygomycoses among wild and captive anurans. Causative agents have included *Mucor* spp. and *Rhizopus* spp. (Taylor, 2001). *Mucor amphibiorum* is a widely distributed zygomycete in Australia. Occasional chronic cases of mucormycosis have been reported in wild cane toads (*Rhinella marina*), green tree frogs (*Litoria caerulea*), white-lipped tree frogs (*Litoria infrafrenata*), and striped marsh frog (*Limnodynastes peronii*) in Queensland, Northern Territory and New South Wales (Speare et al., 1994; Berger et al., 1997; Creeper et al., 1998). Mucormycosis has also caused sub-acute outbreaks in captive slender tree frogs (*Litoria adelensis*) with an 80% mortality rate, and in green tree frogs and dendrobatid frogs in Australia and Germany. Mucormycosis has not been found in wild amphibians outside Australia. *M. amphibiorum* is also a pathogen of free-living platypus in Tasmania, although isolates from platypus differ genetically from anuran isolates (Connolly et al., 2010).

Clinical signs of mucormycosis include lethargy, emaciation, dehydration and occasional large cutaneous papules, however, 74% of infected toads were found without clinical signs. On post-mortem examination, the liver may exhibit massive numbers of small pale, 5 mm diameter nodules. These nodules may also be seen in kidney, lung, mesentery, urinary bladder, subcutaneous sinuses and skin (Berger & Green, 2012). Mild, localised infections are found as incidental findings; however, the development of clinical signs generally leads to mortality in 1-2 weeks (Fowler, 1986; Taylor et al., 1999; Berger & Green, 2012).

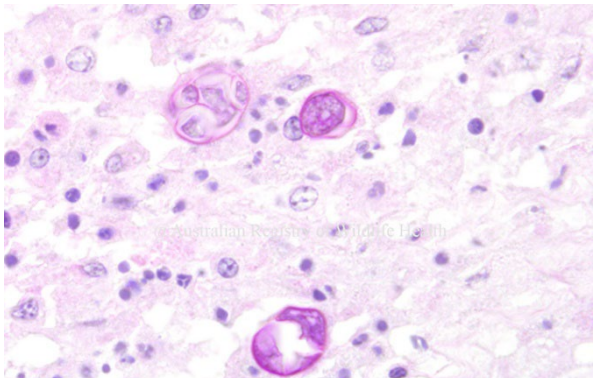

Figure 10 PAS stained muscle tissue from a green tree frog with suspect mucormycosis

Histologically, the fungus is a distinctive yeast-like spherical structure, called a sphaerule, which range in size from 5 to 36 µm. *M. amphibiorum* forms daughter sphaerules inside the mother sphaerule, and these can be seen in histological sections or on wet mounts of infected tissue (Berger & Green, 2012). Fungal sphaerules incite granuloma formation in most organs however, Taylor, et al. (1999) reported nodules in the integument appearing histologically as fungal hyphae without any significant inflammation. Because the Zygomycetes are relatively

ubiquitous in moist environments that are associated with soil and decaying material, infection and development of disease may occur secondary to a compromised immune system and to traumatic introduction through the skin, through ingestion, or through inhalation of fungal spores (Taylor et al., 2001, 1999).

Observation of sphaerules in sections is usually sufficient for diagnosis. PAS and other fungal stains may be useful. *M. amphibiorum* is dimorphic and in culture or soil it forms hyphae. It can be cultured on Saboraud's agar where it forms a mycelium. There are no reports of successful treatment.

6.4 Chromomycosis

Chromomycosis refers to infection by a range of pigmented, septate fungi which have been isolated from a range of exotic captive and wild frogs overseas. Human cases of chromoblastomycosis associated with *F. pedrosi* are occasionally reported in Australia (Weedon et al., 2013). Two forms of disease, cutaneous and disseminated systemic, have been reported in a number of anuran species, in both wild and captive animals. Like other fungal agents, these pathogens commonly exist in soil and dead plant matter. Transmission usually occurs through contamination of the environment, and traumatic injury to the skin may contribute to the infection (Juopperi et al., 2002). Poor husbandry appears to be a cofactor (Berger & Green, 2012).

7 Nutritional diseases

7.1 Dietary deficiencies

Overall dietary deficiency that results in an insufficient caloric intake will lead to malnutrition and starvation. Often, this occurrence is due to a poor understanding of, and failure to provide for, the dietary needs of a particular species and life stage. Although adult amphibians are generally carnivorous, the types and sizes of food they will accept and even the timing of feedings will vary considerably and have a significant impact on their willingness to feed. In addition, many commonly offered food items, including a variety of insects, have inadequate levels or imbalanced ratios of some essential nutrients (Barker et al., 1998). Clinical indications of malnutrition/starvation include anorexia, weight loss, dehydration, and lethargy. Weakness or wasting that mimics starvation may be due to other systemic disease, thus determination of any other causal factors is important (Densmore & Green, 2007). It is often necessary to provide amphibians with nutritional supportive care through assist-feeding. Metabolic bone disease is common in captivity. Juveniles develop spinal deformities and adults may be tetanic after activity (Raphael, 1993; Hulst, 1999). Other nutritional problems include obesity, impaction, iodine deficiency, and oxalate toxicity (Raphael, 1993; Hulst, 1999).

7.2 Metabolic bone disease

Essentially, metabolic bone disease refers to vitamin and mineral imbalances that occur through inappropriate diet and housing. It is common in captive situations, and rare in the wild. Specifically, these imbalances include the failure to ingest or adequately process vitamin D3, calcium (Ca), or phosphorus (P). Elevated levels of vitamin A may also interfere with normal metabolism of vitamin D and contribute to metabolic bone disease. Most often, metabolic bone disease is caused by low levels of calcium or improper Ca:P ratios in the insect prey such as crickets, mealworms, waxworms, earthworms, and fruit flies (Barker, et al., 1998; Wright & Whitaker, 2001). It is possible to correct this deficiency by feeding the insects calcium-rich food or by dusting the insects with a calcium-rich powder. Although proper absorbance of ultraviolet radiation is an important consideration in the prevention of metabolic bone disease among reptiles, this link is not as well established for amphibians.

