Progress report on investigations into chytrid fungal outbreak in Western Australia

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Introduction

This report provides a summary of work undertaken in 1999 at the Western Australian Museum on the subject of chytrid fungal disease of frogs. Many aspects of the investigation remain incomplete on account of the rapidly expanding horizons of the chytrid fungus issue.

The project was initiated when an initial study by the W.A.Museum and James Cook University in late 1998 demonstrated that the chytrid fungus *Batrachochytrium dendrobatis* was present and apparently causing frog mortalities at various localities in southwestern W.A. (Aplin *et al.* unpubl.). Elsewhere in Australia and in North America, *B. dendrobatis* has been implicated in the decline and extinction of various species of frogs (Berger *et al.* 1998), raising similar concerns in relation to the endemic southwestern frog fauna (Aplin *et al.* unpubl.).

The research effort was undertaken in the context of the following unresolved questions in regard to the biology and epidemiology of *B. dendrobatis.*

1. Is the chytrid an exotic (ie, introduced to W.A.) parasite or is it an endemic (ie, naturally occurring) organism?
2. If exotic, how and when was it introduced, and what has been its subsequent pattern of spread?
3. If endemic, has it enjoyed an increase in pathogenicity or has it always been causing frog mortalities at comparable level?
4. Are certain species of frogs more susceptible to individual mortality than others?
5. Are certain species of frog more likely to suffer population declines than others, either due to the mode of infection (eg, via waterways) or because of particular demographic parameters (eg, fecundity, longevity) or other mitigating factors (eg, overall population size, range fragmentation)?
6. Does non-lethal chytrid infection have physiological impacts that might reduce the fitness of infected frogs?
7. Can *B. dendrobatis* exist in the absence of frogs (eg, alternative hosts or as saprophage)?

Project Design and Methods

The work of the W.A. Museum has concentrated on the following issues:

- geographic distribution of *B. dendrobatis*
- history of chytrid outbreak
- prevalence of chytrid infection in 'banana-box' frogs and commercially available tadpoles and axolotyls
- prevalence of chytrid infection within local frog communities
- demographic monitoring of infected populations

Other work undertaken by UWA Honours Zoology Student, Ms Zoe Car in 1999 focussed on:

- attempts to isolate and culture *B. dendrobatis* from locally infected frogs
- susceptibility trials on several local frog species
- alternative methods of diagnosis
- physiological studies of infected frogs

The taxonomic and geographic occurrence of *B. dendrobatis* was investigated from four primary sources

- dead or moribund frogs donated by the public since late 1998
- voucher specimens from the W.A. Museum collection
- sectioned specimens derived from several previous skeletochronology projects at U.W.A. (Thom; Driscoll)
- newly collected specimens derived from targeted survey work during 1999

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The most intensive fieldwork has been undertaken within the wider metropolitan area. However, three wide-ranging sampling trips have also been undertaken: two to the Margaret River area, and one to the south coast between Manjimup and Albany. In some populations, frogs were toe-clipped and then released at the site of capture. In other cases, the frogs were retained as complete voucher specimens. Large pieces of skin were removed from the foot and groin of all voucher specimens, thereby giving an improved chance of accurate diagnosis than through examination of toe-clips alone.

Additional material from outside of Perth has come from donations to the Albany Residency Museum and via the Nannup, Manjimup and Geraldton CALM offices.

Information regarding frog mortalities or declines has been obtained from numerous sources including Alcoa frogwatch members, other members of the public and regional CALM staff.

All diagnosis has been performed through histological examination of thin sections of frog skin. At the time of writing, a total of 1867 specimens have been examined histologically, spanning the last 66 years (see below). 289 of these have been determined as positive for chytrid infection, with a further 46 scored as possible, early stage infections. The overall infection rate is thus around 18%. The infection rate of animals donated dead or moribund is substantially higher, around 70%.

Results

1. *Taxonomic occurrence of chytrid infection*

A total of 19 southwestern frog species have been sampled, plus one from the northwest region (Kumerina), three from the Kimberley district (vicinity of Kununurra), and four from eastern Australia. Pet-shop axolotyls have also been examined for possible infection.

Definite chytrid infection has been detected in 13 locally occurring species, with likely infection in two others (Table 1). At least one species listed as Threatened Fauna (*G. vitellina*) hosts chytrid infection, with likely infections also identified in specimens of *G. alba*. In both of these taxa, the material examined consists of sectioned toe clips stained with haemotoxylin only, and often with very few good sections per slide. The likelihood of detecting chytrid infections from such material must be judged as very slender, hence the infection prevalence data is very likely a substantial underestimate.

