THE MANAGEMENT OF DISEASE IN WILD AMPHIBIAN POPULATIONS IN AUSTRALIA

A REPORT TO THE
NATURAL HERITAGE TRUST THROUGH
QUEENSLAND PARKS AND WILDLIFE SERVICE
AND RAINFOREST CRC

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GLOSSARY

AAHL	CSIRO Australian Animal Health Laboratory, Geelong, Victoria
ARC	Amphibian Research Centre, Melbourne
AQIS	Australian Quarantine and Inspection Service
Chytridiomycosis	The state of being infected with *B. dendrobatidis*. Amphibians can have chytridiomycosis without showing clinical signs (aclinical chytridiomycosis) or can show clinical signs (mild, severe) or death. The term was proposed by Berger *et al.* (1998)
DEH	Commonwealth Department of the Environment and Heritage
Emerging infectious disease	An infectious disease that has newly appeared or is increasing in incidence and geographic range
Endemic	(Used in an epidemiological sense) Incidence of disease is largely stable in an area or population owing to the causative organism being present and sufficient naïve hosts being available to maintain infection
Epidemic	An increase in the incidence of a disease in a population above the level that is "normal" or expected for that population
Epidemiology	The study of disease in populations
Host specificity	The degree to which an infectious agent remains confined to one species of host or taxonomically related hosts. Low host specificity means that the infectious agent can infect many species of host, or species of host that are not closely related taxonomically
Hyperaemic	Red due to a greater amount of blood flowing in area
Immunoperoxidase test	A diagnostic test for *B. dendrobatidis* using specific antibodies that bind to chytrid antigen in histological sections of amphibian skin. The bound antibodies are detected by the immunoperoxidase indicator system and stain *B. dendrobatidis* brown
Incidence	The number of new cases of a disease occurring at a location in a defined period of time
IRCEB	Integrated Research Challenges in Evolutionary Biology. Competitive funding program in USA from National Science Foundation. A consortium based in Arizona were granted funding to research chytridiomycosis and ranavirus
KTP	Key Threatening Process
LIEF	Linkage Infrastructure Equipment and Facilities grant (Australian Research Council)

Morbidity	Clinical disease
Mortality	Death
NHT	Natural Heritage Trust
Pathogenicity	The potential of a pathogen to cause disease
PCR	Polymerase Chain Reaction. A diagnostic test using a molecular biological technique to manufacture additional DNA strands from small numbers of DNA strands in the original specimen
Prevalence	The percent of the population with the disease or condition of interest at a particular point in time
QPWS	Queensland Parks and Wildlife Service
Real-time PCR	A PCR test that is able to quantify the amount of DNA present in the original sample
Resting phase	A stage in the life cycle of some chytrids, which is resistant to dehydration. This stage does not appear to occur in *Batrachochytrium dendrobatidis*
Saprobe	Micro-organism that is capable of living and growing in the environment
Self-cure	The process in which a host cures itself of an infecting agent
Sensitivity	The probability of testing positive if chytridiomycosis is present
Specificity	The probability of testing negative if chytridiomycosis is truly absent
Sporangium	The spherical structure of *B. dendrobatidis* found in epidermis and from which zoospores are released. Interchangeable with “zoosporangium”
Surveillance	The ongoing collection, collation, analysis and interpretation of disease specific data and dissemination to those who need to know to take steps to decrease the impact of the disease
TAP	Threat Abatement Plan
Transmissibility	The ability of a pathogen to transmit to a host or between hosts
Zoosporangium	The spherical structure of *B. dendrobatidis* found in epidermis and from which zoospores are released
Zoospore	The infectious stage of *B. dendrobatidis* that is motile in water

ACKNOWLEDGEMENTS

The role played by Keith McDonald of Queensland Parks and Wildlife Service (QPWS) in providing ideas, guidance on relevance of activities to wildlife managers, and field work was a major one and invaluable. Harry Hines and Alistair Freeman of QPWS also provided valuable assistance.

Deborah Pergolotti of Cairns Frog Hospital provided a constant source of ill and dead amphibians and a valuable link with events in periurban amphibian communities. Gerry Marantelli of the Amphibian Research Centre assisted with experimental infections and provision of ideas.

A large number of collaborators in Australia (Alex Hyatt, Donna Boyle, Veronica Olsen, Richard Retallick, Hamish McCullum, Ray Muller, Lee Skerratt and L. Keegan) and overseas (Che Weldon, Louis du Prez, Joyce Longcore, E. Bonaccorso and Juan Guayasamin) provided valuable assistance and opportunities to test hypotheses.

EXECUTIVE SUMMARY

This Natural Heritage Trust (NHT) funded project aimed to improve the evidence base to inform strategies to manage disease in wild amphibian populations. Communication and research activities were undertaken to address four scope items with nineteen objectives. Scope Item 1 dealt with communication of evidence and strategies via the Amphibian Diseases Home Page on the World Wide Web. All six objectives were met. Scope Item 2 dealt with communication of strategies via written material and public presentations. Of the four objectives under this scope item, three were met. The fourth however, dissemination of information on detection of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in the environment including detection in frog ponds, could not be completed as scientific techniques and knowledge have not yet provided us with a suitable tool. Scope Item 3, dealing with the provision of protocols for management of chytridiomycosis in the field and the laboratory, was fully met and in fact exceeded by provision of evidence of the killing effect of human skin on the amphibian chytrid fungus. Of the eight objectives under Scope Item 4, improving the evidence for management decisions on chytridiomycosis, seven were met. A study on the effect of chytridiomycosis on tadpole mouthparts, Item 4(e) will be completed in 2006.

The project resulted in significant output presented in this report as six appendices. The nine scientific papers published in peer-reviewed journals were a significant contribution to the literature on amphibian diseases, particularly chytridiomycosis. In particular, two papers based on work in Queensland used epidemiological techniques to show that the amphibian chytrid fungus was now endemic and occurred at a prevalence greater than five percent. A northern Queensland study (McDonald *et al.* 2005) provided encouraging evidence that the prevalence of chytridiomycosis had fallen while the population of the surviving frog species had returned to pre-decline levels. A landmark study using archived frogs with collaborators in southern Africa gave epidemiological evidence that the amphibian chytrid fungus had originated in Africa and escaped the continent via the global trade in the African Clawed Frog. Practical protocols for use in field hygiene and to guide laboratory methods were also produced and made available at the Amphibian Diseases Home Page.

A significant impact of this project and the previous NHT funded projects was that the evidence these projects generated provided a firm foundation for development of the Threat Abatement Plan (TAP) for infection of amphibians with the amphibian chytrid fungus resulting in chytridiomycosis. In 2003 and 2004, Richard Speare acted as a consultant to the Commonwealth Department of the Environment and Heritage (DEH) in developing the TAP.

Specific recommendations from this project include:

Scope Item 3: Development of Protocols for the Management of Chytridiomycosis in the Field and the Laboratory

- Extend studies to examine the transfer of *B. dendrobatidis* on types of gloves more commonly used in the field, including plastic freezer bags.
- Evaluate the killing effect in vitro of a range of glove material.

Funds from DEH public tender RFT 16/2004 to develop national hygiene protocols will be used to continue this research and provide evidence on which to base protocols.

Scope Item 4(b): Determine the boundary of the chytrid-positive area in North Queensland

- Four actions are recommended to redefine the northern border of the chytrid-positive zone in Queensland:
  - Continue surveillance of the McIllwraith Range amphibian population.
  - Search for the northern boundary of the distribution of the amphibian chytrid fungus by surveying populations of amphibians in water bodies between Big Tableland and McIllwraith Range. An initial survey should be done in the Endeavour River, roughly thirty kilometres north of O'Keefe Creek.
  - Use realtime-PCR (Polymerase Chain Reaction) as a diagnostic tool, as sensitivity is higher than histology.
  - Develop an action plan for McIllwraith Range if chytridiomycosis is found in this population.
- Extend surveys for *B. dendrobatidis* west to detect the western boundary of the chytrid-positive zone.

Funding for these recommendations was obtained through DEH public tender RFT 63/2003 on mapping protocols for chytridiomycosis.

Scope Item 4(c): Assess the potential role of birds in translocation of the amphibian chytrid

The role of aquatic birds as potential short-term vectors of *B. dendrobatidis* should be investigated using a natural system and living avian hosts.

Scope Item 4(d): Assess the potential role of soil in transmission of chytridiomycosis

- Survival of *B. dendrobatidis* in non-sterile soil should be evaluated.
- Determine where *B. dendrobatidis* grows in the environment and where zoospores and hence infectivity is found in the environment.

Funding for this project and the previous recommendation was obtained in 2005 through DEH public tender RFT 42/2004.

Scope Item 4(h): Using a community based surveillance system, investigate dead and dying wild amphibians or threatened amphibians in captive husbandry for disease and causative agents

- A veterinarian or veterinary student should determine the aetiology and epidemiology of the wasting disease in *L. infrafrenata* and *L. caerulea* in Cairns.
- Additional resources should be obtained to carry out this study for at least one year.

SCOPE ITEMS

The scope items set out in the contract between Rainforest CRC and Queensland Parks and Wildlife Service for this project are listed below.

Item 1: Maintenance and Enhancement of the Amphibian Diseases Home Page

1(a) Implement more professional interface;
1(b) Update existing information;
1(c) Provide protocols for handling frogs;
1(d) Expand links;
1(e) Add overviews of additional amphibian diseases; and
1(f) List amphibian species and whether chytridiomycosis detected.

Item 2: Dissemination of Information to the Community on Amphibian Diseases and Strategies to Lessen the Impact of These

2(a) General diseases of frogs with comment that signs and symptoms are generally non-specific and can be due to a number of causes;
2(b) Detection of the amphibian chytrid in the environment including detection in frog ponds;
2(c) Communication of Protocols to manage the amphibian chytrid in the field and the laboratory; and
2(d) Chytridiomycosis in Australia and relevance to conservation of species (including species already infected and those currently chytrid-free).

Item 3: Development of Protocols for the Management of Chytridiomycosis in the Field and the Laboratory

Item 4: Improving the Evidence for Management Decisions on Chytridiomycosis

4(a) Design a methodologically sound survey strategy to determine the chytrid-infected status of areas (*Getting the Jump on Amphibian Diseases* Recommendations 1.4, 1.13, 2.5);
4(b) Using this methodology, determine the boundary of the chytrid-positive area in North Queensland (*Getting the Jump on Amphibian Diseases* Recommendations 2.16, 2.26);
4(c) Assess the potential role of birds in translocation of the amphibian chytrid (*Getting the Jump on Amphibian Diseases* Recommendation 2.25);
4(d) Assess the potential role of soil in transmission of chytridiomycosis (*Getting the Jump on Amphibian Diseases* Recommendation 2.25);
4(e) Determine the effect of chytrid infection on tadpole mouths (Retallick Recommendation 21);
4(f) Monitor long-term sites in wet tropics to investigate decline in prevalence (Retallick Recommendation 14);
4(g) Implement PCR testing for chytridiomycosis at James Cook University and provide a testing service at cost recovery (Retallick Recommendation 7a); and
4(h) Using a community based surveillance system, investigate dead and dying wild amphibians or threatened amphibians in captive husbandry for disease and causative agents (*Getting the Jump on Amphibian Diseases* Recommendations 2.6, 2.15, 3.21, 4.3, Retallick Recommendation 1).