Figure 11 A green tree frog with metabolic bone disease has mandibular and long bone deformities, and is very thin, and a red-eyed tree frog with long bone and spinal deformities, and abnormal posture, caused by metabolic bone disease

Clinical signs that typify this syndrome are indicative of inadequate bone mineralisation and may include abnormal posture and locomotion, tetany, anasarca, dropsy, subcutaneous oedema, vertebral deformity, mandibular deformity, pathological fractures of long bones, and absence of radio-opaque calcium carbonate in the endolymphatic sacs. It is possible to use radiology to confirm a diagnosis by highlighting the altered bone density and other related indications such as bone deformity or pathological fractures (Densmore & Green, 2007). Treatment is often difficult, particularly in advanced cases. Treatment regimens involve dietary supplementation with calcium and vitamin D3 or, alternatively, topical (bath) or parenteral calcium supplementation (Hadfield & Whitaker, 2005; Wright, 2006). The preferred treatment regime will be largely dependent on the species and life stage in question as well as the severity of disease.

7.3 Vitamin A deficiency

Hypovitaminosis A may be an emerging nutritional disease of concern in captive amphibians in zoos and conservation programs (Wright, 2006). Amphibians do not synthesise carotenoids, including vitamin A (retinol), therefore the animals must obtain these nutrients from food. Carotenoids are essential for maintaining epithelial integrity and bone metabolism.

Clinical signs of hypovitaminosis A in include listlessness, wasting, and reduced ability to capture live prey with the tongue. The principal histological finding is squamous metaplasia of the tongue with consequent loss of mucous cells and sticky secretions on the surface of the tongue that are necessary to apprehend live insects. Parenteral, oral, or topical (bath) treatments with vitamin A may alleviate the deficiency, but recovery time has not been reported and supportive care, including force feeding, may be necessary during treatment (Densmore & Green, 2007).

7.4 Vitamin B deficiency

B vitamin deficiency has been associated with various neurological and musculoskeletal abnormalities, including hindlimb paresis and paralysis, scoliosis, and the spindly leg syndrome in a number of captive species (Wright & Whitaker, 2001; Crawshaw, 2003). In many cases, investigators suspected that leaching of the B vitamins from the diet was a causal factor that B vitamin supplementation of the water could offset (Densmore & Green, 2007).

Thiamine (vitamin B1) clinical signs and lesions include paralysis and peripheral nerve demyelination (Wright & Whitaker, 2001). Treatment with thiamine supplements given topically or parenterally usually reverses the condition, with a feed additive to prevent recurrence (Crawshaw, 2003).

7.5 Dietary excess

Like malnutrition, obesity is most likely to occur in amphibians when care providers do not carefully consider specific dietary requirements and caloric needs (Densmore & Green, 2007). Gastric overload resulting from consumption of an excessive quantity of food such as oversized prey, represents a medical emergency. The resultant overdistension of the stomach may compromise respiration and circulation, placing the animal at risk for hypovolemic shock unless treated immediately. It is usually necessary to remove the food item(s) from the stomach surgically, however in some cases it may be possible to carefully remove the material through the mouth via forceps or endoscopy (Wright & Whitaker, 2001). Similarly, gastric or intestinal impactions may present as surgical emergencies. These occurrences generally result from the ingestion of non-food items such as cage substrate (Densmore & Green, 2007).

Excessive dietary intake of the fat-soluble vitamins A and D has been associated with disease. Hypervitaminosis A is principally a disease of amphibians that are fed mammalian meat. In these circumstances, hypervitaminosis A may play a role in the development of metabolic bone disease and cause anaemia, liver damage, and chronic weight loss (Crawshaw, 2003). Clinical signs included generalised oedema and debilitation, and mineralisation of soft tissues.

7.6 Corneal lipidosis

Figure 12 Green tree frog with corneal lipidosis

Corneal lipidosis is a relatively common eye disorder among aged captive amphibians associated with high levels of dietary cholesterol. Shilton, et al. (2001) documented a correlation between high dietary cholesterol level, corneal lipid deposition, and high serum cholesterol level. Clinically, corneal lipidosis first appears as a haziness that may progress to opacity and blindness (Keller & Shilton, 2002). Temperatures below the preferred optimal temperature zone for the species also seem to contribute to the syndrome. If ocular inflammation or pain is observed, topical anti-inflammatory medication can be administered (Whitaker & Yau, 2022). Treatment is the same as for obesity. To prevent exacerbation of this condition, it is important to modify the diet. There are no reports of successful treatment.

7.7 Oxalate toxicity and renal oxalosis

Wright and Whitaker (2001) describe oxalate toxicity in amphibians on diets containing oxalate-rich plants including spinach and kale. High dietary oxalate levels predispose some species to development of renal calculi and associated urinary disease (Raphael, 1993; Hulst, 1999). Herbivorous aquatic life stages are most at risk. Wright and Whitaker (2001) observed this disease in captive tadpoles and frogs consuming spinach or crickets that fed on an oxalate-rich plants. Clinical signs of renal oxalosis in tadpoles include hydrocoelom and systemic oedema. Presumptive confirmation is made at necropsy with histologic findings. Once clinical signs are observed, treatment is rarely successful (Whitaker & Yau, 2022).