The five species which failed to show evidence of chytrid infection are all poorly sampled and it would be unwise to rule out the likelihood of chytrid infection altogether. For the present, it thus seems best to assume that all frog species are vulnerable to infection, including species which rarely enter water except during tadpole stages (eg, *Heleioporus* spp.).

2. *Prevalence estimates in better-sampled species*

Disease prevalence estimates are provided in Table 2 for the better-sampled species. These should be read as indicative estimates because of the variety of methods involved in both original population sampling and subsequent disease sampling (ie, toe-clips vs large skin pieces). The specimens scored as “possible” are either poorly preserved or represent very light infections in which only one or a few sporangia are visible.

The data show a strong bias towards the more habitually aquatic taxa, with lower overall prevalence figures in taxa which either lack a free-swimming tadpole (eg *Geocrinia alba/vitellina* or spend much of their adult lives away from immediate wetland environments (eg, *Heleioporus eyrei, Limnodynastes dorsalis*).

3. *Geographic distribution of chytrid infection and frog mortality*

Table 3 gives a partial list of localities for which positive diagnoses have been obtained (requires update). The majority fall in the wider metropolitan region, reflecting the concentration of public donations, fieldwork and historical sampling in this area. Outside of the metropolitan area, chytrid infections have been confirmed at numerous, widely scattered localities, both around and distant to major population centres.

To all intents and purposes, *Batrachochytrium dendrobatis* appears to be ubiquitous through the southwest region, at least as far east as Albany and the Porongurup Range, north probably to the vicinity of Geraldton and inland possibly as far as Merredin. The inland and northern parts of its range remain poorly understood.

No indication of chytrid infection has been observed in the admittedly limited samples of northwestern (N = 1; *L. rubella*) and Kimberley (N = 30; *L. coplandi, L. caerulea, L. rothi*) frogs examined to date.

Metropolitan localities where frog mortalities (several to numerous) have been observed include Kingsley, Jackadder Lake, Herdsman Lake, Victoria Park, South Lake, Ferndale, Roleystone, Sawyers Valley, Helena Valley and Gidgegannup. Regional localities where conspicuous numbers of dead or moribund frogs have been observed over a short period of time are Bunbury, Busselton, Nannup and the Geraldton region.

Although the presence of chytrid infection has been confirmed at the majority of these localities, this should not be taken as proof that chytridiomycosis is necessarily responsible for any of the observed deaths. The anecdotal reports typically lack much detail and must be viewed as a population of observations rather than individually.

4. *History of chytrid outbreak in southwest WA*

The history of infection can be pieced together by retrospective sampling of voucher specimens. Initial work focussed on the Perth metropolitan area through to Mandurah, and on the last 10 years. Skin samples were been removed from representative series of the following species: *Litoria moorei, L. adelaidensis, Limnodynastes dorsalis, Heleioporus eyrei, Crinia georgiana, C. insignifera* and *C. glauerti*. Several skeletochronology series covering the period 1992- 1995 were also studied. Zoe Car examined a small number of historical voucher specimens held at UWA Zoology; these slides were re-examined by Kirkpatrick and Aplin so as to ensure consistency of diagnosis.

The earliest WA infections date from October 1985; these were detected initially by Car and confirmed by our re-examination. The specimens in question are *Litoria moorei* from 6.7 km S of Kambellup in the Albany district; the three specimens show heavy chytrid infections. Car's other historical material also revealed infections dating from 1986 at Herdman Lake (*L. moorei* - 2 specimens) and Boyup Brook (*L . moorei* - 1 specimen). Somewhat later (1992) infections had been previously detected at Perry Lakes, Floreat; Gungin Gully, near Kalamunda; two sites near Mandurah; Boulder Rock on the Brookton Highway; Big Brook near Pemberton; Leeming; and Waterford. These records involve a wider range of species including: *L. moorei*, *L. adelaidensis, Crinia insignifera, C. pseudinsignifera, C. georgiana*, *Geocrinia rosea, L. dorsalis* and *Heleioporus eyrei.* In all cases, the infected animals had been collected during the course of general population sampling.

At present the infected *L. moorei* from Kambelup constitute the earliest evidence for chytrid infection of any region in Australia. The earliest record of a chytrid infected frog from southeast Queensland (where frog declines were first noted in 1979) dates from 1989. However, it should be emphasised that only very small numbers of historical specimens have been examined from prior to this date.