OUTPUT AND ACTIVITIES AGAINST SCOPE ITEMS

SCOPE ITEM NUMBER	SCOPE ITEM	STATUS	OUTPUTS
Item 1:	**Web site (Amphibian Diseases Home Page)**		See page 7, site is a valuable resource for wildlife managers and researchers
1(a)	Implementation of a more professional interface.	Completed	See page 8
1(b)	Update of existing information.	Completed	See page 9
1(c)	Provision of protocols for handling frogs.	Completed	See page 9 and Appendix 1
1(d)	Expansion of links.	Completed	See page 9
1(e)	Add overviews of additional amphibian diseases.	Completed	See page 10
1(f)	List amphibian species and whether chytridiomycosis detected.	Completed	See page 10 and Appendix 2
Item 2:	**Dissemination of information to the community.**		See page 11
2(a)	General diseases of frogs with comment that signs and symptoms are generally non-specific and can be due to a number of causes.	Public lectures	Three public lectures given. See page 11 and Appendix 3
2(b)	Detection of the amphibian chytrid in the environment including detection in frog ponds.	Unable to be carried out owing to lack of suitable technique	See page 11
2(c)	Communication of protocols to manage the amphibian chytrid in the field and the laboratory.		See page 11 and Appendix 1
2(d)	Chytridiomycosis in Australia and its relevance to the conservation of species (including species already infected and those currently chytrid-free).	Public lectures	See page 11 and Appendices 3, 4 & 5; Seven scientific papers published or in press; Used to develop chytrid TAP.
Item 3:	**Development of protocols for management of chytridiomycosis in the field and the laboratory**	Completed	See page 13 and Appendix 1
	Role of gloves in field hygiene.	Preliminary work completed.	See page 13

SCOPE ITEM NUMBER	SCOPE ITEM	STATUS	OUTPUTS
Item 4:	**Improving the evidence for management decisions on chytridiomycosis**		See page 17
4(a)	Design a methodologically sound survey strategy to determine the chytrid-infected status of areas (*Getting the Jump on Amphibian Diseases* Recommendations 1.4, 1.13, 2.5).	Commenced	See page 17 Utilised in chytrid TAP and in North Queensland surveys; Two scientific papers published.
4(b)	Use this methodology to determine the boundary of the chytrid-positive area in North Queensland (*Getting the Jump on Amphibian Diseases* Recommendations 2.16, 2.26).	Surveys done	See page 21
4(c)	Assess the potential role of birds in translocation of the amphibian chytrid (*Getting the Jump on Amphibian Diseases* Recommendation 2.25).	Completed	See page 24 Scientific paper published.
4(d)	Assess the potential role of soil in transmission of chytridiomycosis (*Getting the Jump on Amphibian Diseases* Recommendation 2.25).	Completed	See page 25 Scientific paper published.
4(e)	Determine the effect of chytrid infection on tadpole mouths (Retallick Recommendation 21).	Commenced	See page 26 Progress slower than anticipated.
4(f)	Monitor long-term sites in the Wet Tropics to investigate decline in prevalence (Retallick Recommendation 14).	Completed	See page 27 Two scientific papers published.
4(g)	Implement PCR testing for chytridiomycosis at James Cook University and provide a testing service at cost recovery (Retallick Recommendation 7a).	Implemented	See page 29 PCR testing became available at James Cook University in March 2005.
4(h)	Using a community based surveillance system, investigate dead and dying wild amphibians or threatened amphibians in captive husbandry for disease and causative agents. (*Getting the Jump on Amphibian Diseases* Recommendations 2.6, 2.15, 3.21, 4.3, Retallick Recommendation 1)	Implemented	See page 30

SCOPE ITEM 1:
THE AMPHIBIAN DISEASES HOME PAGE

http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm

The Amphibian Diseases Home Page (ADHP) continues to be an authoritative source for information on diseases of wild amphibians both within Australia and internationally. The site now consists of 115 Megabytes of data in 434 files in 12 directories. The entry page has received an average of 1,232 hits per month since July 2004 (Figure 1).

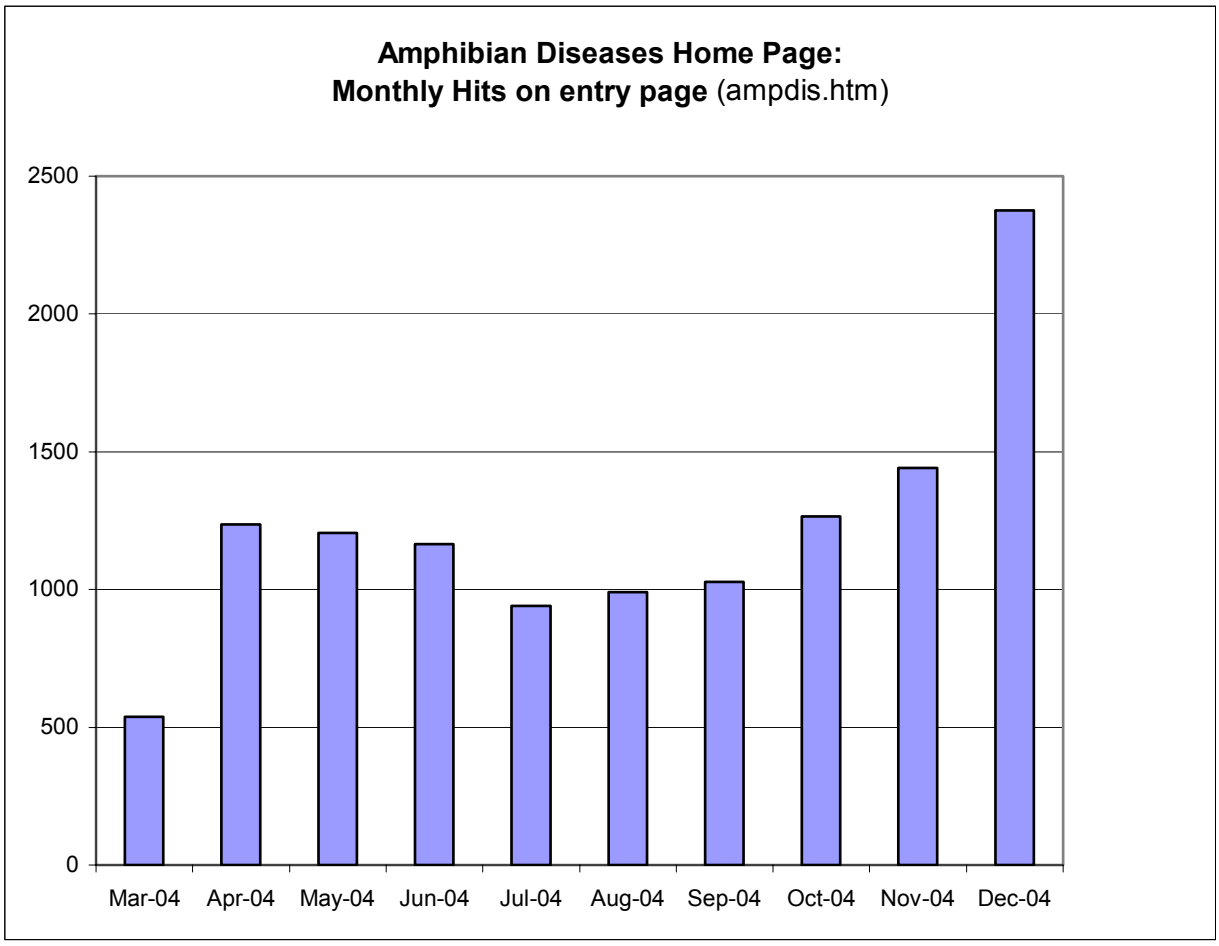

Figure 1: Number of hits per month for the entry page (ampdis.htm) of the Amphibian Diseases Home Page.

Scope Item 1(a) – Implementation of a Professional Interface

Completed. The site remains largely text based as unnecessary graphics slow speed of access. The front page was modified to a more professional appearance (Figure 2).

Figure 2(a): Previous front page for the Amphibian Diseases Home Page.

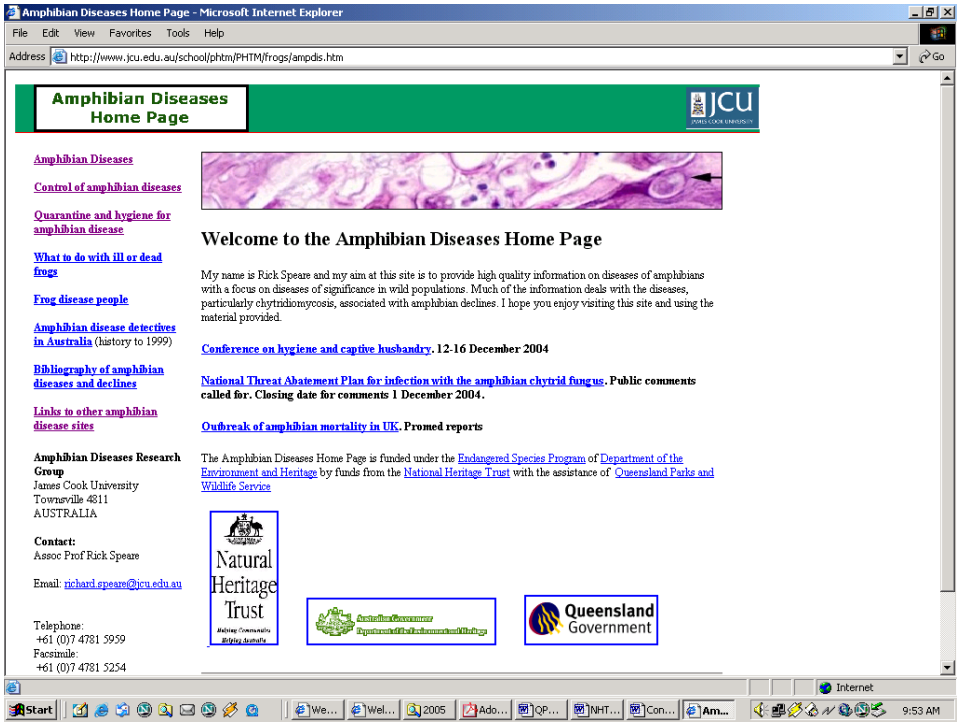

Figure 2(b): New front page for the Amphibian Diseases Home Page.

Scope item 1(b) – Update of Existing Information

Pages with information that is changing are updated at least every six months and monthly for highly dynamic pages (Table 1).

Table 1: Update-schedule for dynamic web pages at Amphibian Diseases Home Page. This does not include pages that are fixed (e.g. papers, protocols, lectures, etc.).

Page	Update schedule
Main page – ampdis.htm	monthly
Chytridiomycosis main page – Batrachochytrium.htm	monthly
Chytridiomycosis publications page – chart.htm	monthly
Chytridiomycosis records in Australia – chyspec.htm	six-monthly
Global chytridiomycosis records – chglobal.htm	six-monthly
Frog disease people – fdpeople.htm	six-monthly
Bibliography of amphibian disease – bibliog.htm	six-monthly

Scope Item 1(c) – Provision of Protocols for Handling Frogs

A hygiene protocol for field-work to prevent the transmission of diseases while handling frogs was developed (See Appendix 1) and made available at the ADHP. Other protocols were also added and include:

- A hygiene protocol for field researchers within sites (http://www.jcu.edu.au/school/phtm/PHTM/frogs/field-hygiene.pdf);
- AQIS requirements for importation of amphibians and their eggs into Australia (http://www.jcu.edu.au/school/phtm/PHTM/frogs/aqis/aqis.htm);
- Protocols used in working with the amphibian chytrid in the laboratory (http://www.jcu.edu.au/school/phtm/PHTM/frogs/bdprotocols.htm); and
- Protocols for use of collection of specimens for PCR diagnosis of chytridiomycosis (http://www.jcu.edu.au/school/phtm/PHTM/frogs/protocols/bd-swabs-protocol.pdf).

Scope Item 1(d) – Expansion of Links

Additional links were added to good quality sites of relevance. Major links included:

- Department of Environment and Heritage (DEH) Key Threatening Process (KTP) and Threat Abatement Plan (TAP) for infection of amphibians with the amphibian chytrid fungus resulting in chytridiomycosis.
- DEH permit requirements for trade in amphibians, particularly importation of foreign amphibians.

Scope Item 1(e) – Overviews of Additional Amphibian Diseases

Additional diseases added to the site included:

- Overview of bacterial diseases of amphibians (http://www.jcu.edu.au/school/phtm/PHTM/frogs/otherdiseases-bacteria.htm)
- Overview of viral diseases of amphibians (http://www.jcu.edu.au/school/phtm/PHTM/frogs/otherdiseases-viruses.htm)
- Overview of fungal diseases of amphibians (http://www.jcu.edu.au/school/phtm/PHTM/frogs/otherdiseases-fungi.htm)
- Overview of parasitic diseases of amphibians (http://www.jcu.edu.au/school/phtm/PHTM/frogs/otherdiseases-parasite.htm)

Scope item 1(f) – Amphibian Species and their Chytridiomycosis Status

A list of Australian species, their status with respect to the detection of chytridiomycosis and their geographical distribution was made available on the Amphibian Diseases Home Page (http://www.jcu.edu.au/school/phtm/PHTM/frogs/TAP-TabB1.pdf) by state and nationally. This is reproduced in Appendix 1.

SCOPE ITEM 2: DISSEMINATION OF INFORMATION TO THE COMMUNITY

Dissemination of information to the community on amphibian diseases and on strategies to lessen their impacts was conducted by public lectures, distribution of written information material, development of protocols, contribution to a national Threat Abatement Plan, and media liaison. Further research on chytridiomycosis is being conducted, for which a project grant could be secured.

Scope Item 2(a) – General Diseases of Frogs

A public lecture was given to the Tablelands Frog Club at Atherton on the night of 12 March 2004. It was attended by approximately fifty people from many parts of North Queensland, including the Atherton Tablelands, Cairns, Innisfail and Mt Garnet. The frog club was given hard copies of the lecture to use in their newsletters. The session generated a lot of media interest in print and on radio. The non-specific nature of clinical presentations of amphibian diseases and the need for laboratory tests to diagnose most frog diseases were emphasized. Two other public lectures were also given, one at James Cook University and one at the University of the North West in South Africa. See Appendix 3 for copies of these presentations.