7.8 Foreign objects

Frogs are often found to have ingested large rocks, pieces of substrate, or foreign objects. This has been observed in both wild and captive frogs to cause gastrointestinal blockages leading to starvation

or sudden death. In a rehabilitation setting, it is important to consider substrate and feeding husbandry to avoid incidental ingestion of these objects.

8 Traumatic injury

Traumatic injuries in amphibians have been attributed to predation events, fighting with conspecifics, wound inflicted by prey, mechanical accidents, or scarring from infections (Martof, 1956; Dubois, 1979; Ouellet, 2000). Most traumatic injuries are minor but can include lacerations, digit, limb, foot and hand amputations, eye enucleation, limb fracture, and internal bleeding. Rapid assessment followed by supportive care is required for a successful outcome. Desiccation is common in captive amphibians that escape their enclosure or do not receive proper care.

For smaller amphibians (<30 grams), most fractures can be managed conservatively with cage rest. For larger amphibians, the use of external or internal fixation may be beneficial. Pain management must be considered in traumatic cases. The presence of opioid receptors suggests administration of opioids may be beneficial (buprenorphine, 0.02 mg/kg, IM, SC, or PO). Nonsteroidal anti-inflammatory drugs may also be administered (meloxicam, 0.2 mg/kg) and seem to provide pain relief (Whitaker & Yau, 2022).

Rostral abrasions are common in captive amphibians. These lesions occur from repeated traumatisation from striking enclosure glass or screens. Treatment requires enclosure modification, and veterinarians should assess stocking density to determine whether conspecific stress could be resulting in increased movement. Topical antimicrobial administration may be necessary to treat secondary bacterial infections. Chronic abrasions typically have substantial granulation tissue or fibrosis along with abnormal pigmentation of the area (Whitaker & Yau, 2022).

9 Neoplasia

A wide range of tumours have been described, but compared with other animals, reports are uncommon. This may be due to neoplasms being overlooked and not reported, or alternatively, amphibians may have some resistance to neoplasms (Berger & Green, 2012). Most reports are of epithelial tumours of the skin, such as squamous cell carcinoma, adenoma, adenocarcinoma, papilloma, and epithelioma (Balls, 1962; Balls & Clothier, 1974; Green & Harshbarger, 2001; Berger, et al., 2004). In Australia, an adenocarcinoma in a wild adult green tree frog (*Litoria caerulea*), a squamous cell carcinoma in a captive adult white lipped tree frog (*L. infrafrenata*) (Berger, et al., 2004), and a neuroblastoma of the hard palate in a captive adult green tree frog (Kishimoto, et al., 2018) are reported. Chromatophoromas have also been diagnosed in northern corroboree frogs (*Pseudophryne pengilleyi*) where complex patterning makes identification difficult (Registry, unpublished). A cluster of green and golden bell frogs (*Litoria aurea*) from an ageing population of captive frogs were reported to consistently have pituitary tumours linked to skin colour changes which may suggest an environmental cause (Registry, unpublished).

Histology is required to confirm diagnosis of neoplasia. Granulomatous inflammatory responses, encysted parasites, and infections by mesomycetozoa are common causes of nonneoplastic tumours which have historically been mistaken for neoplasms (Green & Harshbarger, 2001). Treatment options and prognosis vary considerably depending on the type of neoplasm and progression of the disease.

10 Diseases of unknown aetiology

10.1 Vacuolating and ulcerative dermatitis

In Australia, amphibians were found active or ill with dermatitis ranging in severity from mild to severe with various lesions. The cause has not been determined and there may be more than one aetiology. Species affected include the red-eyed tree frog (*Litoria chloris*), cascade tree frog (*L. pearsoniana*), green tree frog (*L. caerulea*) and the cane toad (*R. marina*). Affected frogs had focal to extensive discolourations, erosions, and ulcers occurring mainly on the dorsal skin of the body and limbs (Berger, 2001). Histological lesions included vacuolation and degeneration of epidermal cells progressing to vesicles and ulcerations, and breakdown of the basement membrane with pigment cells in the epidermis. Chronic lesions with fibrosis of ulcerated tissue were also seen. Some frogs had necrosis and inflammation in the dermis, and degeneration or loss of dermal glands. Bacterial infections of the skin and internal organs in some affected frogs may have been caused by opportunistic invaders. No pathogens were found despite special stains, cell culture, ranavirus PCR and electron microscopy. In two frogs, a few chytrids were found on examination of skin scrapings but were not seen on histology. Further work is required to determine the aetiology of this potentially important syndrome and if there is an association with chytridiomycosis (Berger, 2001).

Figure 13 Green tree frog with ulcerative dermatitis of unknown aetiology

11 Chemical toxicity

11.1 Nitrogenous compounds

The high permeability of amphibian skin renders them very susceptible to toxic insult. A variety of chemicals, especially nitrogen-based chemicals, may cause poisoning in wild or captive animals. Marco, et al. (1999) have associated fertilisers and related products that contaminate natural waters via run-off with debilitation, malformations, and death of tadpoles of pond-breeding amphibians. In closed aquatic culture systems, build-up of nitrogenous waste products such as ammonia and nitrites may be harmful or lethal for amphibians, much as they are for fishes. Disruption of, or failure to establish, adequate biological filtration results in excessive nitrogenous waste build-up. Regular water quality monitoring is an essential preventive measure against disease, and routine testing for nitrogenous wastes is an important component. Unionized ammonia levels greater than 0.02 ppm are potentially problematic (Diana, et al., 2001). Signs of ammonia toxicity include increased mucous production, altered skin pigmentation, abnormal swimming, and other abnormal behaviours. If unresolved, this syndrome is often lethal. Chronic toxicity may also occur with lower levels of ammonia and may produce immunosuppression and increased susceptibility to infectious diseases (Whitaker, 2001). Nitrite toxicity may also occur, and nitrite levels should be maintained below 0.1 ppm (Densmore & Green, 2007). Treatment for nitrogenous waste toxicity involves water changes and correction of any underlying causal factors such as inadequate water filtration and high stocking density. It is also possible to treat amphibians that have methemoglobinemia resulting from nitrite toxicity with aerated baths containing methylene blue (Diana, et al., 2001).