Car's discovery of infection by 1985 prompted sampling of still earlier periods. A total of 117 specimens of *L. moorei* from sites around Perth and Albany (two current 'hot-spots' of infection) were selected from the WA Museum collection. All of these proved negative for chytrids.

The distribution of infected *L. moorei* is broken down by year of collection in Table 4. In total, 117 samples have been examined from the period prior to 1985 without any indication of chytrid infection. Given the overall high prevalence of chytrid infection in this species (>20%), the likelihood of not detecting any cases *in the presence of an endemic disease* appears to be small. However, much additional material will need to be examined before this can be taken as established beyond reasonable doubt.

5. *Prevalence of chytrid infection in 'banana-box' frogs and commercially available tadpoles and axolotyls*

To date, relatively few 'banana-box' frogs have been presented for testing (mainly *Litoria gracilenta*; coming from Perth, Collie and Bunbury). Seven of these have been examined histologically, with definite chytrid infection detected in 1, and one other showing possible infection. Comparable studies in Victoria using large samples of 'banana-box' frogs have shown an infection prevalence of less than 5% (G. Marantelli, *pers. comm.*).

Relatively few pet-shop tadpoles have been made available for testing and only a couple have been examined. Adult *L. moorei* were sampled at the premises of two former tadpole breeders; one of these populations proved to be infected as of September 1999. Two *Heleioporus eyrei* that had been reared in 1998 from pet-shop tadpoles, and died shortly after metamorphosing, both tested positive. These animals had been kept in an aquarium in isolation from other sources of contamination and were almost certainly infected at the time of purchase.

A sample of 15 axolotyls was obtained from local aquarium and pet shops (3 separate vendors). Six of these have been examined histologically and two were found to have chytrid infection. In a similar test on commercially-obtained axolotyls from Townsville, two of three animals were found to be infected (R. Speare, *pers. comm.*). In all cases, the infections are very localised and lack the severity seen in many frogs. This probably reflects differences in skin structure between axolotyls and frogs, and also is consistent with the reported lack of mortality of pet axolotyls. Local axolotyl suppliers indicated Victorian breeders as their major, regular source, although some also mentioned local breeders in Perth and Geraldton. The majority of Australia axolotyls are probably derived from outdoor breeding ponds in northern Victoria (G. Marantelli, *pers*. *comm*.).

6. *Seasonal and longer term variation in chytrid prevalence*

A detailed analysis of temporal trends has not been completed. However, the crude analysis given in Table 5 (based on all taxa and all sampling methods pooled) appears to show a strong and consistent temporal fluctuations operating on a seasonal and a longer time scale. These trends are repeated in smaller subsets of the data relating to individual taxa and sites (eg, see Table 6, data for Perry Lakes or Herdsman Lake).

The repeated seasonal trend appears to involve a peak in chytrid infection (or at least, infection of diagnosable intensity) during Winter-Spring, with a reduction in chytrid activity during Summer-Autumn. This trend is most pronounced for 1999-1999 and 1992, but is also evident for 1993 and 1996 (although in the latter case, the prevalence declines in Spring after a Winter peak).

On a longer time scale, there appear to be major peaks in chytrid activity in 1998-1999 and in 1992, with a hint of a possible earlier peak in 1985. From a quick scrutiny of readily available climatic records, it seems that the early 1990s were a period of higher than average rainfall and reduced temperatures in southwestern Australia, followed by a hot, dry phase, and then returning to cooler, wetter conditions over the last few years. The last decade as a whole has been characterised by major fluctuations in both annual mean temperature and especially annual mean rainfall, especially in comparison with the relative stability of the 1980s. The possibility that these climatic fluctuations have promoted the spread and/or emergence of chytrid disease in southwest WA clearly warrants close examination.

A number of infected metropolitan sites were selected for intensive community sampling (goal of 30 individuals per species). The aim is to establish the prevalence of chytrid infection in a variety of different communities comprised of various suites of species. The results will provide a valuable benchmark for assessment of changes in infection rate through time (seasonal to longer term) and might also yield important insights into the ecological context and mechanisms of infection (i.e., intraspecific contact *vs* substrate).