Scope Item 2(b) – Detection of the Amphibian Chytrid in the Environment

Unfortunately, at the current time there are no tests to detect *B. dendrobatidis* in the environment, including detection in frog ponds. Research is being done by the Australian Animal Health Laboratory (AAHL) on development of these tests. We obtained an Australian Research Council postdoctoral project grant for Dr Lee Berger in 2004 and one of the aims is to investigate the natural history of the amphibian chytrid in artificial environments. This data will be used subsequently to direct searching effort for *B. dendrobatidis* in the field.

Scope Item 2(c) – Communication of Protocols to Manage the Amphibian Chytrid in the Field and the Laboratory

A protocol for use within sites was developed. The protocol is feasible and balances the need to prevent transmission of pathogens within an amphibian population against the practicalities of using hygiene protocols under difficult field conditions. This protocol is available on the World Wide Web at the ADHP at the URL:
http://www.jcu.edu.au/school/phtm/PHTM/frogs/control.htm.

Scope Item 2(d) – Chytridiomycosis in Australia and its Relevance to the Conservation of Species

The distribution of chytridiomycosis in Australia and its relevance to the conservation of amphibian species, including species already infected and those currently chytrid-free, were covered in three public lectures, given by Rick Speare.

The lectures, two of which previously mentioned at Scope Item 2(a), are given in Appendix 3.

A major outcome from our NHT projects has been that the evidence generated has provided a basis for the Threat Abatement Plan for infection of amphibians with the amphibian chytrid resulting in chytridiomycosis (Appendix 5). Rick Speare acted as the consultant to DEH in developing the TAP.

SCOPE ITEM 3: DEVELOPMENT OF PROTOCOLS FOR THE MANAGEMENT OF CHYTRIDIOMYCOSIS IN THE FIELD AND THE LABORATORY

The following protocols for the management of chytridiomycosis in the field and the laboratory have been developed (see Appendices 1 and 3):

- Johnson, Megan. *Working with* Batrachochytrium dendrobatidis*, the amphibian chytrid fungus*.
- Johnson, Megan. *Media for in-vitro cultivation of* Batrachochytrium dendrobatidis.
- Johnson, Megan. *In vitro cultivation of* Batrachochytrium dendrobatidis.
- Johnson, Megan. *Cryopreservation protocol for* Batrachochytrium dendrobatidis.
- Speare, R., Berger, L., Skerratt, L. F., Alford, R., Méndez, D., Cashins, S., Kenyon, N., Hauselberger, K. and Rowley, J. *Hygiene protocol for handling amphibians in field studies*.

All protocols are now available to the public at the Amphibian Diseases Home Page.

Field Hygiene Protocol

Basic principles underlying field hygiene within a site are:

- Protocols must be feasible and not make field work so difficult that studies are impeded;
- The scientist is not expected to reduce the level of contamination below that found in the amphibians' environment; and
- Washing hands in the water in which the amphibians live will bring them to the background level.

The use of gloves in field hygiene protocols, particularly for researchers in the tropics or in physically hazardous streams, is controversial. We attempted to obtain evidence on the potential for bare hands to transmit the amphibian chytrid fungus. The question we aim to answer is: "Does using bare hands increase the risk of transmitting *Batrachochytrium dendrobatidis* between amphibians in the field compared to using disposable gloves?"

Survival of *B. dendrobatidis* on Hands

The first study in this project is to determine the survival of *B. dendrobatidis* on hands. The results are reported here.

Experiment 1:

1. Hands washed in tap water;
2. One dried with paper towel;
3. Other wiped with 70% ethanol and allowed to air dry;
4. Both contaminated by immersion in active *B. dendrobatidis* broth culture;

5. Four fingers of each hand pressed on surface of TGhL agar plate at 0, 5, 10, 15, 20 and 30 minutes;
6. Plates cultured at 23°C.

Results Experiment 1:

Heavy growth both hands at time 0 with no growth thereafter.

Conclusion Experiment 1:

1. Bare skin kills *B. dendrobatidis* within 5 minutes;
2. No difference with pre-treatment with 70% ethanol.

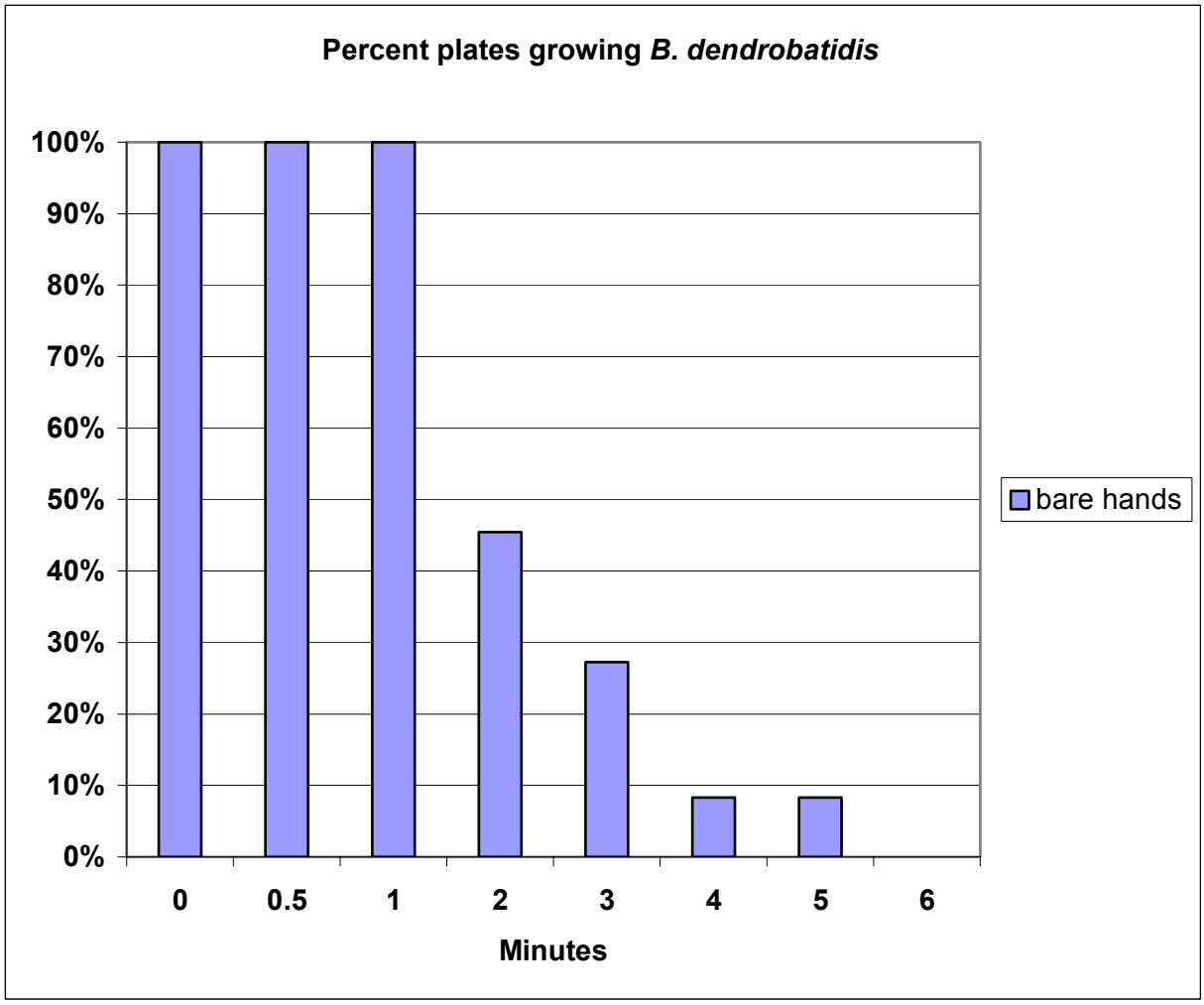

Figure 3: Killing effect of human skin on B. dendrobatidis with time after contamination of fingers.

Experiment 2:

1. Hands washed in tap water and dried with paper towel (2 replicates);
2. Eight fingers contaminated by immersion in active *B. dendrobatidis* broth culture;
3. A single finger pressed on surface of TGhL agar plate at 0, 0.5, 1, 2, 3, 4, 5 and 6 minutes;
4. Each finger used only once;
5. Plates cultured at 23°C.

Results Experiment 2:

1. Heavy growth both hands – time 0 and 0.5 min;
2. Growth thereafter only at 1 min and 5 min in 1 of 2 subjects.

Conclusion Experiment 2:

Bare skin kills *B. dendrobatidis* within 6 min.

Experiment 3:

1. Hands washed in tap water and dried with paper towel;
2. One hand covered with disposable glove;
3. Fingers of each hand (4 bare, 4 gloved) contaminated by immersion in active *B. dendrobatidis* broth culture (>50,000 zoosporangia / ml);
4. A single finger pressed on surface of TGhL agar plate at 0, 0.5, 1, 2, 3, 4, 5 and 6 minutes;
5. Each finger used only once;
6. Plates cultured at 23°C;
7. 10 replicates.

Results Experiment 3:

1. For bare hands 100% growth at 0, 0.5 and 1 min with <50% isolations up to 5 minutes (Figure 3);
2. For nitrile gloves no growth at any time. Sporangia of *B. dendrobatidis* were visible on agar plate.

Conclusion Experiment 3:

1. Bare hands kill *B. dendrobatidis* within 6 minutes;
2. *B. dendrobatidis* does not grow after exposure to nitrile gloves.

Nitrile is a newer product being used to replace latex gloves. Nitrile gloves are used mainly for laboratory, not field-work. The published formal and informal literature contains no reference to antifungal activity of nitrile gloves. The reason for the failure of *B. dendrobatidis* to grow is unknown.

These studies have demonstrated that bare skin kills *B. dendrobatidis* rapidly. Very high zoospore numbers (50,000/ml) contaminating skin became non-infective within six minutes. Use of bare hands in the field would not be expected to comprise a major risk of transmission of the amphibian chytrid fungus between frogs.

Recommendations for Scope Item 3

For further development of protocols for the management of chytridiomycosis in the field and in the laboratory, it is recommended to:

1. Extend studies to examine the transfer of *B. dendrobatidis* on types of gloves more commonly used in the field, including plastic freezer bags; and
2. Evaluate the killing effect *in vitro* of range of glove material.

Funds from DEH public tender RFT 16/2004 to develop national hygiene protocols will be used to continue this research.

SCOPE ITEM 4: IMPROVING THE EVIDENCE FOR MANAGEMENT DECISIONS ON CHYTRIDIOMYCOSIS

Scope Item 4(a) – Design of a Methodologically Sound Survey Strategy to Determine the Chytrid Status of Areas

This scope item was based on Recommendations 1.4, 1.13 and 2.5 from the Cairns 2000 Conference / Workshop *Getting the Jump on Amphibian Diseases* on strategies to lessen the risk of disease in wild amphibians (Speare *et al.* 2001). The principles and methodology developed as part of this Natural Heritage Trust (NHT) project were subsequently used in the draft *Threat Abatement Plan*.

The key points to consider in a survey are:

- Defining the chytrid status;
- Choosing a suitable diagnostic test;
- Determining the number of amphibians to sample;
- Level of survey;
- Logistics of survey, particularly season and sites; and
- Resources required.

Defining the Chytrid Status

Currently we use two terms to define the chytrid status of amphibian populations, water bodies and regions:

- Chytrid-contaminated or chytrid-positive; and
- Chytrid-free.

The first term is straightforward with status determined by a single positive record irrespective of how the record was derived. 'Chytrid-free' is less straightforward as searching effort and sampling technique must have sufficient power to be able to detect an expected leveL. An expected level must be defined as absolute certainty of absence in a population and cannot be achieved unless the complete population is surveyed with a highly sensitive technique. One of the important tasks to be done is to determine what sampling protocols and intensity of survey is adequate.

Three classifications of status can then be used:

- Chytrid-contaminated or chytrid-positive (population has a positive record);
- Chytrid-free (population has been adequately sampled and no positives detected); and
- Chytrid-unknown (population has not been adequately sampled).

'Chytrid-unknown' has not been used. 'Chytrid-free' or 'currently chytrid-free' has been used for areas that are actually 'chytrid-unknown' as the concept of chytrid-free areas is essential for management and a timely response demands decisions based on available evidence even if imperfect.