11.2 Pesticides

Pesticides include many classes of potential toxicants such as organophosphates, carbamates, organochlorines, rotenone, and pyrethroids. Effects may vary with dose, species, life stage, and the chemical in question. Generally, the clinical signs of pesticide toxicity are neurological and may include tremors, seizures, reduced righting reflex and locomotor activity, abnormal posture and behaviour. Rotenone toxicity produces signs consistent with respiratory distress. High doses and prolonged exposure are often lethal and pesticide exposures have been implicated in mortality events among wild populations of frogs (Sparling, et al., 2001). Sublethal environmental exposures have been associated with generalised debilitation, including impaired growth and development and impaired immune function (Blaustein, et al., 2003; Hayes, et al., 2006). Exposure to a number of pesticides has been linked with reproductive impairment, including the development of intersex gonads among amphibians (MacKenzie, et al., 2003; Hayes, et al., 2006).

11.3 Heavy metals

Deleterious effects in association with several metals including copper, lead, aluminium, mercury, zinc, cadmium, arsenic silver, manganese, molybdenum, and antimony are described by Blaustein, et al. (2003). Of these, metals commonly used in cage enclosures or plumbing may harm captive specimens through leaching and toxicity.

11.4 Halogenated compounds

Some cleaning agents or disinfectants used routinely in animal holding facilities are potentially lethal to amphibians. Chlorinated cleaning agents such as bleach are highly toxic, particularly to aquatic life stages. The low-level concentrations usually found in chlorinated tap water may also be harmful over time (Diana, et al., 2001). Higher levels may be acutely harmful or lethal and may produce haemorrhagic and ulcerative skin lesions and death (Crawshaw, 1992). Iodine-based disinfectants, particularly povidone-iodine, may also produce toxicoses that involve generalised debilitation and death (Crawshaw, 2003; Diana, et al., 2001). Soaps and detergents, if not completely rinsed, have a similar effect on tadpoles. Generally, it is wise to assume that any cleaning agent applied to enclosures or equipment to be used for amphibian husbandry is a potential toxin, which requires vigorous and thorough rinsing before use (Densmore & Green, 2007).

12 Species mentioned in text

African clawed frog (*Xenopus laevis*) exotic, introduced sp.
American bullfrog (*Rana catesbeiana*) exotic sp.
Booroolong frog (*Litoria booroolongensis*)
Bumpy rocket frog (*Litoria inermis*)
Cane toad (*Rhinella marina*) (formerly *Bufo marinus*) exotic, introduced sp.
Cascade tree frog (*Litoria pearsoniana*)
Common eastern froglet (*Crinia signifera*)
Common lesser toad (*Rhinella granulosa*) (formerly *Bufo granulosus*) exotic sp.
Dainty tree frog (*Litoria gracilenta*)
Desert tree frog (*Litoria rubella*)
Eastern dwarf tree frog (*Litoria fallax*)
Eastern stony creek frog (*Ranoidea wilcoxii*) (formerly *Litoria wilcoxii*)
European toad (*Bufo bufo*) exotic sp.
Giant barred frog (*Mixophyes iteratus*)
Giant tree frog (*Phyllomedusa bicolor*) exotic sp.
Green-eyed tree frog (*Litoria genimaculata*)
Green and golden bell frog (*Litoria aurea*)
Green poison frog (*Dendrobates auratus*) exotic sp.
Green tree frog (*Litoria caerulea*)
Growling grass frog (*Litoria raniformis*)
Leopard frog (*Rana pipiens*) exotic sp.
Magnificent tree frog (*Litoria splendida*)
Montserrat whistling frog (*Eleutherodactylus johnstonei*) exotic sp.
Northern corroboree frog (*Pseudphryne pengilleyi*)
Northern ornate nursery frog (*Cophixalus ornatus*)
Ornate burrowing frog (*Limnodynastes ornatus*)
Ornate horned frog (*Ceratophrys ornata*) exotic sp.
Peron's tree frog (*Litoria peronii*)
Red-backed toadlet (*Pseudophryne coriacea*)
Red-eyed tree frog (*Litoria chloris*)
Sharp-snouted day frog (*Taudactylus acutirostris*)
Short footed frog (*Cyclorana brevipes*)
Slender tree frog (*Litoria adelensis*)
Smooth toadlet (*Uperoleia laevigata*)
Southern bell frog (*Litoria raniformis*)
Southern corroboree frog (*Pseudophryne corroboree*)
Spotted tree frog (*Litoria spenceri*)
Stony creek frog (*Litoria lesueuri*)
Striped burrowing frog (*Litoria alboguttata*)
Striped marsh frog (*Limnodynastes peronii*)
Túngara frog (*Physalaemus pustulosus*) exotic sp.
White-lipped tree frog (*Litoria infrafrenata*)
Yellow spotted bell frog (*Litoria castanea*)

13 References

Anver, M.R., Pond, C.L., 1984. Biology and diseases of amphibians. *Laboratory Animal Medicine*. Eds J. G. Fox, B. J. Cohen, F. M. Loew. Academic Press, New York, pp. 427-447.

Balls, M., 1962. Spontaneous neoplasms in Amphibia: a review and descriptions of six new cases. *Cancer Res.* 22, pp. 1142–1154.

Balls, M., Clothier, R.H., 1974. Spontaneous tumours in amphibia. A review. *Oncology* 29, pp. 501–519.