The site and taxon specific prevalence data presented in Table 6 are only a subset of the available data. However, they suffice to illustrate a number of important features of the chytrid disease

- chytrid prevalence in large samples drawn from natural, infected populations generally runs at around 5-10%, even outside of the peak Winter-Spring period
- much higher prevalence values can be obtained where populations are sampled during times of observed mass mortality (eg, *Litoria moorei* at Kingsley)
- high prevalence values can be obtained on occasion in natural populations where no mass mortality has been detected [eg, *Litoria adelaidensis* at Gungin Gully in 1992

(75%, N=4); *Crinia georgiana* at Boulder Rock in 1992 (38%, N=21; Dale Roberts *pers. comm*.); *Litoria moorei* at Herdsman Lake in 1999; 60%; N=5]
- disease prevalence can be very different between different sites, even when considering the same species of frog and an identical sampling period (eg *Litoria adelaidensis* at Gwelup vs Perry Lakes, June 1999).

Our 'background' prevalence estimates of 5-10% are somewhat higher than that reported by other workers (usually below 5%; Berger *et al. submitted*; Marantelli, *pers. comm*.). However, this may well reflect the fact that many of the other estimates are based on toe-clip samples, which provide less opportunity for detection of early or light infections.

Our results are perhaps more startling for the number of populations showing significantly higher infection rates of 25% or more. In most cases, these estimates are based on relatively small samples, however there can be no doubt that they represent populations with far higher disease prevalence than the 'background' level. The fact that the majority of these populations were also sampled for other reasons, and were not noted to be undergoing mass die-off, is perhaps also significant. The two obvious interpretations are that the mortality was occurring but was simply not observed, or that the populations involved were able to sustain high rates of infection without suffering mortalities and/or declines.

7. *Demographic monitoring of infected populations*

Monitoring of an infected and rapidly declining population of *L. moorei* has commenced at a Nursery in Kingsley. A monitoring site for *L. adelaidensis* at Perth Zoo has been identified and approval for this project has been obtained. Individuals are sexed, weighed, measured and toe-clipped and released, the toes tested for chytrid infection; an additional toe is sampled on subsequent recaptures. In each case the aim is to document

- the current demographic structure of the population
- the temporal and demographic pattern of survivorship among infected individuals
- the temporal and demographic pattern of new infections
- survivorship among metamorphs and the impact on age structure

The Kingsley Nursery site is important in that it provides the first definite link between a high rate of chytrid infection (64% of captures; N=11) and an observable mass mortality event in a population of *L. moorei*. Importantly, only active, 'healthy' animals have been included in the sample used to estimate prevalence, although several of these animals became moribund and died within 2-3 days of capture. A further 15 mortalities have been collected at the site; two of these have been examined and both had heavy chytrid infections.

Staff at the Kingsley Nursery described finding large numbers of obviously moribund individuals over the period April-July 1999, and also mentioned a marked decrease in the numbers of *L. moorei* over the last three years compared with previously. Unfortunately, they were unable to be more specific about the timing of deaths in previous years, their recent interest having been sparked by the media coverage of the frog fungus issue. However, similar seasonal patterns of mortality in *L. moorei* have been reported by staff of other nurseries around Perth and in the Bunbury-Busselton area, although these have not been specifically linked to chytrid activity.

Taken in combination, these observations suggest that the prevalence of chytrids and/or their effectiveness in producing mortality within a population may be subject to seasonal fluctuations in *L. moorei*, as suggested by Berger *et al.* (submitted) for *Litoria caerulea* in eastern Australia. However, it must also be remembered that occasional carcasses of *L. moorei* with severe infections were presented throughout the summer of 1998/99. Ongoing monitoring of the Kingsley and other sites is essential to provide confirmation of any seasonal pattern.

8. *Attempts to isolate and culture* **B. dendrobatis** *from locally infected frogs*

Zoe Car spent considerable time attempting to culture *B. dendrobatis* from locally infected frogs, using a protocol supplied by Dr. L. Berger (Australian Animal Health Laboratory, Geelong). This work was undertaken in the laboratory of Assoc. Prof K. Sivasithamparam of the Dept of Soil Science and Plant Nutrition, U.W.A, but ultimately proved unsuccessful due to contamination with a myriad of other organisms. She was able to divide and establish a culture of Queensland chytrid supplied by Berger.

9. *Zoe Car's susceptibility trials on several local frog species*

A protocol for susceptibility trials was obtained from Berger. Car perfomed trials on adults of four species (*Litoria moorei, L. adelaidensis*, *Heleioporus eyrei*, *Crinia georgiana*) and used the Queensland culture provided by Berger.