Diagnostic Tests for Surveys

Chytridiomycosis can only be diagnosed by laboratory tests. The technique currently used for surveys is histological examination of toe clips (Berger *et al.* 1999b). This technique has a lower than ideal sensitivity, particularly in amphibians without clinical signs. The real time PCR test developed by AAHL has a greater sensitivity and can be done on an amphibian without removing a digit or sacrificing the animal (Hyatt 2003; Boyle *et al.* 2004). However, it has not yet been thoroughly tested in the field situation. Action to address this is recommended under Objective 3.

Laboratory based testing has the disadvantage that the result is delayed. If one is searching for populations with chytridiomycosis with the presence / absence approach, once one positive frog is detected, no other frog needs to be surveyed. A test with high specificity is obviously needed to rely on one positive test. An ideal test would be one done in the field that gave a result rapidly. The researcher could then declare that site or population as infected with the amphibian chytrid and reduce costs by not testing the full target quota of frogs. However, whilst this comment outlines an ideal situation it must be appreciated that in general "field" assays do not have the same sensitivity and reproducibility as laboratory based tests. In addition the new diagnostic PCR assays incorporate rapid non-destructive sampling regimes and rapid turn around times in a nationally accredited laboratory. It is important that in any national survey data is collected and analysed in a manner whereby the results are not questionable. From this point of view the role of field-testing will have to be carefully defined.

Expected Prevalence in Chronically Infected Populations

Using histological examination of digits, chronically infected populations of frogs showing no clinical disease appear to have prevalences averaged over the year of between 2%-10%. Prevalences have been obtained from Australian surveys in chronically infected populations are far North Queensland (7%) (McDonald *et al.* 2005), central Queensland (>15%) (Retallick *et al.* 2004), corroboree frogs (3-30%) (Méndez, Hunter and Speare, pers. obs.), southwest Western Australia (1-20%) (Aplin and Kirkpatrick 2000) and from overseas – South Africa (3%) (Weldon *et al.* 2004). It is important to realise that prevalence is related to incidence of infection and duration of infection. A species that is highly susceptible to chytridiomycosis may have a low prevalence if infected members of the population die rapidly and hence duration of infection is short. This may be the situation with Corroboree Frogs as the size of the population is showing a steady reduction. The prevalence peaks in winter and is lowest in summer (Aplin and Kirkpatrick 2000, Berger 2001, Berger *et al.* 2004, McDonald *et al.* 2005). However, collecting large numbers of samples in winter is not ideal for most species of amphibians as they are less abundant than in the warmer months of the year. A survey would have the greatest chance of detecting frogs with chytridiomycosis if performed in winter, but the lower numbers of frogs available to examine may decrease the usefulness. A balance between numbers and prevalence needs to be determined for each area and amphibian population.

With a prevalence of 1%, the number of frogs that need to be examined to detect one frog with chytridiomycosis at a likelihood of 0.95 is 298 (DiGiacomo and Koepsell 1986). This is a presence / absence approach. It does not give an estimate of true prevalence, but, if positive, only indicates that chytridiomycosis is present, and, if all results are negative, what the maximum prevalence could be. Numbers needed to detect one frog at other prevalence-rates are given in Table 2.

Table 2: Numbers of frogs that need to be sampled to detect one positive frog with a likelihood of 0.95 at a range of prevalence-rates (DiGiacomo and Koepsell 1986).

Prevalence in population	Number needed to sample to detect one positive case
20%	13
10%	28
5%	58
4%	73
3%	98
2%	148
1%	298
0.5%	598
0.1%	2994

Obviously detecting prevalence-rates of 1% and below requires an immense effort and may not be feasible. If the current prevalence-rates are used as a guide for the prevalence-rates expected in infected populations, a realistic cut-off level in surveys may be 2%, requiring a sample of 150 animals. If the number of frogs in a population is lower than this, only high prevalence-rates can be excluded. However, if the whole population can be sampled, the statistical calculations do not apply.

These prevalence figures are those obtained using histological methods. If the real-time PCR is more sensitive, the "true" prevalence-rates will be higher, and hence fewer frogs may need to be sampled.

Another option for stream-associated frogs may be to sample tadpoles, as the prevalence of chytridiomycosis in tadpoles appears to be higher than in adults and juveniles (Pearl Symmonds pers. comm.). However, this needs to be evaluated (see actions under Objective 3).

Sampling of Species or Localities

Since *B. dendrobatidis* appears to be able to survive in the environment and is usually found in multiple amphibian species at contaminated sites, a more cost-effective survey approach in detecting presence / absence in chytrid-free areas may be to combine results from all species at the same site. So that presence / absence results refers to site rather than species *per se*. Tests for detecting the amphibian chytrid in the environment are being developed by AAHL, but are not currently available.

Wide-scale Survey Protocols

To map the distribution of chytridiomycosis on a wide-scale, the sampling strategy needs to identify appropriate populations of amphibians to sample. The best species to sample are those already known to be susceptible to infection with the amphibian chytrid from other sites, particularly those species that typically have a high prevalence. A list of species in which chytridiomycosis has been detected is given in Appendix 2. The criteria to use in spacing sample sites are unknown.

Resources for National Surveys

A national survey will require appropriate resources for collection of specimens and for laboratory testing. Any laboratory attempting to undertake the diagnostic assays will have to:

- be ISO / NATA accredited;
- have the appropriate equipment;
- have the appropriately trained staff;
- have the ability to process large numbers of samples; and
- have the finances to pay for the reagents.

At present there is only one laboratory, AAHL, which can satisfy the first four of the five criteriA. Funds for testing will have to be obtained. However, the number of expected submissions could not be handled by AAHL since its focus is performing other diagnostic assays of national importance. There is, therefore, a national requirement to establish further infrastructure within Australia to cater for the analyses of large numbers of samples that are not related to commercial livestock and aquaculture samples. A nationally accredited laboratory dedicated to testing for chytridiomycosis should be established. The James Cook University laboratory aims to achieve criteria 1-4.

Tangible Products Related to Survey Techniques

Two scientific papers relevant to national surveys were published during the life of this project, one in late 2003 and another in late 2004:

- Bonaccorso, E., Guayasamin, J. M., Méndez, D. and Speare, R. (2003). Chytridiomycosis in a Venezuelan amphibian (Bufonidae: *Atelopus cruciger*). *Herpetological Review* **34(4):** 331-334; and
- Weldon, C., Du Preez, L., Hyatt, A., Muller, R. and Speare, R. (2004). Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* **10(12):** 2100-2105.

Both used archived specimens from museums to do national surveys. Bonoccorso *et al.* (2003) was a Venezuelan study using archived material from USA and Venezuela targeting a toad, *Atelopus cruciger*, which had become extinct. The study in southern Africa used the stable and widespread *Xenopus* spp. to map *B. dendrobatidis* through time and space in southern AfricA. Both surveys demonstrated how useful historical data could be obtained from archived specimens and the value of museum collections.

Chytridiomycosis in Venezuela

We concluded our Venezuelan study of archived specimens with publication of a paper reporting the first record of chytridiomycosis in Venezuela (Bonaccorso *et al.* 2003). Chytridiomycosis was detected in one of the last specimens of *Atelopus cruciger* collected from the wild. This toad is now regarded as probably extinct in Venezuela although remnant populations still exist in other South American countries.

Chytridiomycosis in South Africa

Weldon *et al.* (2004) is a landmark paper providing epidemiological evidence to support the hypothesis that *B. dendrobatidis* originated in Africa and was originally distributed from South

Africa through the international trade in the African Clawed Frog (*Xenopus laevis*) (Weldon *et al.* 2004). The abstract is reproduced below:

> *The sudden appearance of chytridiomycosis, the cause of amphibian deaths and population declines in several continents, suggests that its etiologic agent, the amphibian chytrid* Batrachochytrium dendrobatidis*, was introduced into the affected regions. However, the origin of this virulent pathogen is unknown. A survey was conducted of 697 archived specimens of 3 species of* Xenopus *collected from 1879 to 1999 in southern Africa in which the histologic features of the interdigital webbing were analysed. The earliest case of chytridiomycosis found was in a* Xenopus laevis *frog in 1938, and overall prevalence was 2.7%. The prevalence showed no significant differences between species, regions, season, or time period. Chytridiomycosis was a stable endemic infection in southern Africa for 23 years before any positive specimen was found outside AfricA. We propose that Africa is the origin of the amphibian chytrid and that the international trade in* X. laevis *that began in the mid-1930s was the means of dissemination.*

Additional details on the ongoing collaboration in southern Africa are provided under Scope Item 4(h).

Scope Item 4(b) – Application of Methodology and Determination of Boundary of the Chytrid-positive Area in North Queensland (*Getting the Jump on Amphibian Diseases* Recommendations 2.16, 2.26)

The most northerly positive record for chytridiomycosis in Australia is Big Tableland (15.7° S 145.2° E) at 400m altitude (Figure 4). In collaboration with Keith McDonald of Queensland Parks and Wildlife Service we have been monitoring frogs at McIllwraith Range in Cape York for the arrival of *B. dendrobatidis* (Table 3). Our previous hypothesis was that the amphibian chytrid fungus moved north in coastal Queensland at about 100 km / year (Laurance *et al.* 1996) and if the front progressed north at this speed it should arrive at McIllwraith Range (14.2° S 143.3° E), roughly 300 km from Big Tableland, 5 years after arrival at Big Tableland; i.e., 1998. We have maintained a monitoring program at McIllwraith since 1999 by sampling at least 60 *Litoria* spp. or *Rana daemelii* every second year. Frogs were sampled from July to September each year. Histology of toe tips was been used as the detection technique (Berger *et al.* 1999).

Table 3: Results of histological surveys for chytridiomycosis in amphibians at McIllwraith Range, Cape York Peninsula.

Year	*Litoria eucnemis*		*Litoria longirostris*		*Rana daemeli*		Total	
	Examined	Positive	Examined	Positive	Examined	Positive	Examined	Positive
1993	5	0	0		0		5	0
1994	4	0	0		0		4	0
1995	5	0	0		0		5	0
1998	20	0	0		0		20	0
1999	21	0	31	0	0		52	0
2000	20	0	0		0		20	0
2002	59	0	31	0	62	0	152	0
Total	134	0	62	0	62	0	258	0

The results have shown all toes to be negative for chytridiomycosis. The number of toes (152) examined in 2002 would have a 0.95 likelihood of detecting one positive frog, if prevalence was 2% (DiGiacomo and Koepsell 1986).

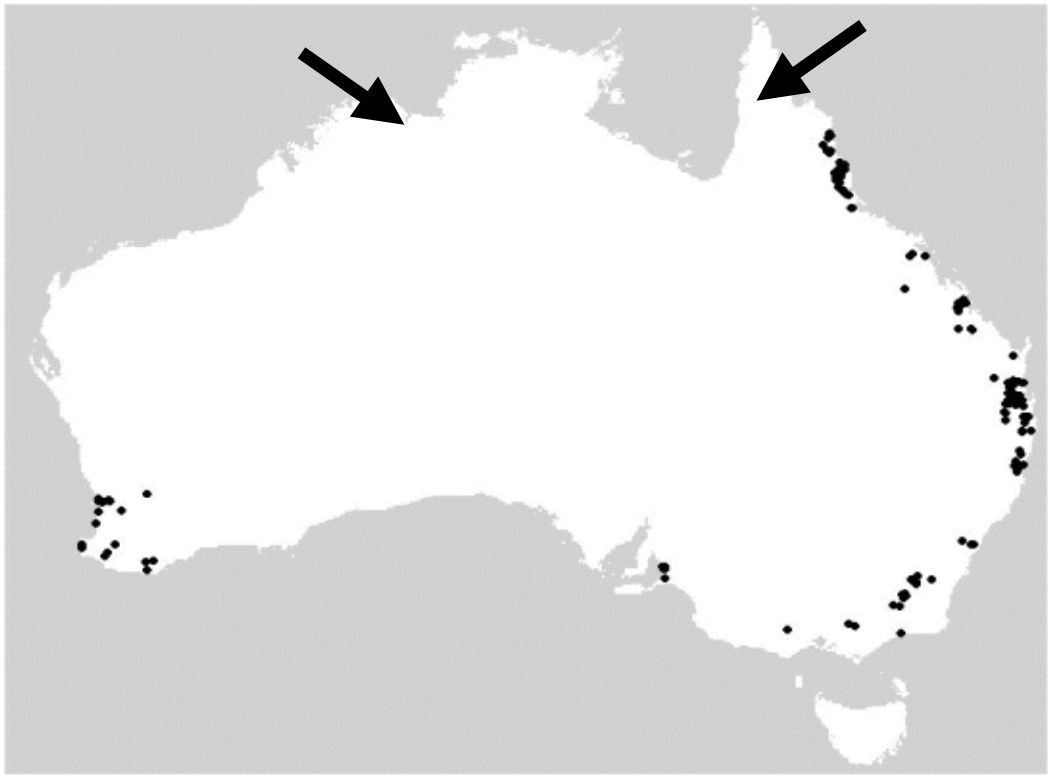

Figure 4: Distribution of chytridiomycosis in Australia (Retallick 2003). Arrows indicate McIllwraith Range monitoring site in far north Queensland (right arrow) and site of the Ord River survey in Western Australia (left arrow). Big Tableland is included in the most northerly black dot.