Barker, D., Fitzpatrick, M.P., Dierenfeld, E.S., 1998. Nutrient composition of selected whole invertebrates. *Zoo Biology*, 17(2), pp. 123-134.

Barton, D., 1994. A checklist of helminth parasites of Australian amphibia. *Records of the South Australian Museum*, 27, pp. 13-30.

Barton, D.P., 1995. The cane toad: a new host for helminth parasites in Australia. *PhD thesis: James Cook University,* Townsville*,* pp. 34-39.

Berger, L., Speare, R., Humphrey, J., 1997. Mucormycosis in a free-ranging green tree frog from Australia. *Journal of Wildlife Disease*, 33(4), pp. 903-907.

Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proceedings of the National Academy of Sciences,* 95(15), pp. 9031-9036.

Berger, L., Volp, K., Mathews, S., Speare, R., Timms, P., 1999. Chlamydia pneumoniae in a free-ranging giant barred frog (*Mixophyes iteratus*) from Australia. *Journal of Clinical Microbiology,* 37(7), pp. 2378-2380.

Berger, L., 2001. Diseases in Australian frogs. *PhD Thesis: James Cook University,* Townsville, pp. 15-16.

Berger, L., Skerratt, L., Zhu, X.Q., Young, S., Speare, R., 2009. Severe sparganosis in Australian tree frogs. *Journal of Wildlife Diseases,* 45(4), pp. 921-929.

Berger, L., Green, D.E., 2012. Amphibian Diseases, *Australian Registry of Wildlife Health Wildlife Pathology Short Course*, Camden, NSW, Australia, February 2012, pp. 26-48 https://arwh.org/wp-content/uploads/2021/03/Berger-L-and-Green-DE.-2012.-Amphibian-Diseases.-In-Wildlife-Pathology-Short-Course-Camden-February-2012-p26-48.-Sydney.-TCSA.pdf

Berger, L., Green, D.E., 2018. Amphibian Diseases, *Australian Registry of Wildlife Health Wildlife Pathology Short Course*, Sydney, NSW, Australia, February 2018, pp. 34-66.

Berger L, Speare R, Middleton D (2004) A squamous cell carcinoma and an adenocarcinoma in Australian treefrogs. Australian Veterinary Journal 82: 96-98.

Beveridge, I., 1978. Helminth parasites of Australian marsupials. Fauna, Proceedings 36 Post-Graduate Committee in Veterinary Science, University of Sydney, Australia, pp. 273–285.

Blaustein, A.R., Hokit, D.G., O'Hara, R.K., Holt, R. A., 1994. Pathogenic fungus contributes to amphibian losses in the pacific northwest. *Biological Conservation*, 67(3), pp. 251-254.

Blaustein, A.R., Romansic, J.M., Kiesecker, J.M., Hatch, A.C., 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity and Distribution*, 9(2), pp. 123-140.

Bowman, D.D., Hendrix, C.M., Lindsay, D.S., Barr, S.C., 2002. Feline clinical parasitology. *Iowa State University Press*, Ames, Iowa, pp. 189–194.

Browne, R.K., Scheltinga, D.M., Pomering, M., Mahony, M., 2002. Testicular myxosporidiasis in anurans, with a description of *Myxobolus fallax* n. sp. *Systematic Parasitology*. 52, pp. 97–110.

Carr, A.H., Amborski, R.L., Culley, D.D., Amborski, G.F., 1976. Aerobic bacteria in the intestinal tracts of bullfrogs (*Rana catesbiana*) maintained at low temperatures. *Herpetologica*, 32(3), pp. 239-244.

Connolly, J.H., Stodart, B.J., Ash, G.J. 2010. Genotypic analysis of *Mucor* from the platypus in Australia. *Journal of Wildlife Diseases*, 46(1), pp. 55-69.

Crawshaw, G.J., 1992. Amphibian medicine. Kirk, R.W., Bonagura, J.D., Osborne, C.A., eds. *Current Veterinary Therapy*. XI. Small Animal Practice. Philadelphia: W.B. Saunders Company. Pp. 1219-1230.

Crawshaw, G., 2003. Anurans (anura, salienta): frogs, toads. Fowler, M.E., Miller, R.E., eds. *Zoo and Wild Animal Medicine*. 5th ed. St. Louis: Elsevier Science, pp. 22-33.

Creeper, J.H., Main, D.C., Berger, L., Huntress, S., Boardman, W., 1998. An outbreak of mucormycosis in slender tree frogs (*Litoria adelensis*) and white-lipped tree frogs (*Litoria infrafrenata*). Australian Veterinary Journal, 76(11), pp. 761-762.

Cullen, B.R., Owens, L., 2002. Experimental challenge and clinical cases of Bohle iridovirus (BIV) in native Australian anurans. *Diseases of Aquatic Organisms,* 49(2), pp. 83-92.

Cunningham, A.A., Langton, T.E., Bennett, P.M., Lewin, J.F., Drury, S.E., Gough, R.E., Macgregor, S.K., 1996a. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Philosophical Transactions of the Royal Society of London, Biological Sciences*, 351(1347), pp. 1539-1557.

da Silva ICO, Carvalho SS de, Ceron K, Santana DJ, Tavares LER. 2019. *Phyllomedusa* 18(2):283–292.

Delvinquier, B.L.J., Freeland WJ. 1988. Protozoan parasites of the cane toad, *Bufo-marinus*, in Australia. *Australian Journal of Zoology*. 36(3), pp. 301-316.

Delvinquier, B.L.J., Freeland, W.J., 1989. On some trypanosomes of the Australian anura. *Proceedings - The Royal Society of Queensland*, 100, pp. 79-87.

Densmore, C.L., Green, D.E., 2007. Diseases of Amphibians. *ILAR Journal*, 48(3), pp. 235-254.