Car's infection trials produced a complex set of results that are hard to interpret. This was primarily due to a high rate of natural infection in each of the source populations, and the failure to increase infection rates over that seen in the control populations. In other words, the exposure to the Queensland culture did not result in any measurable increase in disease prevalence. This might be explained in various ways, not of which can be explicitly tested at present.

The pattern of mortality/survivorship among Car's test animals provides some valuable insights into progress of the disease. In many case, control frogs suffering from heavy chytrid infections were still alive at the conclusion of the experiment, several months after capture. This clearly demonstrates that chytrid infection alone is not automatically lethal to individual frogs, even in the case of species that have been observed to be suffering significant mortalities in the wild. The possible interpretations of this result are that the individual frogs concerned have an acquired immune resistance to the disease, or that the disease has little impact on the health of a frog until certain external or internal environmental parameters are met or exceeded.

Other possibilities interpretations might include the fact that the infection culture was sterile on account of its being derived from a ‘plate’, and therefore may have been lacking in some associated microbial organism that might otherwise gain entry into a frog with chytrid spore penetration.

It was unfortunate that Car was unable to test metamorph stage animals, as that appears to be one of the most sensitive stages of the overall developmental process. Further testing is probably warranted for tadpoles and additional species, most notably the Threatened *Geocrinia* and *Spicospina.* Should it be considered desirable to conduct trials on these species, it will be necessary to make arrangements to obtain a new chytrid culture, either by local work or import from Berger. Far greater caution should also be applied to usage of the culture, which could perhaps be varified in a small pilot trial.

10. *Zoe Car’s physiological studies of infected frogs*

Car's physiological studies of infected frogs demonstrated clearly that the presence of chytrid in the epidermis does not result in any great functional impairment. Previous suggestions that frogs die through disruption of respiratory function, water balance or ionic balance are therefore made implausible, with the alternative hypothesis of toxic impact getting a boost in the process. The exact cause of death (alternatively, of survival in some individuals) remains mysterious.

11. *Assessing the possible scale and extent of frog declines in WA*

This is undoubtedly the most controversial aspect of the whole chytrid issue, at least insofar as it pertains to WA. Opinion in the frog community is clearly divided between those who believe that populations are essentially stable, and those who believe that there is an ongoing and progressive decline affecting many species. The issue of whether chytrid fungus has contributed to any decline is of course secondary to whether there has in fact been any decline to speak of.

The fundamental problem on both sides of this debate is the absence of a sufficient empirical base in the form of long-term, quantitative monitoring sites. This absence makes it necessary either to form an opinion on personal experience, which generally rests on repeated visits to a relatively small number of favourite localities, or to rely to some extent on various, wider sources of anecdotal information.

My personal feeling based on 10 years observation of various sites in the wider Perth region and beyond is that many frog populations have suffered an overall decline in abundance over this period. This impression is supported by a large body of anecdotal information submitted by Alcoa frogwatch members and other community members since 1995, many of whom recall times when local frogs were significantly more abundant and broadly distributed. What this larger body of information also suggests is that while frog populations have declined over much of the wider metropolitan region, this has occurred at different times in different areas. Oftentimes, the earliest noted declines are often back at least ten years; yet in some areas changes have been noted during last five years. In several of these cases, the more recent events are documented by semi-quantitative monitoring data for specific sites. Occasionally, informants report that an initial period of decline has been followed by a slow recovery of frog numbers, though not to original levels of abundance, but nonetheless suggesting some cyclical process.

Reports from various areas in the southwest suggest that similar local declines have occurred in various parts of the Darling Range, in areas around Collie, Albany and Nannup, along the Deep River near Manjimup, in the Margaret River and Merredin districts. As in the local area, the suggested timing of these declines ranges from the immediate past back to as much as 7-8 years.

Although much more needs to be done before this body of anecdotal records can be accorded any strong meaning, my gut feeling is that the last decade has been witness to a series of regionally localised declines, some more severe than others, but each having a broad and lasting impact on frog numbers. In some cases, this process has clearly been exacerbated by major urban development or other detrimental activity. However, in others, there is no obvious development or contamination, leaving it difficult to ignore the possibility that an additional factor like epidemic outbreaks of chytrid fungal disease has contributed to the decline. Under the banner of Alcoa frogWAtch, we will be working in 2000 to compile a far more extensive body of anecdotal information relevant to the wider southwest region.