Conclusion on Scope Item 4(b)

B. dendrobatidis has not been detected in the McIllwraith Range amphibian population. This could be explained in three ways:

1. *B. dendrobatidis* is now present, but prevalence is too low to be detected by the number of frogs sampled;
2. *B. dendrobatidis* is still advancing northward, but rate of advance has decreased below 100 km / year to less than 30 km / year (300 km / 10 years) and it has not yet arrived at the McIllwraith Range; or
3. *B. dendrobatidis* has stopped advancing northward.

Explanation 1:
The number of frogs sampled in 2002 would allow one positive to be detected if prevalence was 3%. Prevalence determined by histology appears to be 3% or greater in populations where chytridiomycosis is endemic. Hence, since the 2002 sample size appears adequate to detect the expected prevalence, the amphibian chytrid fungus was not present at McIllwraith Range in 2002.

Explanation 2:
In Panama the rate of advance of the amphibian chytrid fungus is roughly 30 km / year (Lipps 2004), at least a third that seen in coastal Queensland. If this is the rate of advance north of Big Tableland, we predicted that *B. dendrobatidis* would arrive at McIllwraith Range about 2004. Subsequent surveys over the next few years are essential to test this.

Explanation 3:
A study done by Richard Retallick using BIOCLIM on our database of positive and negative records (Retallick 2003) predicted that *B. dendrobatidis* had a low chance of being found on Cape York Peninsula if temperature parameters were used for the model (Figure 5). Testing this model by locating the current northern border of the distribution of *B. dendrobatidis* is essential.

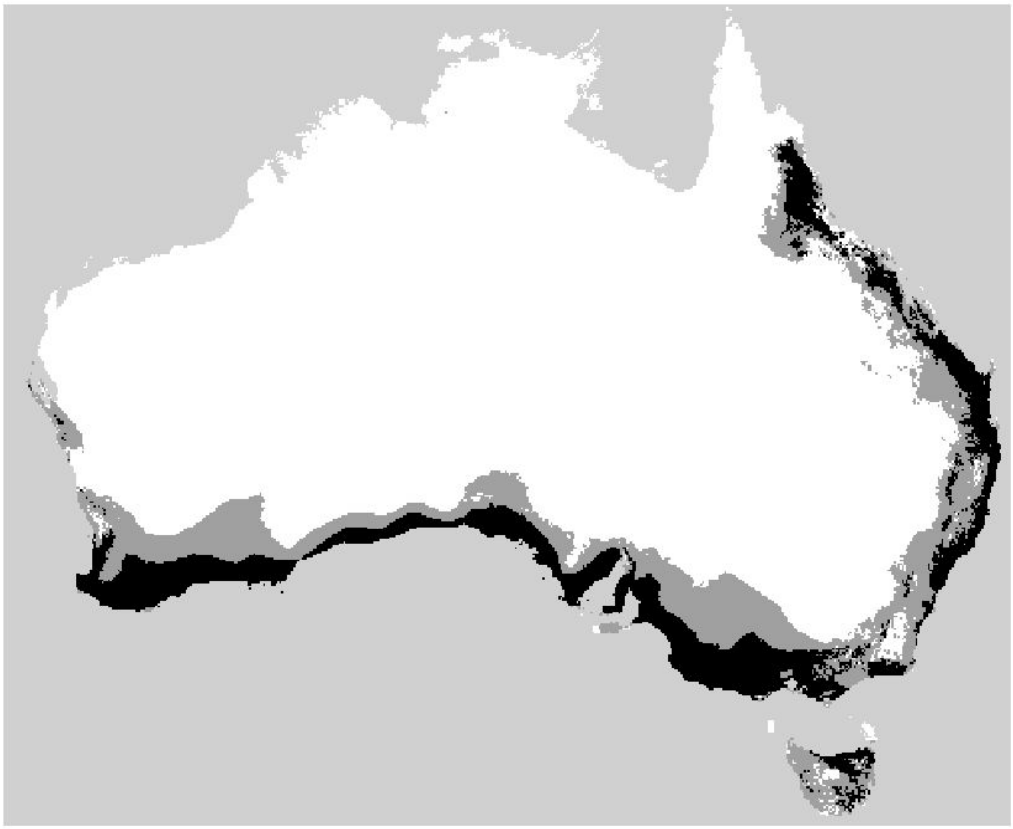

Figure 5: Predicted distribution of *B. dendrobatidis* in Australia using seven temperature parameters (Retallick 2003).

Recommendations for Scope Item 4(b)

The McIllwraith site is an important site as it is the last remaining chytrid-free upland site in coastal North Queensland. Chytridiomycosis has not been detected at this site. Ongoing monitoring is important to determine whether *B. dendrobatidis* is advancing north and, if it is, what the rate of advance is. Four actions are recommended:

1. Continue surveillance of the McIllwraith Range amphibian population;
2. Search for the northern boundary of the distribution of the amphibian chytrid fungus by doing surveys of populations of amphibians in water bodies between Big Tableland and McIllwraith Range. An initial survey should be done in the Endeavour River, roughly 30km north of O'Keefe Creek;
3. Use realtime-PCR as a diagnostic tool as sensitivity is higher than histology; and
4. Develop an action plan for McIllwraith Range if chytridiomycosis is found in this population.

Survey in Western Australia

We assisted HLA-Envirosciences Pty Ltd to conduct a survey of amphibians from the Ord River Region in Northwest Western Australia. Histology of toe tips was used as a diagnostic technique. Toes from 550 amphibians from fifteen species were examined. All were negative for chytridiomycosis. The model by Retallick using seven temperature parameters predicts that the amphibian chytrid fungus will not establish in northwest Western Australia (Retallick 2003) (Figure 5).

Scope Item 4(c) – Assessment of the Potential Role of Birds in Translocation of the Amphibian Chytrid (*Getting the Jump on Amphibian Diseases* Recommendation 2.25)

Results of this experiment on feathers and on soil (page 25) as inanimate vehicles for transport of *B. dendrobatidis* are in press:

Johnson, M. L. and Speare, R. (2005). Possible modes of dissemination of the amphibian chytrid, *Batrachochytrium dendrobatidis*, in the environment. *Diseases of Aquatic Organisms* 2005; **65:** 181-186.

In summary, using a sterile *in vitro* system we demonstrated that:

1. *B. dendrobatidis* zoospores could attach to feathers and sporangia could develop normally attached to feathers, and
2. If these feathers were dried in air, infectivity was retained for one hour for zoospores and up to three hours for zoosporangia.

Conclusions on Scope Item 4(c)

These results suggest that aquatic birds could be potential short-term vectors of *B. dendrobatidis*. A flight time of one hour and in some cases up to three hours could possibly allow *B. dendrobatidis* to be transferred successfully to a new site. However, the work was done on a sterile *in vitro* system and needs to be evaluated in a natural system.

Recommendations for Scope Item 4(c)

The role of aquatic birds as potential short term vectors of *B. dendrobatidis* should be investigated using a natural system and living avian hosts.

In 2005 funds were obtained from DEH via public tender RFT 42/2004 to continue research on this recommendation.

Scope Item 4(d) – Assessment of the Potential Role of Soil in Transmission of Chytridiomycosis (*Getting the Jump on Amphibian Diseases* Recommendation 2.25)

A tangible product that has come out of this assessment is the following publication:

Johnson, M. L. and Speare, R. (2005). Possible modes of dissemination of the amphibian chytrid, *Batrachochytrium dendrobatidis*, in the environment. *Diseases of Aquatic Organisms* 2005; **65:** 181-186.

In summary we demonstrated:

1. *B. dendrobatidis* can grow in sterile soil for many cycles;
2. Moist river sand (pH 5.8) was an suitable media for growth; and
3. Soil pH appeared to be a critical factor for this to occur with *B. dendrobatidis* failing to grow in potting mix with a pH of 4.1.

Conclusions on Scope Item 4(d)

Moist river sand may be a vehicle for movement of the amphibian chytrid fungus. However, in nature *B. dendrobatidis* may not be found in river sand. At present the natural history of *B. dendrobatidis* in the environment is unknown.

Recommendations for Scope Item 4(d)

This work needs to be done using a natural non-sterile system. The following should be done:

1. Survival of *B. dendrobatidis* in non-sterile soil should be evaluated; and
2. It should be determined where *B. dendrobatidis* grows in the environment and where zoospores and hence infectivity is found in the environment.

In 2005 funding to investigate the survival of the amphibian chytrid fungus in soil and aquatic environments was obtained from DEH via public tender RFT 42/2004.

Scope Item 4(e) – Determine the effect of chytrid infection on tadpole mouths (Retallick Recommendation 21).

A paper describing the distribution of *B. dendrobatidis* on the skin of tadpoles was published in 2004:

Marantelli, G., Berger, L., Speare, R. and Keegan, L. (2004). Changes in distribution of *Batrachochytrium dendrobatidis* and keratin during tadpole development leading to high mortality after metamorphosis. *Pacific Conservation Biology* **10(1):** 173-179.

This paper described how the amphibian chytrid fungus grew only in the keratinised tissue of the mouthparts of *Mixophyes fasciolatus* tadpoles for most of the larval development, appearing on the feet and resorbing tail just prior to metamorphosis. At this stage the jaw sheaths and denticles were shed. The study did not examine morphological changes to the teeth. Studies in USA suggested that mouthparts of tadpoles infected with *B. dendrobatidis* were damaged and that loss of depigmentation in mouthparts was a useful tool to survey amphibian populations for chytridiomycosis (Fellers *et al.* 2001). This project was designed to evaluate the usefulness of changes in tadpole mouthparts in detecting chytrid positive populations by examination of mouthparts using a magnifying glass, a non-destructive screening tool able to be applied in the field.

To determine the effect of infection with *B. dendrobatidis* we need populations of tadpoles that are chytrid-free and chytrid positive. We are examining two populations of tadpoles:

1. *L. genimaculata* tadpoles collected from O'Keefe Creek, Big Tableland, before, during and after the 1993 population crash (McDonald and Alford 1999), and
2. Tadpoles from the Northern Territory in the Darwin region. These were collected by Marion Ansters in 2003.

Criteria have been developed in collaboration with Marion Ansters, the study has commenced (Figure 6), but will not conclude until 2006.

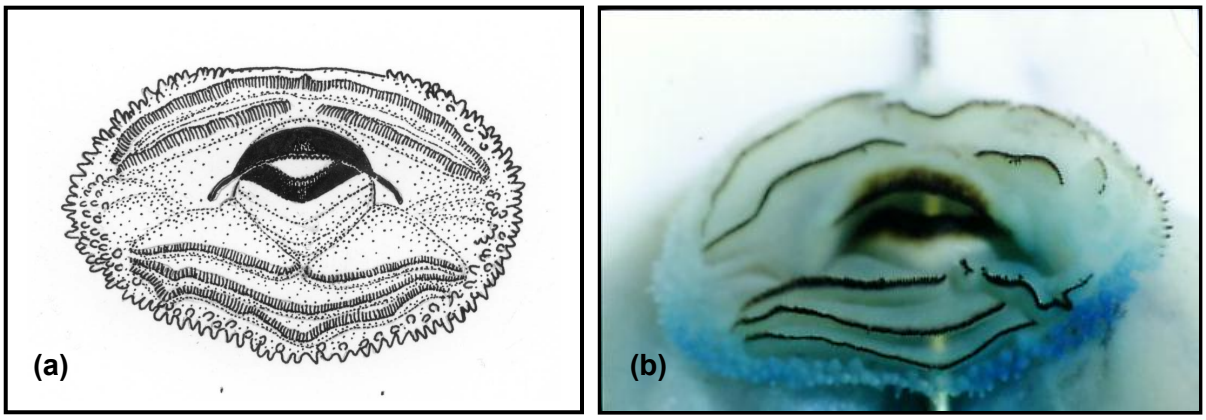

Figure 6: Oral disc of *Litoria genimaculata*; (a) diagram of normal disc; (b) damaged disc from Big Tableland, North Queensland, showing missing teeth, deformities of the tooth rows, and depigmentation of the jaws.

Scope Item 4(f) – Monitoring of Long-term Sites in the Wet Tropics to Investigate Decline in Prevalence (Retallick Recommendation 14)

Tangible products for this scope item include hardcopy and online publications:

McDonald, K. R., Méndez, D., Müller, R., Freeman, A. B. and Speare, R. (2005). Decline in the prevalence of chytridiomycosis in upland frog populations in North Queensland, AustraliA. *Pacific Conservation Biology* 2005; **11(2):** 114-120.