Department of Agriculture, Fisheries and Forestry, Australian Government, 2012. 6: Diseases of Amphibians. Viral diseases of amphibians. Infection with Ranavirus. *Aquatic Animal Diseases Significant to Australia: Identification Field Guide*. 4th Edition, DAFF, Canberra, pp. 268-271.

Diana, S.G., Beasley, V.B., Wright, K.M., 2001. Clinical toxicology. Wright, K.M., Whitaker, B.R., eds. *Amphibian Medicine and Captive Husbandry*. Malabar, F.L., Krieger Publishing Company, pp. 223-232.

Dickman, C.R., 1996. Overview of the impacts of feral cats on Australian native fauna. *Australian Nature Conservation Agency*, Canberra.

Dubois, A., 1979. Anomalies and mutations in natural populations of the Rana ‘‘esculenta’’ complex (Amphibia, Anura). *Mitteilungen aus dem zoologischen Museum,* Berlin, 55(1), pp. 59–87.

Elkan, E., 1965. Myiasis in Australian frogs. *Annals of Tropical Medicine and Parasitology,* 59, pp. 51-54.

Flynn, R.J., 1973. Parasites of Laboratory Reptiles and Amphibians. *Parasites of Laboratory Animals*. Iowa State University Press. Ames, USA, pp. 507-642.

Fowler, M.E., 1986. Amphibians. Fowler, M.E., ed. Zoo and Wild Animal Medicine. 2nd ed. Philadelphia: W.B. Saunders Company, pp. 99-184.

Frenkel, J.K., Harman, W.J., 1985. *Allodera hylae*, an endoparasitic worm (Naididae, Oligochaeta, Annelida) from a Colombian poison dart frog, Dendrobates histrionicus: Ureteral and cloacal habitat and visceral migration. Proc Am Soc Parasitology. Abstr 58.

Glorioso JC, Amborski RL, Amborski GF, Culley DC. 1974. Microbiological studies on septicemic bullfrogs (*Rana catesbiana*). American Journal of Veterinary Research 35:1241-1245.

Gordon H McL, Forsyth BA. 1954. Sparganosis in feral pigs in New South Wales. Australian Veterinary Journal 30(5):135-138

Gould AR, Hyatt AD, Hengstberger SH, Whittington RJ, Coupar BEH. 1995. A polymerase chain reaction (PCR) to detect epizootic haematopoietic necrosis virus and Bohle iridovirus. Dis Aquat Organ 22:211-215.

Graczyk TK, Cranfield, MR, Bicknese EJ, Wisnieski AP. 1996. Progressive ulcerative dermatitis in a captive, wild-caught South American giant tree frog (*Phyllomedusa bicolor*) with microsporidial septicaemia. J Zoo Wildl Med 27: 522-527.

Gray M, Chinchar V. 2015. 'Ranaviruses.' (Springer: Switerland).

Green SE, Bouley DM, Tolwani RJ, Waggie KS, Lifland BD, Otto GM, Ferrell Jr JE. 1999. Identification and management of an outbreak of *Flavobacterium meningosepticum* infection in a colony of South African clawed frogs (*Xenopus laevis*). J Am Vet Med Assoc 214: 1833-1838.

Green DE. 2001. Pathology of amphibia. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger Publishing Company. p 401-485.

Green DE, Harshbarger JC. 2001. Spontaneous neoplasia in amphibia. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger Publishing Company. p 335-400.

Hadfield CA, Whitaker BR 2005. Amphibian emergency medicine and care. Semin Avian Exot Pet Med 14:79-89.

Harman WJ, Lawler AR. 1975. *Dero* (*Allodera*) *hylae*, an oligochaete symbiont of hylid frogs in Mississippi. Trans Am Micro Soc 94: 38-42.

Hartigan A, Phalen, DN, Šlapeta, J. 2010. Museum material reveals a frog parasite emergence after the invasion of the Cane toad in Australia. Parasit. Vectors 3, 50.

Hartigan A, Fiala I, Dyková I, Jirků M, Okimoto B, Rose K, Phalen DN, Šlapeta J. 2011. A suspected parasite spill-back of two novel *Myxidium* spp. (Myxosporea) causing disease in Australian endemic frogs found in the invasive Cane toad. PLoS ONE, e18871.

Hartigan A, Dhand NK, Rose K, Šlapeta J, Phalen, DN. 2012a. Comparative pathology and ecological implications of two myxosporean parasites in native Australian frogs and the invasive Cane toad. PLoS One 7, e43780.

Hartigan A, Fiala I, Dykova I, Rose K, Phalen DN, Šlapeta J. 2012b. New species of myxosporea from frogs and resurrection of the genus Cystodiscus Lutz, 1889 for species with myxospores in gallbladders of amphibians. Parasitology 139, 478–496.

Hartigan A, Peacock L, Rosenwax A, Phalen DN, Šlapeta J. 2012c. Emerging myxosporean parasites of Australian frogs take a ride with fresh fruit transport. Parasit. Vectors 5, 208.

Hartigan A, Phalen DN, Šlapeta J. 2013. Myxosporean parasites in Australian frogs: Importance, implications and future directions. International Journal for Parasitology: Parasites and Wildlife 2 62-68.

Hayes TB, Case P, Chui S, Chung D, Haeffele C, Haston K, Lee M, Mai VP, Marjuoa Y, Parker J, Tsui M. 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: Are we underestimating the impact? Environ Health Perspect 114(Suppl 1):40-50.

Hemingway V, Brunner J, Speare R, Berger L. 2009. Viral and bacterial diseases in amphibians. In Amphibian Biology: Amphibian Decline: Disease, Parasites, Maladies, and Pollution. (Eds H Heatwole, JW Wilkinson.) Vol. 8 (Surrey Beatty & Sons: Chipping Norton, NSW).