SUMMARY AND RECOMMENDATIONS

The preliminary findings presented and discussed here already add greatly to our knowledge of chytrid fungal disease in W.A. In southwest WA we now have the longest, continuous record of chytrid infection of anywhere in Australia, and the most detailed picture of geographic and taxonomic distribution of any region of the world. The number of species shown to be affected in natural populations has been increased to between 12 and 15, including several gazetted Threatened Species. The strong indication that chytrid fungus is absent in WA prior to the 1980s is a finding of potential international significance, as it stands to help resolve the persistent issue of 'endemic' vs 'exotic'. The implication that the fungus is a 'novel' disease capable of having profound impact on initially naive frog communities has major immediate implications for management, even more so if the preliminary indication of a still chytrid-free Kimberley is confirmed. These historical and distributional issues clearly need to be resolved through targeted investigation of series from critical time periods and localities.

The historical findings also give a clear indication of fluctuating severity of infections, with short periods of peak infection separated by potentially longer periods of reduced fungal prevalence. The possibility that this cyclic activity might be associated with climatic fluctuations needs to be tested through more comprehensive historical sampling, both in WA and elsewhere in Australia. A link to climatic events clearly raises exciting prospects of prediction and increased vigilance during periods of peak threat. The nature of the relationship between disease prevalence and mortality will obviously need to be addressed as part of this process.

The intensive population sampling in the metropolitan region has provided the first definite link in W.A. between a high rate of chytrid infection and an ongoing, heavy mortality event in at least one population of *Litoria moorei*. By continuing with a monitoring and sampling regime at this and other key localities, much of valuable can be learned regarding the demographic response of populations to the continued presence of disease.

The rapidly accumulating population prevalence data provides the following valuable insights

- confirmation of a likely 'endemic' infection rate of around 5-20%, with this value higher overall in some species than others, perhaps related to the degree of aquatic adaptation.
- discovery of far higher prevalence values (50% or more) in natural populations, some of which show no obvious catastrophic decline.
- strong indication that infection (and mortality in at least one species; *L. moorei*) follows a seasonal pattern, with most heavy infections developing during the Winter and into Spring.

<u>Proposed continuation of study</u>

The potential threat posed by the spread and/or emergence of chytrid fungal disease around the world has been recognised by the scientific community and lately by wildlife managers abroad. In the USA, significant funds have been committed to the study of frog disease including chytridiomycosis and its role in the worldwide decline of amphibians.

In Australia, the importance of this issue has been recognised by Environment Australia and the World Wide Fund for Nature in their decision to jointly sponsor a major international conference/workshop on chytrid disease in Cairns, mid-2000. The intention of the conference steering committee, of which Aplin is a member, is to focus the programme on issues and discussion that lead to a new phase of integrated research and to immediate management recommendations.

The ongoing investigation in WA is recognised both nationally and internationally as a critically important test of our ability to document and understand the process of dispersal and/or emergence of chytrid fungal disease within a discrete, isolated amphibian fauna. The unique combination of geographic isolation, a biologically well-known fauna and a large Museum collection base provides what is almost certainly one of the best opportunities world-wide to answer fundamental questions to do with origins and rates of spread of the fungal organism across a varied landscape and through an ecologically diverse fauna. By contributing this critical body of information to the growing scientific framework, WA will stand to benefit vastly more as a result of the ensuing boost to international chytrid research.

Postscript

Funding for the W.A. Museum's chytrid research terminated in January 2000. Although samples are still being collected from various populations, no further histological examinatin will be possible until further funds become available.

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HYLIDAE	**Chytrids**	**N**
Litoria adelaidensis	+	160
Litoria moorei	+	441
MYOBATRACHIDAE		
Crinia georgiana	+	203
Crinia glauerti	+	104
Crinia insignifera	+	142
Crinia pseudinsignifera	+	37
Crinia subinsignifera	+	3
Geocrinia alba	+?	179
Geocrinia leai	-	3
Geocrinia rosea	+	21
Geocrinia vitellina	+	43
Heleioporus albopuncatatus	-	10
Heleioporus barycragus	+	2
Heleioporus eyrei	+	282
Limnodynastes dorsalis	+	162
Neobatrachus kunapalari	-	10
Neobatrachus pelobatoides	+?	10
Myobatrachus gouldi	-	3
Pseudophryne guentheri	-	1
Spicospina flammocaerulea	-	2

Table 1. Recorded distribution of chytrid infection among southwestern WA frogs. Species are scored as + (definite cases of chytrid infection), +? (possible to likely cases of chytrid infection) and – (no indication of chytrid infection). N is the total number of individual frogs examined per species.