Retallick, R. W. R., McCallum, H. and Speare, R. (2004). Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLoS Biology* **2(11):** e351.

Abstract from McDonald et al. *(2005)*

In the early 1990s stream-associated amphibian populations in tropical upland North Queensland experienced severe declines resulting in extinction of three species, local elimination of four species, marked reductions in one species and apparently no declines in other species. Chytridiomycosis, a disease due to the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, was the likely cause of this epidemic. We conducted a monitoring study for chytridiomycosis in four species of frogs in North Queensland from October 1998 to October 2002 by collecting specimens in the field and using histology of removed digits to diagnose chytridiomycosis. Chytridiomycosis was diagnosed in 112 (7.1%) of the 1,578 specimens and prevalence was significantly associated with season and altitude, with higher prevalence-rates in winter and above 300 metres altitude. A multivariate model adjusting for potential confounding effects arising from the sampling process demonstrated a significant decline in the time trend of prevalence of chytridiomycosis. The study supports the hypothesis that *B. dendrobatidis* becomes endemic after the initial epidemic wave. Since the surviving species of stream-associated frog, *Litoria genimaculata*, has increased to pre-decline numbers, the decline in prevalence of chytridiomycosis is evidence of a changed pathogen-host relationship. The reasons for this change are speculative but could be due to an increase in innate host resistance in response to selection pressure by *B. dendrobatidis* or to lower rainfall associated with an El Nino effect. These findings justify management strategies that assist susceptible amphibian species to survive an initial epidemic wave of chytridiomycosis.

Abstract of Retallick et al. *(2004)*

The chytrid fungus *Batrachochytrium dendrobatidis* has been implicated in the decline and extinction of numerous frog species worldwide. In Queensland, Australia, it has been proposed as the cause of the decline or apparent extinction of at least 14 high-elevation rainforest frog species. One of these, *Taudactylus eungellensis*, disappeared from rainforest streams in Eungella National Park in 1985-1986, but a few remnant populations were subsequently discovered. Here, we report the analysis of *B. dendrobatidis* infections in toe tips of *T. eungellensis* and sympatric species collected in a mark-recapture study between 1994 and 1998. This longitudinal study of the fungus in individually marked frogs sheds new light on the effect of this threatening infectious process in field, as distinct from laboratory, conditions. We found a seasonal peak of infection in the cooler months, with no evidence of interannual variation. The overall prevalence of infection was 18% in *T. eungellensis* and 28% in *Litoria wilcoxii/jungguy*, a sympatric frog that appeared not to decline in 1985-1986. No infection was found in any of the other sympatric species. Most importantly, we found no consistent evidence of lower survival in *T. eungellensis* that were infected at the time of first capture, compared with uninfected individuals. These results refute the hypothesis that remnant populations of *T. eungellensis* recovered after a *B. dendrobatidis* epidemic because

the pathogen had disappeared. They show that populations of *T. eungellensis* now persist with stable, endemic infections of *B. dendrobatidis*.

Figure 7: Image of *Taudactylus eungellensis* used on the cover of the issue of PLOS Biology in which Retallick *et al.* (2004) was published.

Conclusion on Scope Item 4(f)

Both these studies show that *B. dendrobatidis* became endemic after it arrived in a population of amphibians. In the Far North Queensland population one species of frog, *L. genimaculata*, had recovered to its former level and the prevalence of chytridiomycosis has declined. In central Queensland the prevalence of chytridiomycosis is higher than in the far north and prevalence of chytridiomycosis did not decline. In this population the critical species, *Taudactylus eungellensis*, has not recovered to its former status, but remains as remnant populations in its former range. The two studies illustrate that the outcome of the interaction between amphibian host, amphibian chytrid fungus and the environment varies. These results highlight that to gain an understanding of the impact of the amphibian chytrid fungus on a particular host population in particular areas, targeted studies are required as extrapolation from studies at other sites will not be completely accurate.

Scope Item 4(g) – Implementation of PCR Testing for Chytridiomycosis at James Cook University and Provision of a Testing Service at Cost Recovery (Retallick Recommendation 7a)

The real time PCR test for *B. dendrobatidis* developed by the Australian Animal Health Laboratory (Boyle *et al.* 2004) using material extracted from swabs has a greater sensitivity than histology of toe tips in diagnosing chytridiomycosis. Over 2004 in collaboration with AAHL we implemented a technology transfer to reach the point of being able to offer the real time PCR test at James Cook University (JCU) from January 2005. Three grants were used to fund various aspects of this Scope Item; Australian Research Council Linking Infrastructure Equipment and Facilities (LIEF) Grant and DEH chytrid mapping tender for equipment, DEH chytrid mapping grant for some employment costs and this NHT grant for disposable costs and some employment costs.

The steps in achieving this were:

1. Real-time PCR equipment, including robotic system, obtained through a LEIF grant in early 2004.
2. Sarah Canyon, employed under this NHT grant, attended AAHL in Geelong to be trained in the technique – June 2004.
3. Ancillary equipment (e.g. bead beater, centrifuge, pipettes) and chemicals purchased in August 2004 using this NHT grant and DEH chytrid mapping tender funds (RFT 63/2003).
4. Research assistant, Ruth Campbell was employed by DEH chytrid mapping tender funds to implement testing at JCU and to adapt test to different equipment at JCU – November 2004.
5. DNA standards obtained from AAHL in December 2004.
6. Preliminary testing completed at JCU in December 2004.
7. Testing of samples from JCU amphibians will commence in January 2005.
8. Testing of other samples will commence in February 2005.

Scope Item 4(h) – Community Based Surveillance for Dead and Dying Wild Amphibians or Threatened Amphibians in Captive Husbandry for Disease and Causative Agents (*Getting the Jump on Amphibian Diseases* Recommendations 2.6, 2.15, 3.21, 4.3, Retallick Recommendation 1)

We have continued to assist in the investigation of disease in wild amphibians. However, our capacity to meet demand has been inadequate.

We have prioritised our investigations to:

1. declines and diseases in wild populations in rural and remote areas,
2. diseases in urban amphibians, and
3. diseases in captive amphibians.

Our primary focus has been on chytridiomycosis and its impact on wild populations, but we have investigated other diseases.

A tangible product outcome is the following publication:

Berger, L., Speare, R. and Middleton, D. (2004). A squamous cell carcinoma and an adenocarcinoma in Australian tree frogs. *Australian Veterinary Journal* **82:** 88-90.

Australian specimens: Chytridiomycosis

Chytridiomycosis was found in specimens of *Litoria caerulea* from Kuranda and Atherton on the Atherton Tableland submitted by Deborah Pergolotti of the Cairns Frog HospitaL. In other specimens from Cairns frogs we have been unable to find chytridiomycosis. Swabs to collect specimens for PCR testing have been sent to Deborah to enable a more comprehensive survey of Cairns frogs to be carried out.

Australian Specimens: Wasting syndrome in Litoria infrafrenata

No additional work was done on the wasting syndrome in *L. infrafrenata* identified by Deborah Pergolotti at the Cairns Frog Hospital (http://www.fdrproject.org/pages/disease.htm).

White Lipped Tree Frogs (*L. infrafrenata*) and Green Tree Frogs (*L. caerulea*) show extensive wasting of voluntary muscle and usually die of opportunistic bacterial or parasitic disease. In Cairns, the larval stages of the dog and cat tapeworm, *Spirametra erinacei*, which infect frogs, cause much more serious pathology than in normal frogs (Figures 8-10).

Figure 8: Wasted and lethargic White Lipped Tree Frog from Cairns (specimen submitted by Deborah Pergolotti).

Figure 9: White Lipped Tree Frog with wasting and holes in skin made by escaping larvae (pleurocercoids) of the cat and dog tapeworm, *Spirametra erinacei*.

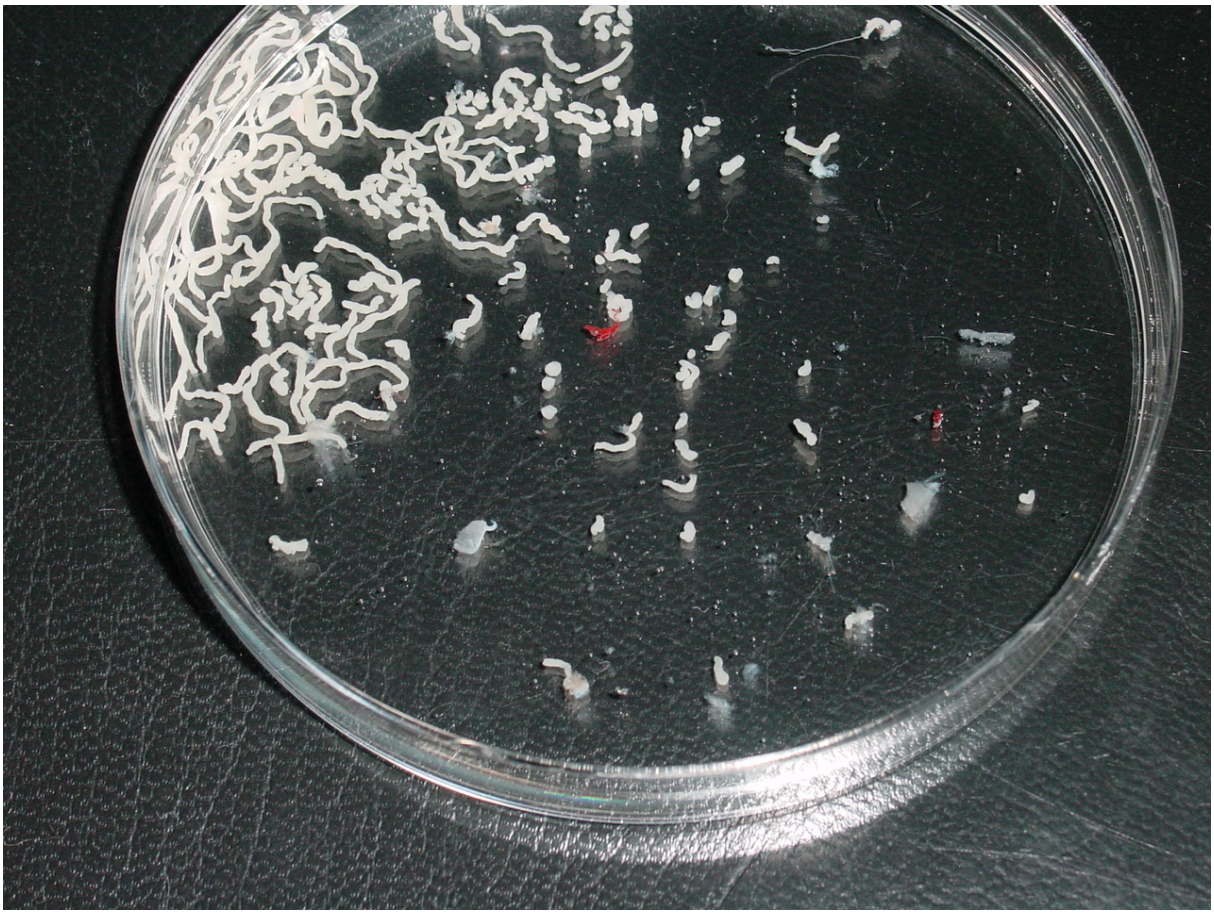

Figure 10: One hundred and seventeen pleurocercoids of *Spirametra erinacei* were found in one White Lipped Tree Frog.

In conclusion, both species of Green Tree Frogs in Cairns appear to have a disease which has hallmarks of an immunodeficiency – wasting and death by opportunistic pathogens. In a survey of dead and dying amphibians in Australia (Berger 2001) only one of 285 wild adult or juvenile amphibians had evidence of a serious opportunistic infection. Hence, the wasting disease of Green Tree Frogs in Cairns is unusuaL. Unfortunately, investigating this problem further is complex and requires time and a researcher dedicated to elucidating the aetiology. During 2004 we did not have sufficient time or resources to deal adequately with this problem.

Recommendations are:

1. A veterinarian or veterinary student should determine the aetiology and epidemiology of the wasting disease in *L. infrafrenata* and *L. caerulea* in Cairns; and
2. Additional resources should be obtained to carry out this study for at least one year.

Australian Specimens: Cancers in Amphibians

A paper on three case reports on cancers in wild amphibians was published in 2004 (Berger *et al.* 2004). This work had been funded by previous NHT grants. Cancers have also been noted in Cairns amphibians, but were not described in Berger *et al.* 2004.

Overseas Specimens: South Africa

Our collaboration with South African colleagues continued to be productively highlighted by publication of a landmark paper providing epidemiological evidence to support the hypothesis that *B. dendrobatidis* originated in Africa and was originally distributed from South Africa through the international trade in the African Clawed Frog (*Xenopus laevis*) (Weldon *et al.* 2004).