Hill BD, Green PE, Lucke HA. 1997. Hepatitis in the green tree frog (*Litoria caerulea*) associated with infection by a species of *Myxidium*. Aust Vet J. 75:910-1.

Hird DW, Diesch SL, McKinnell RG, Gorham E, Martin FB, Kurtz SW, Dubrovolny C. 1981. *Aeromonas hydrophila* in wild-caught frogs and tadpoles (*Rana pipiens*) in Minnesota. *Laboratory Animal Science, 31*(2), 166-169. http://purl.umn.edu/147965

Hubbard GB. 1981. *Aeromonas hydrophila* infection in *Xenopus laevis*. Laboratory Animal Science 31:297-300.

Hulst F. 1999. Amphibian care and medical management. In: Wildlife Health in Australia, Healthcare and Management. Proceedings 327, Postgraduate Foundation in Veterinary Science, University of Sydney. Pp 119-146.

Hyatt A, Williamson M, Coupar B, Middleton D, Hengstberger S, Gould A, Selleck P, Wise T, Kattenbelt J, Cunningham A (2002). First identification of a ranavirus from green pythons (*Chondropython viridis*). Journal of Wildlife Diseases 38 239-252.

Jerrett IV, Whittington RJ, Weir RP (2015) Pathology of a Boehle-like Virus Infection in Two Australian Frog Species (*Litoria splenddida* and *Litoria caerulea*). Journal of Comparative Pathology 152 248-259.

Johnson ML, Berger L, Philips L, Speare R. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. Dis Aquat Organ 57:255-260.

Johnston, T.H., Bancroft, M.J., 1918. A parasite, Myxobolus hylae sp. nov. of the reproductive organs of the golden swamp frog, Hyla aurea. Aust. Zool. 1, 171.

Johnson ML, Speare R. 2003. Survival of *Batrachochytrium dendrobatidis* in water: Quarantine and disease control implications. Emerg Infect Dis 9:922-925.

Johnson ML, Speare R. 2005. Possible modes of dissemination of the amphibian chytrid *Batrachochytrium dendrobatidis* in the environment. Dis Aquat Organ 65:181-186.

Juopperi T, Karli K, DeVoe R, Grindem CB. 2002. Granulomatous dermatitis in a spadefoot toad (*Scaphiopus holbrooki*). Vet Clin Path 31: 137-139

Kattenbelt JA, Hyatt AD, Gould AR. 2000. Recovery of ranavirus dsDNA from formalin-fixed archival material. Dis Aquat Organ 39:151-154.

Keller CB, Shilton CM. 2002. The amphibian eye. Vet Clin Exot Anim Pract 5:261-274.

Kishimoto TE, Chambers JK, Miyama K, V Son N, Kok MK, Uneyama M, Nakayama H, Uchida K. 2018. Neuromastoma of the hard palate mucosa in an Australian green tree frog (*Litoria caerulea*).
J Vet Med Sci 80(10): 1511-1514

Lemckert, F. 2000. Parasitism of the common eastern froglet *Crinia signifera* by flies of the *Batrachomyia* (Diptera: Chloropidae): parasitism rates and the influence on frog condition. *Australian Zoologist 31:* 492–495.

MacKenzie CA, Berrill M, Metcalfe C, Pauli BD. 2003. Gonadal differentiation in frogs exposed to estrogenic and antiestrogenic compounds. Environ Toxicol Chem 22:2466-2475.

Mann, R.M., Hyne, R.V., Choung, C.B., 2010. Hormonal induction of spermiation, courting behavior and spawning in the Southern bell frog *Litoria raniformis*. Zoo Biol. 29, 774–782

Mao J, Tham TN, Gentry GA, Aubertin A, Chinchar VG. 1996. Cloning, sequence analysis and expression of the major capsid protein of the iridovirus frog virus 3. Virology 216:431-436.

Marco A, Quilchano C, Blaustein AR. 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest. Environ Toxicol Chem 18:2836-2839.

Martof B. 1956. Factors influencing size and composition of populations of *Rana clamitans*. American Midland Naturalist 56:224–245

Mauel MJ, Miller DL, Frazier KS, Hines ME II. 2002. Bacterial pathogens isolated from cultured bullfrogs (*Rana catesbeiana*). J Vet Diag Invest 14: 431-433.

Mazzoni R, Cunningham AA, Daszak P, Apolo A, Perdomo E, Speranza G. 2003. Emerging pathogen of wild amphibians in farms (*Rana catesbeiana*) farmed for international trade. Emerg Infect Dis 9:995-998.

Mendez D, Freeman AB, Spratt DM, Speare R. 2010. Pathology of cutaneous trombidiosis caused by larval trombiculid mites in a wild Lesueur's tree frog (*Litoria wilcoxii*). Aust Vet J 88: 328-330.

Oakwood M, Spratt D. 2000. Parasites of the northern quoll, *Dasyurus hallucatus* (Marsupialia: Dasyuridae) in tropical savanna, Northern Territory. Australian Journal of Zoology 48: 79–90.

OIE. 2016. 'OIE listed diseases.' Available at http://www.oie.int/animal-health-in-the-world/oie-listeddiseases-2016/ [Accessed 15 September 2016].

Olson ME, Gard S, Brown M, Hampton R, Morck DW. 1992. *Flavobacterium indologenes* infection in leopard frogs. Journal of the American Veterinary Medical Association 201:1766-1770.

O'Shea P, Speare R, Thomas AD. 1990. Salmonellas from the cane toad, *Bufo marinus.* Aust Vet J 67: 310.

Ouellet M. 2000. Amphibian deformities: current state of knowledge. In D. W. Sparling, G. Linder, and C. A. Bishop (eds.), Ecotoxicology of Amphibians and Reptiles, pp. 617–661. SETAC, Pensacola, FL.

Parker JM, Mikalian I, Hahn N, Diggs HE. 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). Comp Med 52:265-268.

Pessier AP, Nichols DK, Longcore JE, Fuller MS. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). J Vet Diag Invest 11:194-199

Pessier AP. 2002. An overview of amphibian skin disease. Semin Avian Exot Pet Med 11:162-174.

Poynton SL, Whitaker BR. 2001. Protozoa and metazoan infecting amphibians. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger Publishing Company. p 193-221.

Raphael BL. 1993. Amphibians. Veterinary Clinics of North America; Small Animal Practice 23: 1271-1286.

Reichenbach-Klinke H, Elkan E. Amphibia. 1965. In: The Principal Diseases of Lower Vertebrates. Academic Press, New York. Pp 209-384.

Schell CB, Burgin S (2001). *Batrachomyia strigapes* (Diptera) parasitism of *Uperoleia laevigata* (Anura) Journal of Parasitology 87(5): 1215-1216.

Sharma VK, Kaura YK, Singh IP. 1974. Frogs as carriers of *Salmonella* and *Edwardsiella*. Antonie von Leeuwenhoek 40:171-175.

Shilton CM, Crawshaw GJ, Keller CB, Connelly PW. 2001. Corneal lipid deposition in Cuban tree frogs (*Osteopilus septentrionalis*) and its relationship to serum lipids: An experimental study. J Zoo Wildl Med 32:305-319.

Shilton CM, Brown GP, Benedict S, Shine R. 2008. Spinal arthropathy associated with *Ochrobactrum anthropi* in free-ranging cane toads (*Chaunus [Bufo] marinus*) in Australia. Vet Path 45: 85-94.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4: 125-134.

Sparling DW, Fellers GM, McConnell LL. 2001. Pesticides and amphibian population declines in California, USA. Environ Tox Chem 20:1591-1595.

Speare R, Smith J. 1992. An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia. Diseases of Aquatic Organisms 14: 51-57.

Speare R, Thomas AD, O'Shea P, Shipton WA. 1994. *Mucor amphibiorum* in the toad, *Bufo marinus* in Australia. J Wildl Dis 30: 399-407.

Speare R. 2000. Developing management strategies to control amphibian diseases: decreasing the risks due to communicable diseases. Report to Environment Australia by the Steering Committee of Getting the Jump on Amphibian Disease. 209.

Taylor FR, Simmonds RC, Loeffler DG. 1993. Isolation of *Flavobacterium meningosepticum* in a colony of leopard frogs (*Rana pipiens*). Laboratory Animal Science 43:105.

Taylor SK, Williams ES, Pier AC, Mills KW, Bock MD. 1999. Mucormycotic dermatitis in captive adult Wyoming toads. J Wildl Dis 35: 70-74.

Taylor R, Sloan D, Cooper T, Morton B, Hunter I. 2000. A waterborne outbreak of *Salmonella Saintpaul*. Communicable Diseases Intelligence 24(11):336-340.

Taylor SK, Green DE, Wright KM, Whitaker BR. 2001. Bacterial diseases. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger Publishing Company. p 159-179.

Tinsley RC. 1995. Parasitic disease in amphibians: control by the regulation of worm burdens. Parasitology 111 Suppl:153-178.

Vogelnest L. 1994. Miasis in a green tree frog (*Litoria caerulea*). Association of Reptile and Amphibian Veterinarians 4: 4.

Weedon D, van Deurse M, Allison S, Rosendahl C. 2013. Chromoblastomycosis in Australia: an historical perspective. Pathology 45(5): 489-491.

Weir R, Moody N, Hyatt A, Crameri S, Voysey R, Pallister J, Jerrett I. 2012. Isolation and characterisation of a novel Bohle-like virus from two frog species in the Darwin rural area, Australia. Diseases of Aquatic Organisms 99: 169-177.

Whitaker BR. 2001. Water quality. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger Publishing Company. p 147-157.

Whitaker BR, Yau TJ. 2022. Non-infectious Disorders of Amphibians. Trauma of Amphibians. In: MSD Veterinary Manual. https://www.msdvetmanual.com/exotic-and-laboratory-animals/amphibians/noninfectious-disorders-of-amphibians accessed 28 April 2022.

Whittington R, Middleton D, Spratt DM, Muntz F, Carmel B, McCracken HE, Strakosch MR, Stephanson-Shaw J, Harper PA, Hartley WJ. 1992. Sparganosis in the monotremes *Tachyglossus aculeatus* and *Ornithorhynchus anatinus* in Australia. Journal of Wildlife Diseases 28: 636–640.

Williams RW. 1960. Observations on the life history of *Rhabdias sphaerocephala* Goodey, 1924 from *Bufo marinus* L., in the Bermuda Islands. Journal of Helminthology 34(1/2):93-98.

Wolf K, Bullock GL, Dunbar CE, Quimby MC. 1968. Tadpole edema virus: A viscerotropic pathogen for anuran amphibians. J Infect Dis 118:253-262.

Wright KM, Whitaker BR. 2001. Nutritional disorders. In: Wright KM, Whitaker BR, eds. Amphibian Medicine and Captive Husbandry. Malabar FL: Krieger Publishing Company. p 73-87.

Wright KM. 2006. Overview of amphibian medicine. In: Mader DR, ed. Reptile Medicine and Surgery. 2nd ed. St. Louis: Saunders, Elsevier. p 941-971.

Zupanovic Z, Lopez G, Hyatt A, Green B, Bartran G, Parkes H, Whittington R, Speare R. 1998. Giant toads *Bufo marinus* in Australia and Venezuela have antibodies against 'ranaviruses'. Diseases of Aquatic Organisms 32: 1-8.