Species	**% Positive**	**% Poss/Likely**	**% Negative**	**N**
Litoria moorei	20.6	3.9	75.5	441
Litoria adelaidensis	17.5	3.1	79.4	160
Crinia georgiana	30.0	2.5	67.5	203
Crinia glauerti	21.2	1.9	76.9	104
Crinia insignifera	29.6	2.1	68.3	142
Crinia pseudinsignifera	5.4	0	94.6	37
Geocrinia alba	0	1.1	98.9	179
Geocrinia vitellina	7.0	4.7	88.3	43
Limnodynastes dorsalis	6.8	2.5	79.0	162
Heleioporus eyrei	7.1	2.1	90.8	282

Table 2. Disease prevalence estimates for a variety of better-sampled frog species. N is the total number of individual frogs examined per species.

	DATE	*Lm*	*La*	*Cg*	*Cp*	*Gr*	*He*	*Ld*	*Np*
Perth Metropolitan Area									
Perry Lakes, Floreat	1992-99		+				+	+	
Waterford	Oct 92						+		
Ken Hurst Park, Leeming	Nov 92						+		
Norman Rd, Cardup	Oct 96						+		
Brickwood Reserve, Byford	Oct 96						+		
Rushton Pk, Kelmscott	Oct 96						+		
Ferndale	Oct 98	+							
Perth Zoo	Aug 98		+						
University of W.A.	1998	+							
Herdsman Lake, Osborne Park	July 99	+							
Woodlands	1998-99	+						+	
Lake Gwelup, North Beach	June 99		+						
East Victoria Park	1998-99	+							
Kingsley	July 99	+							
Darling Range									
Sawyers Valley area	1998-99	+			+			+	
Gungin Gully, near Kalamunda	April 92		+						
Mt Helena	1998-99	+							
Boulder Rock, Brookton Highway*	Aug 92			+					
Southern Coastal Plain									
Golden Bay	Sep 92	+	+						
Tim's Thicket, Yalgorup NP	Oct 92							+	
Southern Coastal									
Albany	1998-99	+							
Ellecker	Apr 99			+			+		
Lower Calgun River	1999							+	
Southwest Forest									
Big Brook*	Aug 92					+			
Witchcliffe	Nov 98	+							
Manjimup	Nov 98	+							
Central Wheatbelt									
Kellerberin*	May 95								+

Table 3. Selected list of localities where chytrid infections have been detected in W.A. The date of collection and the range of infected frog species are also shown for each locality. Records marked with an asterisk are based on material sectioned by Thom (1995). Key to species: *Lm - Litoria moorei*; *La - Litoria adelaidensis*; *Cg - Crinia georgiana*; *Cp - Crinia pseudinsignifera*; *Gr - Geocrinia rosea*; *He - Heleioporus eyrei*; *Ld - Limnodynastes dorsalis*; *Np - Neobatrachus pelobatoides*.

YEAR	N	% INFECTED
1999	165	41.8
1998	53	26.4
1995	9	0
1994	6	0
1993	27	3.7
1992	24	45.8
1990	1	0
1986	21	9.5
1985	8	12.5
1984	3	0
1981	1	0
1980	17	0
1979	16	0
1978	2	0
1977	1	0
1976	14	0
1975	10	0
1970	3	0
1965	20	0
1963	1	0
1956	12	0
1933	5	0

Table 4. *Litoria moorei* - % infected for each yearly intervals

YEAR	DEC-FEB	MAR-MAY	JUN-AUG	SEP-NOV	YEARLY
1999	**4.8% (63)**	**4.8% (353)**	**51.6% (310)**	**24.8% (117)**	**28.4% (843)**
1998	0 % (11)	-	100% (1)	52.6% (19)	55% (31)
1997	0 % (3)	0% (27)	40% (5)	-	6.1% (35)
1996	**0 % (9)**	**0% (15)**	**2.4 % (42)**	**12.3% (65)**	**6.8% (131)**
1995	-	4.8% (21)	0% (10)	0% (1)	2.4% (41)
1994	**0% (4)**	**9.1% (11)**	**0% (2)**	**6.3% (64)**	**6.2% (81)**
1993	**0% (51)**	**4.3% (47)**	**22.2% (27)**	**8.7% (23)**	**6.1% (179)**
1992	**30% (10)**	**8.9% (45)**	**19.1% (89)**	**43% (100)**	**26.4% (244)**
1991	-	-	0% (1)	0% (1)	0% (2)
1990	-	0% (14)	-	0% (1)	0% (15)
1989	**50% (2)**	**100% (1)**	0% (3)	0% (2)	**13.6% (8)**
1988	0% (1)	-	-	-	0% (1)
1986	0% (5)	**18.8% (16)**	-	0% (1)	0% (22)
1985	-	**100% (1)**	-	**57.1 (7)**	**62.5% (8)**
1984	-	0% (2)	-	-	0% (2)
1981	0% (1)	-	-	-	0% (1)
1980	0% (16)	0% (1)	-	-	0% (17)
1979	0% (12)	-	-	-	0% (12)
1978	-	0% (2)	-	-	0% (2)
1977	-	0% (1)	-	-	0% (1)
1976	0% (8)	-	0% (1)	0% (6)	0% (15)
1975	0% (6)	0% (1)	0% (2)	0% (2)	0% (11)
1970	-	0% (3)	-	-	0% (3)
1965	-	0% (22)	-	-	0% (22)
1963	0% (1)	-	-	-	0% (1)
1956	0% (22)	-	-	-	0% (22)

Table 5. Seasonal and longer term trends in chytrid prevalence, based on pooling of all taxa and all kinds of population samples. The values in each cell are percentage infected (total sample examined). The year is broken roughly into Summer (December-February), Autumn (March-May), Winter (June-August) and Spring (September to November).

Locality + taxa sampled	Date	Infected/Total	% Infected
Kangaroo Gully, Brookton Hwy			
Crinia georgiana	Jun 92	0/3*	0
Crinia pseudinsignifera	Jun 92	0/1*	0
Neobatrachus pelobatoides	Jun 92	0/2*	0
Big Brook			
Geocrinia rosea	Aug 92	1/21*	5
Boulder Rock, Brookton Hwy			
Crinia georgiana	Aug 92	8/21*	38
	Aug 99	6/18	33
Perry Lakes			
Litoria adelaidensis	Apr 92	1/4	25
“ “	May 92	0/4	0
“ “	Sep 92	2/3	67
“ “	Mar 99	0/3	0
“ “	Apr 99	0/14	0
“ “	July 99	2/3	67
“ “	Aug 99	2/4	50
Heleioporus eyrei	May 92	1/16	6
Limnodynastes dorsalis	Sep 92	1/1	100
“ “	May 99	2/29	7
Crinia insignifera	May 92	0/2	0
“ “	June 92	7/8	86
“ “	July 99	7/11	64
“ “	Aug 99	12/16	75
Golden Bay			
Litoria moorei	Sep 92	1/1	100
Litoria adelaidensis	Sep 92	3/6	50
Tim’s Thicket, Yalgorup			
Litoria moorei	Nov 92	0/9*	0
Limnodynastes dorsalis	Nov 92	2/4*	50
Gungin Gully, nr Kalamunda			
Litoria adelaidensis	Nov 92	3/4	75
Crinia glauerti	Nov 99	4/13	31
Brookton Hwy			

Heleioporus albopunctatus	May 95	0/10*	0
Kellerberin			
Neobatrachus pelobatoides	May 95	1/10*	10
Wyalkatchem			
Neobatrachus kunapalari	Jul 95	0/10*	0
Dianella			
Litoria moorei	Sept 98	0/18#	0
Herdsman Lake, Osborne Pk			
Litoria moorei	Apr 86	1/10	10
" "	May 86	1/2	50
" "	Apr 92	1/4	25
" "	May 99	3/5#	60
" "	June 99	8/15	53
Elleker, near Albany			
Crinia georgiana	May 99	1/6	17
Heleioporus eyrei	May 99	1/19	5
Wildflower Nursery, Kingsley			
Litoria moorei	Jun 99	7/11	64
Lake Gwelup, Gwelup			
Litoria adelaidensis#	Jun 99	1/13	8
Crinia insignifera#	Jun 99	0/15	0

Table 6. Preliminary results of chytrid prevalence survey.
Localities are arranged by date of first sampling. Samples marked with * are those based on one to a few transverse sections of a single toe-clip. Samples marked with # are based on multiple transverse or longitudinal sections of toe-clippings. The remainder are based on large pieces of skin from the foot and groin (sometimes, the entire foot).