Genetic analysis of strains from North and Central America and Australia supported the hypothesis that *B. dendrobatidis* is a recently emerged clone (Morehouse *et al.* 2003). To provide additional support for this Out-of-Africa hypothesis we predicted that *B. dendrobatidis* would show a greater genetic diversity in AfricA. Our African colleagues at the University of the North West were unable to isolate and culture *B. dendrobatidis* from infected amphibians. I facilitated the participation of Joyce Longcore, the world's expert on the taxonomy and biology of *B. dendrobatidis*, to go to South Africa to isolate strains and to transfer her skills in culture of the amphibian chytrid fungus (Figure 11).

Figure 11: Collaborators discuss chytridiomycosis during the South African trip in August 2004. From left: Joyce Longcore (University of Maine, USA), Che Weldon and Louis Du Prez (University of North West, Potschefstroom, South Africa).

During a productive ten days in August we collected three species of amphibians and isolated *B. dendrobatidis* from alL. Although these initial cultures were subsequently lost, the transfer of technology was so successful that Che Weldon was able to reisolate the chytrid and establish cultures for DNA analysis. An interesting finding was that all three species of amphibians collected, *X. laevis*, *Afrana fuscigula* and *Bufo robinsoni*, were infected and that

prevalence at 80% was much greater than in AustraliA. Depigmentation of mouthparts and loss of mouthpart structures were also noted in tadpoles of *A. fuscigula* and were associated with clustering of sporangia at these sites (Figure 12). However, sporangia also occurred in areas of the mouthparts that were not depigmented.

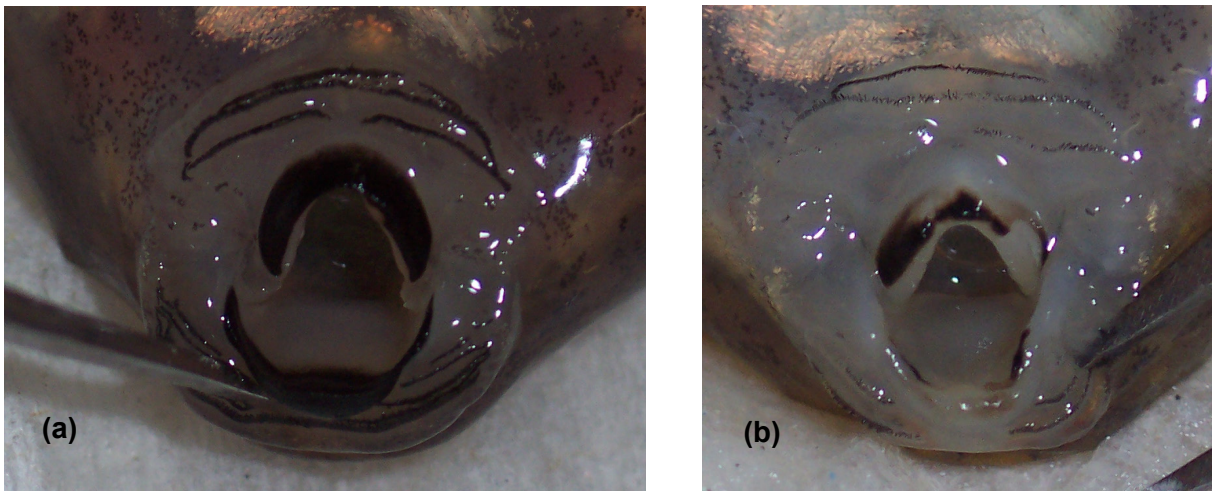

Figure 12: Mouthparts of *A. fuscigula* tadpoles collected in Namaqualand, South AfricA. Specimen (a) is normal while specimen (b) shows extensive depigmentation associated with infection with *B. dendrobatidis*.

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APPENDIX 1:
HYGIENE PROTOCOL FOR HANDLING AMPHIBIANS IN FIELD STUDIES

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General Principles and Background

1. Effective wildlife management is based on sound scientific evidence and collection of this evidence sometimes requires handling, measurement and manipulation of wild amphibians.
2. Hygiene protocols should be guided by the best available scientific evidence.
3. Hygiene protocols must be practical to carry out under field conditions.
4. Wild amphibians are naturally at risk of exposure to infectious disease via contact with the environment such as water and moist substrates and other amphibians. The number and level of pathogens encountered through these pathways represent the background risk of transmission of pathogens to amphibians.
5. The most severe diseases of wild amphibians are chytridiomycosis, caused by the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), and to a lesser extent, ranaviral disease caused by Ranaviruses. Outbreaks of chytridiomycosis have been documented in wild amphibians in eastern Australia, environs of Adelaide and southwest Western AustraliA. No outbreaks of ranaviral disease in wild amphibians have been detected in Australia although one ranavirus, the Bohle iridiovirus, occurs in the wild in Australia.
6. Once the amphibian chytrid fungus is present in a water body it appears to naturally spread throughout that water body.
7. Handling of amphibians should be done in a manner that does not significantly increase their risks of exposure to infectious disease above those normally experienced in the absence of handling. People handling amphibians should not be expected to reduce risks below the natural level for those amphibians.
8. Current data do not indicate that scientific activities have played a significant role in the transmission of chytridiomycosis or other pathogens of amphibians in the wild in Australia or any other country.

9. There is no evidence that the amphibian chytrid fungus or other pathogens of amphibians have been transmitted between water catchments by vehicles, footwear or clothing
10. As the amphibian chytrid fungus is extremely sensitive to temperatures above 29°C and will die at 32°C, *B. dendrobatidis* will not grow on human skin. Ranaviruses, the other major pathogen of amphibians, also show sensitivity to temperature, being unable to grow above 33°C.
11. Complete drying will kill the amphibian chytrid fungus, but will not kill ranaviruses.
12. The greatest risk of transmission of infectious agents is when amphibians are placed together in contact or in the same container or in containers reused for holding amphibians without disinfection between amphibians.
13. Effective disinfection strategies (See Table 1), based on scientific evidence, are available for a range of purposes to reduce risks associated with the amphibian chytrid fungus and with ranaviruses.
14. Amphibians have a range of powerful natural anti-microbial agents in their skin which may be responsible for the low incidence of infection after toe tip clipping.
15. Amphibians do not appear to show signs of stress after handling; however, unnecessary handling should be avoided.
16. The duration of handling should be as short as possible as handling procedures that are quick, even if they are potentially painful, may have less affect on stress levels than longer procedures.

Specific Actions

1. Amphibians can be handled using bare hands as long as the handler washes their hands between amphibians in water to which the animals would normally be exposed; this will ensure that the risks to frogs of exposure are not increased above environmental levels.
2. If no water is available for washing hands between amphibians, the handler should wear unused disposable gloves, or wear an unused plastic bag, or wipe their hands with a sterilising alcohol-based hand disinfectant between amphibians.
3. If amphibians are held in a container prior to return to the wild, the container should not have previously have been used for holding other amphibians, or if previously used, the container should be disinfected prior to use using methods given in Table 1.
4. Surgical instruments, such as scissors used for toe tip clipping, should be sterilised between amphibians by chemical disinfection using 70% ethanol or other chemicals listed in Table 1.
5. When toe tip clipping is used, no more than 50% of the free length of the digit should be removed.
6. Amphibians should be handled and released as quickly as possible.
7. Amphibians should be released at the site from which they were captured.
8. No more than one terrestrial individual should ever be held in the same container simultaneously.
9. Tadpoles normally share water and placing them in a common container does not increase their rates of physical contact. They can therefore be held in groups in containers, as long as all members of the group are from the same site.
10. Tadpoles for release should not be held with batches of tadpoles collected from other sites in the same or different water bodies.
11. Non-surgical equipment used in a stream or water body should be disinfected using one of the methods listed in Table 1 prior to use in any other water bodies.

12. Footwear should be washed to remove any mud and disinfected using one of the methods listed in Table 1 prior to being used in a separate water catchment or water body isolated from the initial water body.
13. As there is no evidence that vehicles play a role in dissemination of the amphibian chytrid fungus, no action is required at this time.
14. Dead amphibians or amphibians that are obviously ill should be regarded as a higher infection risk than clinically normal amphibians and should be handled with gloves or plastic bags. If a sick or freshly dead wild amphibian is found, it should be collected, preserved and submitted for disease diagnosis.

Table 1: Disinfection strategies suitable for killing *Batrachochytrium dendrobatidis* and ranaviruses in field studies. Where concentrations and time are given, these are minimum shown to be effective. For *B. dendrobatidis* based on Berger (2001) and Johnson *et al.* (2003) and for ranaviruses on Langdon (1989) and Miocevic *et al.* (1993).

Purpose	Disinfectant	Concentration	Time	Pathogen killed
Disinfecting surgical equipment and other instruments (e.g. scales)	Ethanol	70%	1 min	*B. dendrobatidis* Ranaviruses
	Vircon	1 mg/ml	1 min	*B. dendrobatidis* Ranaviruses
	Benzalkonium chloride	1 mg/ml	1 min	*B. dendrobatidis*
Disinfecting collection equipment and containers	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	0.5 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
	Heat	60°C	*5 min*	*B. dendrobatidis*
			15 min	Ranaviruses
	Heat	37°C	4 hrs	*B. dendrobatidis*
	Sterilising UV light		1 min	Ranaviruses only
Disinfecting footwear	Sodium hypochlorite (bleach)	1%	1 min	*B. dendrobatidis*
	Sodium hypochlorite (bleach)	4%	15 min	Ranaviruses
	Didecyl dimethyl ammonium chloride	1 in 1000 dilution	1 min	*B. dendrobatidis*
	Complete drying		3 hrs or greater	*B. dendrobatidis*
Disinfecting cloth (e.g. bags, clothes)	Hot wash	60°C or greater	5 min	*B. dendrobatidis*
			15 min	Ranaviruses

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APPENDIX 2:
CHYTRIDIOMYCOSIS RECORDS FOR SPECIES OF AMPHIBIANS IN AUSTRALIA BY SPECIES OF AMPHIBIAN AND STATE

Australian frog species and chytridiomycosis infection status of wild populations by state and nationally. For a particular species in a particular state, if one case of chytridiomycosis has been found, the species in that state is listed as infected (Inf). Occurrence of frog in state = Y. (For completeness, species which have been infected only in captivity (C-Inf) are listed, but not counted). Shaded cells = species does not occur.

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
Hylidae																		
Cyclorana	*alboguttatata*			Y		Y		Y										
	Australis					Y		Y								Y		
	Brevipes			Y				Y										
	Cryptotis					Y										Y		
	Cultripes			Y		Y		Y		Y						Y		
	longipes					Y										Y		
	maculosa					Y		Y										
	maini					Y		Y		Y						Y		
	manya							Y										
	novaehollandiae			Y				Y										
	platycephala			Y		Y		Y		Y						Y		
	vagita					Y										Y		
	verrucosus			Y				Y										
	adelaidensis															Y	Inf	Inf
Litoria	*andiirrrmalin*							Y										
	aurea[T]	Y		Y	Inf									Y				Inf
	*barringtonensis**			Y	Inf			Y	Inf									Inf
	bicolor					Y		Y								Y		
	booroolongensis			Y	Inf													Inf
	brevipalmata			Y				Y										
	burrowsae											Y						C-Inf

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
Litoria	*caerulea*	Y		Y	Inf	Y		Y	Inf	Y						Y		Inf
	cavernicola															Y		
	castanea^{T}	Y		Y														
	chloris			Y	Inf			Y	Inf									Inf
	citropa			Y	Inf													Inf
	cooloolensis							Y										
	coplandi					Y		Y								Y		
	cyclorhynchus															Y		
	dahlii					Y		Y								Y		
	daviesae			Y										Y				
	dentata			Y				Y										
	electrica							Y										
	eucnemis							Y										
	ewingii			Y						Y	Inf	Y		Y	Inf			Inf
	fallax			Y				Y										
	freycineti			Y				Y										
	genimaculata							Y	Inf									Inf
	gilleni					Y												
	gracilenta			Y				Y	Inf									Inf
	inermis					Y		Y								Y		
	infrafrenata							Y	Inf									Inf
	jervisiensis			Y														
	latopalmata	Y		Y				Y		Y								
	lesueuri	Y		Y	Inf			Y	Inf					Y	Inf			Inf
	littlejohni^{T}			Y										Y				
	longirostris							Y										
	lorica^{T}							Y										
	meiriana					Y										Y		
	microbelos					Y		Y								Y		
	moorei															Y	Inf	Inf

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
Litoria	*nannotis*^{T}							Y	Inf									Inf
	nasuta			Y		Y		Y	Inf							Y		Inf
	nigrofrenata							Y										
	nudidigitus			Y										Y				
	nyakalensis^{T}							Y										
	olongburensis^{T}			Y				Y										
	pallida					Y		Y								Y		
	paraewingi			Y										Y				
	pearsoniana			Y	Inf			Y	Inf									Inf
	peronii	Y		Y	Inf			Y		Y				Y				Inf
	personata					Y												
	phyllochroa	Y		Y	Inf													Inf
	piperata^{T}			Y														
	raniformis^{T}	Y		Y						Y	Inf	Y		Y				Inf
	revelata			Y				Y										
	rheocola^{T}							Y	Inf									Inf
	rothi					Y		Y								Y		
	rubella			Y		Y		Y		Y						Y		
	spenceri^{T}			Y										Y	Inf			Inf
	splendida					Y										Y		
	subglundulosa			Y				Y										
	tornieri					Y										Y		
	tyleri			Y				Y										C-Inf
	verreauxii	Y		Y	Inf			Y						Y				Inf
	v. alpina^{T}			Y										Y				
	wotjulumensis					Y		Y								Y		
	xanthomera							Y										
Nyctimystes	*dayi*							Y	Inf									Inf

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
Microhylidae																		
Austrochaperina	*adelphe*					Y												
Austrochaperina	*fryi*							Y										
	gracilipes							Y										
	pluvialis							Y										
	robusta							Y										
Cophixalus	*bombiens*							Y										
	concinnus							Y										
	crepitans							Y										
	exiguus							Y										
	hosmeri							Y										
	infacetus							Y										
	mcdonaldi							Y										
	monticola							Y										
	neglectus							Y										
	ornatus							Y										
	peninsularis							Y										
	saxatilis							Y										
	zweifeli							Y										
Myobactrachidae																		
Adelotus	*brevis*			Y	Inf			Y	Inf									Inf
Arenophryne	*rotunda*															Y		
Assa	*darlingtoni*			Y				Y								Y		
Crinia	*bilingua*					Y										Y		
	deserticola			Y		Y		Y		Y								
	georgiana															Y	Inf	Inf
Crinia	*glauerti*															Y	Inf	Inf
	insignifera															Y	Inf	Inf
	nimbus											Y						

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
	parinsignifera	Y		Y				Y		Y				Y				
	pseudinsignifera															Y	Inf	Inf
	remota					Y		Y										
	ripara									Y								
	signifera	Y		Y				Y		Y		Y		Y				
	sloanei			Y										Y				
	subinsignifera															Y		
	tasmaniensis											Y						
	tinnula			Y				Y										
Geocrinia	*alba*[T]															Y		
	laevis									Y		Y		Y				
	leai															Y		
Geocrinia	*lutea*															Y		
	rosea															Y	Inf	Inf
	victoriana			Y										Y				
	vitellina[T]															Y	Inf	Inf
Heleioporus	*albopunctatus*															Y		
	australiacus[T]			Y	Inf									Y				Inf
	barycragus															Y	Inf	Inf
	eyrei															Y	Inf	Inf
Heleioporus	*inornatus*															Y		
	psammophilus															Y		
Kyarranus	*kudagungan*			Y				Y										
	loveridgei			Y				Y										
	sphagnicolis			Y				Y										
Lechriodus	*fletcheri*			Y				Y	Inf									Inf
Limnodynastes	*convexiusculus*					Y		Y								Y		
	depressus					Y										Y		
	dorsalis															Y	Inf	Inf
	dumerili	Y		Y				Y	Inf	Y	Inf	Y						Inf

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
	fletcheri			Y				Y		Y				Y				
	interioris			Y										Y				
	lignarius					Y										Y		
Limnodynastes	*ornatus*			Y		Y		Y								Y		
	peronii	Y?		Y				Y		Y		Y		Y				
	salmini			Y				Y										
	spenceri					Y		Y		Y						Y		
	tasmaniensis	Y		Y				Y		Y	Inf	Y		Y				Inf
	terraereginae			Y	Inf			Y										Inf
Metacrinia	*nichollsi*															Y		
Mixophyes	*balbus*^{T}			Y	Inf									Y				Inf
	fasciolatus			Y	Inf			Y	Inf									Inf
	fleayi^{T}			Y	Inf			Y	Inf									Inf
Mixophyes	*iteratus*^{T}			Y	Inf			Y										Inf
	schevilli							Y										
Myobatrachus	*gouldii*															Y		
Neobatrachus	*albipes*															Y		
Neobatrachus	*aquilonius*					Y		Y								Y		
	centralis					Y		Y								Y		
	fulvus															Y		
	kunapalari															Y		C-Inf
	pelobatoides															Y	Inf	Inf
	pictus			Y						Y				Y				
	sudelli	Y		Y				Y		Y				Y				
	sutor									Y						Y		
	wilsmorei					Y				Y						Y		
Notaden	*bennettii*			Y				Y										
	melanoscaphus					Y		Y								Y		
	nichollsi					Y		Y								Y		
	weigeli															Y		

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
Paracrinia	*haswelli*			Y										Y				
Philoria	*frosti*[T]													Y				
	kundagungan			Y				Y										
Philoria	*loveridgei*			Y				Y										
	sphagnicola			Y														
Pseudophryne	*australis*			Y														
Pseudophryne	*bibronii*	Y		Y						Y				Y				
	coriacea			Y				Y										
	corroboree[T]			Y	Inf													Inf
	covacevichae[T]							Y										
	dendyi	Y		Y										Y				
	douglasi															Y		
	guentheri															Y		
	major							Y										
	occidentalis									Y						Y		
	pengilleyi[T]	Y	Inf	Y	Inf													Inf
	raveni							Y										
	semimarmorata									Y		Y		Y				
Rheobatrachus	*silus*[E]							Y										
	vitellinus[E]							Y										
Spicospina	*flammocaerulea*[T]															Y		
Taudactylus	*acutirostris*[E]							Y	Inf									Inf
	diurnus[E]							Y										
	eungellensis[T]							Y	Inf									Inf
	liemi							Y										
	pleioni[T]							Y										
	rheophilus[T]							Y										Inf
Uperoleia	*altissma*							Y										
	arenicola					Y												
Uperoleia	*aspera*															Y		

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
	borealis					Y										Y		
	capitulata			Y				Y		Y								
	crassa															Y		
Uperoleia	*fusca*			Y				Y										
	glandulosa															Y		
	inundata					Y		Y										
	laevigata			Y				Y	Inf					Y				Inf
	lithomoda					Y		Y								Y		
	littlejohni							Y										
	marmorata															Y		
	martini			Y										Y				
	micromeles					Y										Y		
	mimula							Y										
	minima															Y		
	mjobergi															Y		
	orientalis					Y												
	rugosa			Y				Y										
	russelli															Y		
	talpa															Y		
	trachyderma					Y		Y								Y		
	tyleri			Y										Y				
Ranidae																		
Rana	*daemeli*					Y		Y										

		ACT		NSW		NT		Qld		SA		Tas		Vic		WA		Aust
		Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Occur	Infect	Infect
Bufonidae																		
Bufo	*marinus*			Y		Y		Y	Inf									Inf
State	**Number (%)**	**18**	**1 5.6%**	**84**	**16 19.0%**	**47**	**0**	**123**	**21 17.1%**	**27**	**4 14.8%**	**10 10.0%**	**1**	**33**	**2 6.1%**	**77**	**12 15.6%**	

* *Litoria barringtonensis* has not been formally recognized. The Qld population at Kroombit is genetically different from L. *pearsoniana* and sometimes is given the title *barringtonensis*. The difficulty is determining if *barringtonensis* is a prior name for *L. pearsonianA.* (Keith McDonald pers com 2003).
T = Threatened species (endangered or vulnerable) (See Table C.1); E = Extinct species (See Table C.2).

APPENDIX 3:
PUBLIC LECTURES ON CHYTRIDIOMYCOSIS (SCOPE ITEM 2)

The following documents can be downloaded in PDF-format from the Amphibian Diseases Home Page at http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm

Speare, R. (2004). Chytridiomycosis, a formidable infectious disease of amphibians. *Lecture for the Tablelands Frog Club*, Atherton, 12 March 2004.

Longcore, J. and Speare, R. (2004). Chytrids and chytridiomycosis. *Lecture at the School of Environmental Sciences and Development*, North West University, Potschrefstroom, South Africa, 24 August 2004.

Speare, R. (2004). Putting diseases in wild amphibians into perspective. *Lecture at the School of Tropical Biology, Department of Zoology, James Cook University*, Townsville, 16 December 2004.

APPENDIX 4:
SCIENTIFIC PUBLICATIONS AND CONFERENCE PRESENTATIONS

The following documents can be downloaded in PDF-format from the Amphibian Diseases Home Page at http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm

SCIENTIFIC PUBLICATIONS

Berger, L., Speare, R., Hines, H. B., Marantelli, G., Hyatt, A. D., McDonald, K. R., Skerratt, L. F., Olsen, V., Clarke, J. M., Gillespie, G., Mahony, M., Sheppard, N., Williams, C. and Tyler, M. J. (2004). Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal* **82(7):** 31-36.

Berger, L., Speare, R. and Middleton, D. (2004). A squamous cell carcinoma and an adenocarcinoma in Australian tree frogs. *Australian Veterinary Journal* **82:** 88-90.

Bonaccorso, E., Guayasamin, J. M., Méndez, D. and Speare, R. (2003). Chytridiomycosis in a Venezuelan amphibian (Bufonidae: *Atelopus cruciger*). *Herpetological Review* **34(4):** 331-334.

Johnson, M. L. and Speare, R. (2005). Possible modes of dissemination of the amphibian chytrid, *Batrachochytrium dendrobatidis*, in the environment. *Diseases of Aquatic Organisms* ***65:*** *181-186*.

Marantelli, G., Berger, L., Speare, R. and Keegan, L. (2004). Distribution of the amphibian chytrid *Batrachochytrium dendrobatidis* and keratin during tadpole development. *Pacific Conservation Biology* **10(1):** 173-179.

McDonald, K. R., Méndez, D., Müller, R., Freeman, A. B. and Speare, R. (2005). Decline in the prevalence of chytridiomycosis in upland frog populations in North Queensland, AustraliA. *Pacific Conservation Biology* **11(2):** 114-120.

Olsen, V., Hyatt, A. D., Boyle, D. G. and Méndez, D. (2004). Co-localisation of *Batrachochytrium dendrobatidis* and keratin for enhanced diagnosis of chytridiomycosis in frogs. *Diseases of Aquatic Organisms* **61:** 85-88.

Retallick, R. W. R., McCallum, H. and Speare, R. (2004) Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLoS Biology* **2(11):** e351. (online at http://www.plos.org/).

Weldon, C., Du Preez, L., Hyatt, A., Müller, R. and Speare, R. (2004). Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* **10(12):** 2100-2105.

CONFERENCE PRESENTATIONS

McDonald, K., Méndez, D., Müller, R., Freeman, A. and Speare, R. (2004). Decline in the prevalence of chytridiomycosis in frog populations in far North Queensland. *Integrated Research Challenges in Evolutionary Biology Meeting*, Arizona State University, Phoenix, USA, 11 November 2004.

Speare, R. (2004). Field Hygiene Protocols: Some early evidence. *Integrated Research Challenges in Evolutionary Biology Meeting*, Arizona State University, Phoenix, USA, 12 November 2004.

Speare, R. (2004). Threat Abatement Plan for infection of amphibians with chytrid fungus resulting in chytridiomycosis. *Hygiene Conference / Workshop, Amphibian Research Centre*, Werribee, Victoria, 13 December 2004.

Speare, R. and Berger, L. (2004). Threat Abatement Plan for infection of amphibians with chytrid fungus resulting in chytridiomycosis. *Australian Veterinary Association National Conference*, Australasian Association of Veterinary Conservation Biologists, Canberra, ACT, 4 May 2004.

Speare, R., Berger, L., Skerratt, L., Alford, R., Méndez, D., Cashins, S., Kenyon, N., Hauselberger, K. and Rowley, J. (2004). Hygiene protocols for handling amphibians in field studies. *Hygiene Conference / Workshop*, Amphibian Research Centre, Werribee, Victoria, 13 December 2004.

Speare, R., Méndez, D. and Berger, L. (2004). Hygiene protocols to prevent amphibian disease: from theory to evidence. *Hygiene Conference / Workshop*, Amphibian Research Centre, Werribee, Victoria, 13 December 2004.

APPENDIX 5:
NATIONAL THREAT ABATEMENT PLAN FOR INFECTION OF AMPHIBIANS WITH THE AMPHIBIAN CHYTRID FUNGUS RESULTING IN CHYTRIDIOMYCOSIS

This document can be downloaded in PDF-format from the Amphibian Diseases Home Page at http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm