BIOLOGICAL CONTROL OF CANE TOADS

February 26-27th, 2004
Brisbane

*Bufo marinus* (Photo Dr Ross Alford)

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(A) Example of the massive number of eggs (arrow) laid by one female cane toad (Photo CSIRO). (B) A cane toad exuding venom (arrows) from the parotoid glands (Photo Dr Ross Alford)
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Executive Summary

On 26-27 February 2004 a workshop on “Biological Control of Cane Toads” was held in Brisbane, sponsored by the Australian Government Department of Environment and Heritage under the National Threat Abatement Component of the Natural Heritage Trust.

The purpose of the workshop was to provide information to the Australian scientific community and public interest groups about the current CSIRO biocontrol project and to discuss key issues relating to the impact and control of cane toads (Bufo marinus). The thirty-five attendees represented a wide range of expertise and interests and included scientists, policy-makers, conservationists and land managers. Participants reached agreement on a series of options to advance research into the control of cane toads.

An important outcome of the workshop was the agreement that there is a need to control cane toads through a combination of short-term, local, methods as well as long-term, nation-wide methods. Several short-term and long-term options are outlined in this report with the latter generating most discussion among participants. For long-term control, issues relating to disseminating infectious agents versus non-disseminating agents require further discussion and agreement.

The general recommendations arising from the workshop were:

- Establish a national cane toad group to coordinate research.
- Collate and document all current knowledge on the short and long-term impacts of cane toads.
- Identify gaps in impact knowledge and support further research.
- Identify and implement short-term control and damage mitigation measures.
- Identify research gaps in short and long-term control methods.
- Provide support for research into short and long-term control measures.

It was also agreed that (a) a risk assessment of short-term and long-term impacts is necessary as further control strategies will be difficult to justify without such an assessment and (b) a major research effort is required if Australia is to reduce significantly the numbers of cane toads. This type of research needs to be multi-faceted and coordinated by a national body.

More details on these recommendations are listed on the following page.
Recommendations

1. Establish a national cane toad group to coordinate research.

*Future research projects should be multi-disciplinary and coordinated by a national body.*

Effective research should be multi-disciplinary (e.g. including the disciplines of ecology, modelling, pathology, microbiology) and integrated so that data from all areas facilitates the effective delivery of a control strategy. As such, large-scale studies should be administered via a National Cane Toad group.

2. Collate and document all current knowledge on the short and long-term impacts of cane toads.

*Identify short-term and long-term impacts and conduct a risk assessment.*

The short-term (approximately five years) impacts of cane toads on some animals, such as quolls, are significant. The long-term (greater than 10 years) impacts of cane toads on any species or ecosystem are not known. This limited knowledge is attributed to the lack of research.

Although there are data gaps, it is critical that an initial risk assessment be undertaken. Based on the outcome of this assessment the Australian Government may proceed with listing *B. marinus* as a ‘Key Threatening Process’ and developing and implementing a ‘Threat Abatement Plan’. At the least, this initial assessment will identify what data are needed before a full risk assessment can be performed. Because any control measure that may ultimately be developed will have costs as well as benefits, it will be necessary to understand the impacts of cane toads so that the tradeoff between these costs and benefits can be understood, and rational decisions reached regarding the application of control measures. Future research into the control of cane toads should only continue if it is demonstrated that cane toads have a significant impact on biodiversity and/or aspects of the social-community-cultural structure and/or economics.

3. Identify and implement short-term control and damage mitigation measures.

*(a) Support both short and long-term impact studies.*

The incursion of cane toads into unoccupied areas is known to have short-term impacts and is suspected to have long-term impacts. Any funded control strategies must address both of these areas.

*(b) Support short-term strategies.*

If cane toad populations are to be significantly reduced at specific geographical locations (and before the development of trans-continental biological control options) then a range of short-term control strategies must be developed. Some short-term control strategies might be feasible to limit the short-term impacts of toads. Such strategies (listed in Recommendation 6)
5) could be used to control toad numbers in particularly sensitive areas, such as World Heritage areas, national parks, and urban areas.

(c) Short-term control strategies must be uniform across the country.

All states and territories need to agree on methods for collecting and dispatching toads. Traps or collection protocols and euthanasia must be humane. A suggested euthanasia protocol is (a) collect toads into plastic bags, (b) cool animals to 4°C and then freeze in a -20°C freezer (conventional freezer). Animals can then be buried or incinerated.

(d) Conduct cost-benefit analysis for both short-term and long-term control strategies.

The impact of toads needs to be determined and quantified so that a cost-benefit analysis of potential control options can be assessed. Clearly, if an eradication program requires significant funding compared to the impact of the toad then other strategies should be considered. One of the challenges for the implementation of this important recommendation is to identify how the cost of cane toads can be measured.

4. Identify research gaps in short and long-term control methods.

It is envisaged that a role for the National Cane Toad Group would be to identify research gaps. This would need to be done in consultation with scientists, natural resource managers and other interested parties.

5. Adopt the following strategies for addressing the short and long-term impacts of cane toads.

<table>
<thead>
<tr>
<th>SHORT-TERM STRATEGIES</th>
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<tbody>
<tr>
<td><strong>Ecological, social and economic</strong></td>
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<tr>
<td>a) Implement cane toad impact studies incorporating biodiversity, social and economic aspects of the impact of cane toads.</td>
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<thead>
<tr>
<th>Biological</th>
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<tbody>
<tr>
<td>a) Devise and assess effectiveness of traps or other methods to eradicate toads from specific geographical areas.</td>
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<tr>
<td>b) Identify and assess attractants, such as pheromones, that would draw toads to traps.</td>
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<tr>
<td>c) Research the feasibility of physical barriers, where locally appropriate, and assess effectiveness following construction.</td>
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<tr>
<td>d) Relocate valued at-risk species to cane toad-free areas, where feasible</td>
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<tr>
<th>LONG-TERM STRATEGIES</th>
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<tbody>
<tr>
<td><strong>Ecological, social and economic</strong></td>
</tr>
<tr>
<td>a) Implement cane toad impact studies incorporating biodiversity, social and economic aspects of their ecological impact over several decades.</td>
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<tr>
<th>Biological</th>
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<tr>
<td>a) Research biological control via recombinant viruses targeting toad-specific ‘vital’ proteins</td>
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<td>(i). continue the exploration of the use of genetically modified organisms (GMOs) to determine if ‘concept’ is valid</td>
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<tr>
<td>(ii). encompass the use of non-disseminating agents in research</td>
</tr>
<tr>
<td>(iii). explore the use of other gene targets.</td>
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</table>
b) Explore the concept of **sterile males** to reduce the number of fertile males.

c) Explore the concept of “**daughterless**” **technology for toads**, (restricting all offspring to males).

d) Resume the search for **cane toad-specific pathogens**, similar to rabbit myxoma virus and RHDV (known as rabbit calicivirus). The search should also include other infectious agents of toads (for other potential vectors for GMOs).

6. **Fund a major long-term coordinated program to address the above recommendations, prioritising short and long-term strategies from the above list.**
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1.0 Background to current CSIRO research and the Workshop

Since their introduction in 1935, cane toads (*Bufo marinus*) have spread rapidly across northern Australia. Recent estimates (CSIRO, 2002) indicate the toads are spreading at around 27 kilometres each year (east to west, the spread from north to south is slower) and now extend from northern New South Wales to the Northern Territory. The toads threaten many World Heritage areas including Kakadu National Park.

The general public, ecologists and natural resource managers perceive cane toads as a significant ecological and social pest. In response to these concerns, Australian researchers embarked on a $1.25 million project from 1990 to 1993 to investigate the ecology of cane toads in Venezuela, Brazil and Australia. The project also included protocols for the isolation of cane toad-specific pathogens from toads in Venezuela. The work succeeded in isolating a number of viruses from the virus family *Iridoviridae*, genus *Ranavirus*. From 1993 to 1997, a $2 million Cane Toad Study completed the ecological work in Venezuela and continued a more intensive investigation of the potential of the isolated viruses as biological control agents. The research found that the Venezuelan viruses killed from 80 to 100 per cent of cane toads and modelling predicted this kill rate would be sufficient to control toad numbers in Australia. However, subsequent work at CSIRO’s Australian Animal Health Laboratory demonstrated the viruses were not cane-toad specific.

In early 2000, Senator Robert Hill, the then Minister for the Environment and Heritage, supported ongoing funding of research into possible biological controls for cane toads ($1 million over two years). This latest research has involved the development of a modified recombinant ranavirus for the potential control of Australian cane toads. This work is on-going and if successful will require more years of research. The plan is to design a recombinant virus that will have the following characteristics (a) it will be an infective recombinant virus, (b) the wild type virus will be endemic to Australia, (c) the virus will be non-pathogenic to all Australian species, (d) the virus will carry a cane toad specific gene that will be lethal to cane toads only and (e) the virus will be self-disseminating. The initial phases of this research have been completed (production of a recombinant virus, attenuation of the virus and establishment of a technology where cane toad (larval and adult) active genes can be identified).

The current CSIRO research was reviewed externally to provide both the Australian Government Department of Environment and Heritage (DEH) and CSIRO with a plan for future research directions.

The recommendations from this review were:

1. The project should proceed for three years subject to the considerations raised in the review.
2. DEH should investigate and execute complimentary research activities outlined in the review.
3. DEH should establish processes to consult with relevant stakeholders on the project’s progress, cane toad control issues, complementary research, communication and research coordination.

The review emphasised the importance of informing the broader scientific community of the progress of the research through forums such as conferences, workshops and scientific literature. The review also noted that the project is clearly of interest to a diverse range of scientists and practitioners who could assist in the project if they are kept informed of its progress. This led to the establishment of the “Biological Control of Cane Toads” Workshop as a means of informing the main stakeholders of progress with the research and to allow them input into future directions.
2.0  Workshop objectives

The specific agreed objectives of the workshop were to:

(1) Explore current and proposed approaches to cane toad control and identify research gaps.
(2) Inform the scientific community of the current CSIRO research.
(3) Scope issues associated with current CSIRO cane toad research. The issues should be identified via the forum but should include quarantine, virus specificity, biology of the virus and feasibility as a long-term control strategy.
(4) Explore areas for national integration and collaboration.
(5) Submit recommendations for the effective control of cane toads in Australia.

3.0  Program

Thursday, 26 February, 2004

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>10.00 am</td>
<td>Registration and Morning Tea</td>
</tr>
<tr>
<td>10.30 am</td>
<td>Welcome and background to the workshop</td>
</tr>
<tr>
<td>10.45 am</td>
<td>Overview of the workshop and introductions</td>
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<tr>
<td>11.30 am</td>
<td>The cane toad risk</td>
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<tr>
<td>12.15 pm</td>
<td>Lunch</td>
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<tr>
<td>1.00 pm</td>
<td>Cane toad control options - is there a need to get cane toads on the Key Threatening Processes agenda?</td>
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<tr>
<td>2.30 pm</td>
<td>Break</td>
</tr>
<tr>
<td>3.00 pm</td>
<td>Research options</td>
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<tr>
<td>5.00 pm</td>
<td>Close</td>
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Friday, 27 February, 2004

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<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>8.30 am</td>
<td>Review</td>
</tr>
<tr>
<td>9.15 am</td>
<td>Overview of current CSIRO research</td>
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<tr>
<td>10.30 am</td>
<td>Break</td>
</tr>
<tr>
<td>11.00 am</td>
<td>Discussion and identification of key issues</td>
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<tr>
<td>12.15 pm</td>
<td>Lunch</td>
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<tr>
<td>1.00 pm</td>
<td>Future directions and actions</td>
</tr>
<tr>
<td>3.00 pm</td>
<td>Summary and close</td>
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<tr>
<td>3.30 pm</td>
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4.0 The cane toad risk

The following sections were edited from presentations given at the workshop.

(i) Professor Rick Shine, University of Sydney

Remarkably little is known about the nature, magnitude and duration of the ecological impacts exerted by cane toads. A general consensus exists that toads cause population declines in some, but not all, native fauna via predation, competition, disease and their extreme toxicity to predators. However, the details of the impacts are obscure and the initial impact may be reduced through time if toad numbers decline after invasion, thereby allowing local systems to recover. A better understanding of cane toad impacts might suggest new strategies to reduce the intensity of these impacts.

Research at the University of Sydney has investigated several facets of toad impact. Physiological tolerance of various reptilian predators and toad toxins has been quantified and the accumulation of anti-toad adaptations in predator populations after long-term exposure has been explored. A proposal to list cane toads as a ‘Key Threatening Process’ has been written. Funding has been obtained from the Australian Research Council to examine the ecological impact of toads on snakes at Fogg Dam near Darwin, the site of a 20-year mark-recapture and radiotelemetry study. Most recently, DEH has provided funding to assess the feasibility of controlling cane toads by exploiting aggregative responses in tadpoles and metamorphs.

Overall, it is clear that although there is agreement among most researchers that the ecological impact of toads need to be better understood, progress in gaining that understanding has been frustratingly slow. The logistical impediments are severe, but the need for information is critical.

(ii) Professor Gordon Grigg and Associate Professor Hamish McCallum, University of Queensland, Dr Andrew Taylor, University of New South Wales

Lack of reliable data about the impact of cane toads on native fauna prompted the University of Queensland to develop a novel automated method which can monitor the calling activity of native frogs every night for a year or more without constant attention to the monitoring equipment. The software relies on machine learning technology and identifies frogs to species by their calls, logging this information, along with temperature and rainfall, to a flash card. Ten solar-powered and tamper-proof systems were installed along 100 kilometres of the Roper Valley Highway east of Mataranka, Northern Territory, in 1997-98. These sites were overrun by cane toads by early 2000. At every site the number of frog species recorded decreased markedly over the five-year period. There have been significant and substantial declines in the number of days of records for at least seven of the 21 monitored species, with possible increases in two species. Although these data suggest that toads may have a detrimental effect on frogs, these declines cannot yet be unequivocally attributed to the impact of toads. Data collection from these sites is continuing and may resolve the cause and extent of the declines. Data is also being collected from six additional sites within Kakadu National Park, just now being invaded by toads.

(iii) Dr Rob Taylor, Northern Territory Department of Infrastructure, Planning and Environment

The level of current knowledge of the impacts of toads on fauna is poor. Some species are going to be affected but uncertainties remain about many species. Contrary to events in Queensland and New South Wales, in the Northern Territory studies have been set up to compare populations of species
in areas before and after the colonisation of cane toads. Snakes, goannas, quolls and frogs are some examples of the groups being studied. The impacts have been well studied in Kakadu National Park. This work has shown that goanna populations have declined. Quoll populations have become locally extinct within 12 months of the arrival of cane toads. From anecdotal information derived from the Queensland experience it seems that even after 20 years quoll populations have not recovered.

In the Northern Territory other categories of impact – lifestyle and native food resources for Aboriginal people- are often talked about. A significant number of indigenous communities in the Northern Territory depend on 'bush tucker'. Cane toads have affected these indigenous communities by reducing their bush food supplies.

In the Northern Territory cane toads were recorded in Katherine with little political outcry, and are expected to arrive in Darwin within the next two years. Following a Northern Territory Government inquiry, the Government supported a national coordination effort and national task force on cane toad control and are willing to support this through the Natural Resource Management Ministerial Council. Other suggestions to find solutions for control included a national conference and erection of a cane toad proof fence, although the effectiveness of such a fence is uncertain.

The Northern Territory Government receives letters from all over Australia expressing views on cane toad control and on top of the list is implementing a bounty system. However work in the Northern Territory and in Queensland has shown that bounties achieve very little.

Island Ark, is a cane toad response program funded by the Northern Territory Government and has four components:

(1) **Public education.** The Department of Infrastructure, Planning and Environment has produced posters and brochures and education kits for schools. The brochures include information on how to identify cane toads, how to kill them humanely and how to keep them out of your backyard.

(2) **Biosecurity of islands.** Cane toads are likely to reach some islands naturally, such as the Pellew Island in the Gulf. Cane toads reached those island two years ago when there was massive flooding in the river system adjacent to the islands. However, there are some islands that cane toads will not be able to reach naturally. For these islands biosecurity procedures are being implemented that include minimising the chance of human transportation of toads via boats, barges and airplanes. In these island situations traps may have a role in luring and detecting early colonisers.

(3) **Development of aboriginal land management capacity.** Aboriginal people own nearly all Northern Territory islands. If these areas are to remain cane toad-free we will need the support of the owners and they will need the capacity to manage their islands and the cane toad threat.

(4) **Translocation of species threatened by cane toads.** To date quolls have been translocated onto two islands because of the strong evidence that quolls are likely to undergo a massive decline in areas where cane toads colonise.
4.1 Discussion on presentations

While there was no clear indication of the extent of the cane toad risk, participants agreed that cane toads have a significant impact on the environment and recognized the need for further research. It was agreed that cane toads had a significant short-term impact but the extent of the long-term impact still needs to be quantified.

Whilst the long-term impact still needs to be quantified, a risk assessment of the impact should be attempted. To do this, the risk should be assessed in reference to the (a) impact on Australia’s biodiversity, (b) social impact (e.g. Aboriginal culture) and (c) economics (e.g. loss of ecotourism).

To understand more clearly the risk of cane toads, participants recommended the following strategies:

a) Determine existing evidence of impact and continue to collect data.

b) Distinguish between perceived impact and measured impact.

c) Gain an understanding of cane toad biology and behaviour.

d) Identify the consequences of immediate impact and long-term impact.

e) Be aware of the community’s expectations regarding cane toad control.

f) Recognise that cane toad control is both a scientific and political question.
5.0 Control Options
The following sections were edited from presentations given at the workshop.

5.1 Physical Controls

5.1.1 Cane toad fence

Mr Dave Linder, Kakadu National Park

The purpose of a cane toad fence is:

a. To prevent toad invasion or occupation of land suitable for toad habitat and prevent their impact on previously toad-free habitat.

b. To allow toad eradication to proceed in toad-occupied land with protection from re-infestation.

c. To isolate toad populations in the wild for toad research.

Basis and history of the concept:
A cane toad barrier was first suggested in the Northern Territory in 1969 to protect the Coburg Peninsula from cane toad invasion. The proposal was raised again in the mid 1990’s and in 2003. Each proposal was unsuccessful due to a variety of reasons including; lack of government support, high cost and high potential failure of the structure.

How would a cane toad barrier work?

a) Land must be suitable. Fresh water drainage across barrier alignment should be avoided – barrier should be installed at the top of the watershed. Drainage from within toad-free enclosure can be directed over a toad-inaccessible weir top. Inflow of water has not been contemplated, as a toad barrier with fresh water inflow would be difficult. Barrier alignment at top of watershed having manageable outflow only from toad exclusion area.

b) Techniques. Barrier alignment to be cleared and formed to provide a seasonally stable (ie. weatherproof) alignment for barrier fence. A formed up and drained bush road would approximate the required work with trees kept below height allowing barrier impact from blow-downs each side of formed alignment.

c) Robust barriers – for remote bush locations (eg. Coburg Penninsula) salvage railway line posts, and top rail with bolted on welded sheet metal panels, such as heavily galvanised squatter tank sheet metal providing a 1.2 metre high barrier imbedded approximately 30cm into ground. Lesser structures may be acceptable for easily serviced non-remote enclosures (eg. urban areas or rural area adjacent to large towns).

d) Toad-excluding vehicle access. For Coburg, an oil-filled shallow bath (10cm or less) under a rain-excluding roof. Toad detection such as tracker dog for incoming vehicles. Toad traps along outside of barrier such as lights over drop trap structures. Coburg barrier site was a very poor toad habitat proposition and invasion numbers would be very low across this landscape.

e) Sea frontages onto toad exclusion prospects must not provide low salinity corridors for road drift from toad infected adjacent land. Toads die quickly in salinity - 28,000ppm and higher and were dead within 40 minutes - otherwise non-stressed immersion.

The barriers require infinite variation on the above to meet individual project requirements. They are achievable for sensibly chosen sites. Ongoing maintenance and surveillance commitment would be significant and can never be relaxed or subject to compromise without full prior risk clearance.
This is the biggest challenge. For remote and large projects, private sponsorship and opportunistic salvage materials could be used to provide superior structures; these structures must be used where costs would be otherwise prohibitive (e.g. Coburg).

It was also noted that the national threat abatement component of the Natural Heritage Trust has recently funded Mr Peter Whitehead, Northern Territory University, to undertake a project that will examine the role of exclusion, whether employed as a dominant strategy or in combination with other methods to control cane toads. In particular, the project seeks to provide robust estimates of the cost of isolating long-term viable populations of susceptible native fauna in sites managed to remain free of cane toads.

5.1.2 Traps
A submission from Auszeal Plastics was submitted after the workshop. The document is included as Appendix 3.

It was felt that the approach had merit for local removal but need an indication of the numbers potentially removed versus population size.

The national threat abatement component of the Natural Heritage Trust has recently funded two projects that are related to this topic. The first project is being undertaken by Professor Rick Shine, University of Sydney and will examine the effectiveness and feasibility of three methods to reduce toad abundances. These three subprojects involve field research to: (1) identifying attributes of waterbodies used by toads, with a view to modifying them to discourage toad use; (2) developing methods to trap tadpoles; and (3) developing methods to trap juvenile (metamorph) toads. The second project is being undertaken by Dr Ross Alford, James Cook University, and will quantify the responses of toads to acoustic and olfactory cues, to determine whether such signals, alone or in combination, can be used to lure them into traps or disrupt their reproductive biology. If the project team finds signals that attract toads, it will use them to design traps and evaluate their effectiveness using field trials.

5.1.3 Bounties
Bounties were not discussed in detail. It was concluded that as other bounty exercises had failed (e.g. foxes) then a similar exercise would not work for cane toads. This strategy was not discussed further.

5.2 Use of Attractants

5.2.1 Pheromones

Michael Tyler, Associate Professor, University of Adelaide

Pheromones can work in either water or air. In the Great Lakes (USA) the salmon industry was decimated with the introduction of lampreys, however the plight of the industry was reversed when the lampreys were controlled with the use of pheromones. The pheromones (produced by juvenile lampreys) were used to attract lampreys into ditches leading away from the lakes and from there they were eradicated.

Cane toads as well as other amphibians produce pheromones. Research has demonstrated that some of these pheromones can be used as effective attractants. For example, if a minute quantity of the
pheromone splendiferum (from male *Litoria splendida*) is placed on a small pad at one end of a pool and a female is placed at the other, the female would sit for a couple of minutes then leap up and move to the other end of the pool where she would sit on this pad.

Pheromones were also reported to have the potential to function as repellants. An example was given in respect to cane toad tadpoles. When tadpole homogenates were added to a school of cane toad tadpoles it resulted in the dispersal of the tadpoles. It was also conceivable that repellents could work at distances of up to several hundred metres. Pheromones are likely candidates for collecting large number of tadpoles or adults particularly in areas inhabited by people and it is an area of potential control that requires further exploration.

5.3 Ecological Controls

5.3.1 Control of cane toads by the sterile male approach

*Dr Michael Mahony, University of Newcastle*

**Background**

The concept of population control by the release of sterile males is based on the basic principle that underpins any form of biological control - the control method must be specific to the organism that is targeted. A feature that is specific to any organism is that males and females mate usually only with members of their own species. If there is a means by which the majority of males can be rendered sterile then most matings will fail to produce offspring.

The release of sterile males to control populations has been most effectively applied to insects (e.g. screw worm fly, mosquitoes). The general approach is to swamp a population with sterile males so that the eggs of females will not be fertilised.

This method works most effectively in organisms that are not highly mobile, where reproduction is restricted to one copulation, where reproductive output is high, and the life cycle relatively short. The method has not been applied to vertebrate pests because they often do not meet these criteria. However, the cane toad meets several of these criteria, and in these features is more akin to the insect models than other vertebrates. It has a high reproductive output, reproduction for the female, as far as is known, is restricted to one single mating with a single partner per season, and adults are relatively sedentary around established breeding sites.

It is claimed that an effective way to control a highly fecund species, such as the cane toad, would be to reduce their reproductive potential. Research is needed to investigate genetic methods to produce sterile male cane toads that have libidos equal to or greater than normal males. However, this project does not aim to study whether the population dynamics of the toad are amenable to this approach. We have taken the position that it is first necessary to determine whether sterile males can be produced, before this question should be considered.

**Advantages of this approach**

- It does not involve introducing viral pathogens or the testing of specificities of any pathogens (i.e. it does not involve introducing a disease to kill toads or the need to test a large array of native animals to ascertain whether the disease is harmless to them).
- It does not require a vector (i.e. there is no need to have a means to spread an introduced disease).
- It does not involve genetically engineered pathogens.
- The method of producing sterility (by producing constitutive triploid male toads) does not require any harmful reagents.
• It is humane, safe and cost effective.

There were some disadvantages to this approach – ie sterile males still pose a threat to species such as quolls.

**How would a Sterile Male Release work?**
Large numbers of tadpoles that grow into sterile males would be released into known breeding ponds. These would grow and develop into sterile males. The release would be in the advancing zone of the toad to act as a buffer. Previous studies have shown that the first cane toad tadpoles in a pond in the breeding season predate on the eggs and tadpoles of subsequent matings.

**Objectives**
Proof of Concept: Can sterile male cane toads with normal libido be produced?
The research objective is to determine whether this can be achieved by producing triploid males that grow normally and have normal testes with respect to the production of male hormones, but which produce abnormal sperm. When these males mate with a female their sperm are either not capable of fertilising eggs or if they do, development will not proceed. Triploidy is known to result in sterility in numerous animal groups. Triploids have three sets of chromosomes rather than two. It results in sterility because not all chromosomes can find a pair during cell division in the testes. The result is abnormal sperm and sterility. However, somatic development (body tissues and organs) of a triploid is normal, thus hormones controlling libido should be the same as in a diploid.

To achieve this outcome a number of steps must be shown to be possible in the cane toad.

1) **Can triploid cane toads be produced?** Triploid toads can be produced by using the simple method of cooling toad eggs immediately after fertilisation. The technology needed to gear up to produce the necessary numbers for a sterile male release program is already applied in some sections of the aquaculture industry.

2) **Do triploid toads grow and develop normally?** A small number of triploid toads have been grown through the larval stage to beyond metamorphosis and there appears to be no major impediment to the concept at this stage of the life cycle. Young toads have not been grown through to adulthood to confirm that this is possible.

3) **Do triploid toads have normal libido?** If triploid toads grow to adulthood we would need to assess whether they have normal libido (hormone profiles and microscopic examination of testes).

4) **Are triploid adult males sterile?** Demonstrate that any sperm produced are abnormal.

Another related matter to be clarified in these studies, and one that offers considerable potential for other means of biological control, is elucidating the means of sex determination in the cane toad. For the bio-control method it is critical that only sterile males are produced. The sex determination mechanism in toads is not known, but it would be determined in step three above. Understanding the sex determining mechanism would also provide an avenue for other approaches to bio-control such as the daughterless male approach. By elucidating the sex determining mechanism of toads, combined with molecular studies of the sex-determining gene in lower vertebrates, there is the potential for a powerful means to target sterility and produce a disseminating means of control.

5.3.2 Daughterless technology
A presentation on ‘Daughterless Technology” was not given. It should, however, be noted that this concept where all offspring are restricted to males is also a theoretical possibility for controlling cane toad populations. The principle behind this technology is that only the specific toad gene
would be affected whereby over many generations, less and less females would be produced thereby driving the overall population down. The advantage of integrative research programs could be the incorporation of the current CSIRO project with this type of approach thereby obviating the need to release large numbers of transgenic animals into the environment. The PAC CRC/MDBC’s ‘daughterless’ approach being explored as a control technique for Carp should be closely monitored to assist in determining its applicability of the concept to toads.

5.4 Biological control

5.4.1 History of biological control in relation to cane toads

Dr Rick Speare, Associate Professor, James Cook University

The first phase biological control of cane toads in Australia began around the early 1980s. Funding was provided by the Commonwealth Government through Council Of Native Conservation Ministers (CONCOM) in 1987. At that stage little was known about the diseases of cane toads. Funding was provided to James Cook University at the School of Tropical Veterinary Science to look at cane toad diseases in Australia. The aim was to discover any diseases that could be used for biological control. The initial objectives were to identify some agents and some very basic pathology of cane toads resulting in the type of the diseases that were present in the Australian population of cane toads. The end result of that project, (from 1987 to 1989), was the identification of a number of pathogens, but none of them appeared to be particularly useful as a control agent. At the same time, a ranavirus was identified in frogs, which was called the Bohle iridovirus. Experimentally that was then put back into cane toads and it killed them. However, subsequent work discovered it also affected three classes of vertebrates, namely amphibia, pisces and reptilia. The interesting thing about the ranavirus group is that they also infect mammalian cells and in culture, keeping the cells at a lower temperature, ranaviruses will kill mammalian cells quite effectively. The factor that may protect mammals in the natural situation is that ranaviruses have a temperature limit and will not grow above 34°C. Presumably if that temperature barrier is broken it may also cross to mammals and homeotherms.

During this project, a visit was made to Costa Rica and some apparently normal cane toads sent to the University. Pathology found another iridovirus, one of the large iridoviruses. This was the only work done outside Australia in that particular project. By the end of this stage the project leaders decided nothing existed in Australia to control cane toads, so the next step was to search outside Australia.

The next project was funded by the Commonwealth Government through CSIRO Wildlife and Ecology (now CSIRO Sustainable Ecosystems). Again this looked at disease aspects as well as biological aspects. Disease work was carried out in Venezuela. The next project started in 1990 and would have continued until about 1994. Unfortunately, it concentrated on surveying normal cane toads and some ill cane toads to find mainly another group of ranaviruses. No work was done on diseases of sympatric Bufo species or on pathogens in Brazil where the ecological work was being done as local political processes prevented the collection of toads from this area. The ranaviruses were then taken from Venezuela and brought back to AAHL. Experimental work was done on these ranaviruses to look at host range. The next stage looked at taking ranaviruses, which are DNA viruses, potentially a lot more stable than RNA-based viruses and that is where the subsequent project emerged.
5.4.2 Use of viruses in cane toad control

Dr Tony Robinson, CSIRO

The current CSIRO project exists in two parts, at the Division of Livestock Industries in Geelong (Australian Animal Health Laboratory) and at the Division of Sustainable Ecosystems in Canberra. The key principle of the work is based on engineering a virus which will interfere with metamorphosis in cane toads and that will disseminate throughout the environment. The idea of using a disseminating agent was preferred to a baiting regime as the latter could not operate over the scale in which the cane toad exists. This type of approach was the principle behind the introduction of myxomatosis and rabbit calicivirus. The current research differs from the rabbit control measures in that a virus must be identified and engineered to interfere with metamorphosis. The virus chosen is an iridovirus. It has, however, a problem associated with it in that it can, under experimental conditions, kill a range of fish and amphibians. Based on this information, research at AAHL is twofold (1) attenuate the virus so that it does not cause diseases and (2) engineer the attenuated virus such that it expresses a gene that would have a detrimental effect on metamorphosis.

The research based at Sustainable Ecosystems, CSIRO, Canberra, is related to the question "what gene can be used to interfere with metamorphosis"? The researchers screened a large number of genes, expressing them in bacteria or in culture systems and physically inoculating tadpoles to determine whether they can interfere with metamorphosis. At this point in time it was reported that there is some evidence metamorphosis can be interfered with via this approach. It was acknowledged that this type of research is complex and many related issues need further discussion. In the end controlling cane toads on a continental scale will require a disseminating infectious agent such as a virus.
6.0 Current CSIRO Research

6.1 Overview

Previous research has shown that ranaviruses (endemic and exotic) can kill cane toad tadpoles.

In 1990, because of mounting concern about the impact of the cane toad on Australian fauna, funding was provided by the Australian Government for a large research project into the biology and impact of cane toads in Australia. As part of this, Australia funded a project in Venezuela in association with IVIC (Centro de Microbiologia y Biologia Celular, Instituto Venezolano de Investigaciones Cientificas) to identify disease-causing agents present in toads in their original habitat. From this study seven viruses were isolated and imported into Australia for further study. These viruses, in addition to Bohle iridovirus (BIV), were assessed for their ability to infect and kill cane toads and/or life stages.

In experiments where tadpoles were infected, mortalities ranged from 72 per cent to 100 per cent. An important aspect of this work was that tadpoles could be targeted for biological control. The basis of this statement comes from work by Dr M. Lampo who developed an age-structured population model for cane toads. The model predicts that control strategies targeted towards female fecundity or tadpole survival would have very little impact on adult population density because a reduction in the number of tadpoles would be compensated for by a density-dependent increase in their survival. However, very large tadpole mortalities would have a significant effect on adult numbers. The model estimates a maximum tadpole survival of 0.349, and with an additional morality source (the virus) of 0.8, then the predicted total maximum survival is 0.07, or 7 per cent of eggs surviving to metamorphosis. Using the model, this could reduce adult densities by 80 per cent (see Appendix 2), but it would require consistent and continuing high tadpole mortality, which was not achieved under laboratory conditions. The earlier work with exotic viruses and BIV showed that experimental infections could induce high levels of tadpole mortalities and therefore the strategy to target tadpoles is plausible. The use of these viruses was not, however, recommended because they were indiscriminate in their host specificity.

The concept

The concept for the CSIRO approach is based on the physiological processes of metamorphosis and the characteristics of ranaviruses.

Significance of metamorphosis: The toad is an amphibian. As such it will undergo metamorphosis. Early research demonstrated there may be an immune memory throughout the developing phases associated with metamorphosis. If, for example, tadpoles are injected with an adult protein such as adult haemoglobin then when the tadpole undergoes metamorphosis there will be an immune response whereby the adult form of the protein will not be produced, or it will be removed by the immune system.

Significance of ranaviruses: The characteristics of ranaviruses are that they are double-stranded DNA viruses, genetically stable, stable (antigenically and structurally) under some adverse conditions (e.g. temperature and states of desiccation) can be spread in the water table/bodies and via fomites and have a very large genome. Ranaviruses are also present in the environment and are lethal to tadpoles. It is therefore an ideal vector as it is water borne and will strike the most susceptible stage of the toad. Its one weakness however is that it is not species-specific.
The concept is as follows:

*Engineer an endemic, attenuated ranavirus (known to be infectious to *B. marinus* tadpoles) to carry genes critical to metamorphosis and/or survival of the adult toad but benign to native species.*

To achieve ‘proof of concept’ the key objectives of the research project were:

1. Establish and maintain a breeding colony of cane toads to ensure supply of all stages of life cycle for experimental work.
2. Demonstrate that ranaviruses can be attenuated.
3. Demonstrate the ability to create recombinant ranaviruses.
4. Identify genes critical to distinct developmental stages and access their potential to disrupt development.

**SUMMARY**

1. Genes from cane toads can be identified and used to alter or interfere with the gene expression in the adult.
   - Adult β-globin has been cloned and injected into tadpoles.
   - Injection of adult β-globin into tadpoles changes the profile of haemoglobin in the adult.
   - Other genes have been discovered via the application of microarray technology.

2. An attenuated enzootic ranavirus has the potential to act as a delivery system for foreign genes.
   - Ranaviruses have been successfully manipulated.
   - Non-essential regions of the virus identified (x4).
   - Foreign genes have been inserted into these regions in plasmids.
   - Recombinant virus has been generated using these plasmids.
   - Attenuation of *BIV* has been achieved.

### 6.1.1 Summary of research currently being undertaken at AHHL

**Dr Jackie Pallister, AHHL, CSIRO**

Viral delivery was chosen because a virus is a self-disseminating agent, and cane toads inhabit remote and inaccessible areas. This is one way of getting the control into the vast areas occupied by cane toads. The project aims to create a virus whose effect is specific to the cane toad. The virus itself must be harmless to any species it infects so that its effect will only be due to the genes it is expressing that are specific to the cane toad. Ideally a virus released into the environment would also be native to Australia to avoid the added complication of introducing an exotic virus into the environment.

Bohle iridovirus (*BIV*) fulfilled a couple of these criteria; it was isolated in Australia, in Bohle, North Queensland, and so far is the only virus that has been isolated from amphibians in Australia. It infects cane toads as both adults and tadpoles. It is also a double-stranded DNA virus, which makes its genome easier to manipulate in the lab.

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1. Virus does not cause severe morbidity or death

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However, BIV is less than ideal in a number of ways. It is not specific to cane toads. As well as amphibians it also infects fish and reptiles and, experimentally, it causes high mortality rates in barramundi fingerlings and tortoise hatchlings. In addition little is known about the virus - its genetic organisation and how it behaves in the environment. In spite of this lack of knowledge BIV was determined to be the best option we have at the moment.

AAHL has been attempting to establish proof of principle in these early stages of the project. It needs to be determined if BIV can act as a delivery system for foreign DNA. In addition, the virus by itself must not cause disease in any species it is able to infect - a crucial requirement for BIV as a delivery system.

There are a couple of ways of attenuating viruses. One is the traditional method of growing the virus under non-ideal conditions, for example, at a non-ideal temperature, or in different cells. BIV was passaged 99 times in African green monkey kidney cells, then tested its pathogenicity in frogs. The wild type virus (unpassaged) killed all frogs while the passaged virus only killed one out of 12 frogs. When the surviving frogs were challenged with the wild type virus, none of them got sick or died. Even though the frogs infected with the passaged virus appeared unaffected by the virus, the virus had actually produced an immune response that protected them from challenge.

Another method of attenuating a virus is called rational attenuation, so called because a known gene is altered. The previous attenuation of the virus is a random process. But in rational attenuation a specific gene is deleted or altered, usually one that is not essential for virus growth. Often these genes are ones that may be involved, for example, in DNA metabolism and while not essential for the virus, they might actually help the virus in its replication. Attenuation in this manner is a real possibility and has been done with other viral pathogens.

Because BIV is poorly characterised it was first necessary to identify non-essential genes by comparison with other large double-stranded DNA viruses. Four different non-essential genes in BIV were identified then cloned into plasmids (workhorses for manipulating genes). This allowed removal of a part of the non-essential gene (rendering it inactive) and insertion of an antibiotic resistance gene (foreign DNA). To make a recombinant virus the viral DNA (containing an intact version of the non-essential gene) was introduced into a cell with the plasmid containing the altered non-essential gene that now carries the antibiotic resistance gene. The aim is to achieve recombination, or crossing over, between these similar areas of DNA in the non-essential gene.

The desired result of this process is a whole virus genome with the antibiotic resistance gene inserted into the non-essential gene. This has been shown to work with two different non-essential regions so far and work is continuing on the other two. At least two sites have been identified where foreign DNA can be inserted. The next step is to test the recombinant viruses to see if the deletion of this gene has attenuated them.

There is now proof of principle for the virus development part of the project. Foreign DNA can be inserted into BIV and it is expressed. We know that the antibiotic resistance gene is expressed because recombinant viruses carrying the gene are able to grow in the presence of the antibiotic, while the wild type virus cannot. We have also shown that BIV can be attenuated by passage in culture.

This is the beginning of work on BIV - in the lab and in the environment. There are big questions about BIV in the environment, for example, how to test the effect of BIV on other species. This is part of the reason for this workshop - to get input from other people. Little is known about the ecology of BIV. How widespread it is in the wild? How does it disseminate? Would it disseminate
in a way that would allow it to be an effective biological control? How would an attenuated virus behave in the environment? Would it be different to the wild type virus?

And last, but definitely not least, some very good studies of environments that have been affected by cane toads are needed, as well as those not yet affected, before the effectiveness of a biological control agent can be measured.

Refer to Appendix 2 for details of the work and the review.

6.2 Issues where more information/work is required

The issues discussed below were raised at the workshop and throughout the research review. Each section lists the issues and identifies the immediate next steps.

6.2.1 Will it work? – proof of concept

Many of the issues raised in this section have also been addressed within others.

Identified Issues/Comments

- Will genetically modified organisms (GMOs), or indeed any infectious organism, be permitted to be tested in the field?
- What alternatives exist?
- What are the ranges of options?
- Does it have to be virally delivered?
- The virus must be non-disease causing in native animals
- Do we continue to advocate impact studies?

Next Steps

(i) Proof of concept must be achieved at ecological, evolutionary and molecular levels. Critical failure at any level will render the approach useless.

(ii) Consultation is required with Government to determine whether GMO’s and/or infectious organisms will be permitted to be tested in the field. Note that PACCRC/GRDC/UWA/CSIRO’s current mouse immunocontraception work, where extensive liaison will be required with the OTGR, could be used as a ‘pathfinder’ on this issue.

(iii) Variations of the concept must be identified, discussed and evaluated.

(iv) Current bio-control delivery mechanism.

- What level of infection is required for effective delivery?
- What will be the impact on the toad? If it is reduced fitness, then what per cent reduction is required?

6.2.2 Laboratory work and/or field work?

Identified Issues/Comments

- GMOs and delivery research should be done at the same time.
- Any control strategy will need to be multifaceted to succeed, therefore research at the molecular and ecological level should be carried out concurrently.
- Understanding of the virus in the environment is poor.
- Don’t go too far down dead-end directions.
6.2.3 Modification of the research to produce a GMO that is non-disseminating and has multiple targets

This section identifies a major concern of workshop delegates regarding the feasibility/advisability of using a disseminating GMO. Future research must acknowledge these comments and address them in research options.

Identified Issues/Comments
- A non-disseminating agent overcomes problems of ‘escape’ from Australia.
- What is the global trend in relation to the use of GMOs (disseminating and non-disseminating) for pest control and vaccines?
- How is a non-disseminating ‘agent’ spread? Can this approach be used as a trans-continental control agent? How would repeated doses be administered?
- What is the cost of constant deployment of a non-disseminating control mechanism, and for how long would it be needed for effective control of cane toads?
- Is a dead toad better than a sterile toad (toxicity, consumption of biomass etc)?

Next Steps
(i) Government guidelines must be obtained on the use of disseminating and non-disseminating agents (including GMOs and transgenic animals) within the Australian environment.
(ii) Review literature on non disseminating viruses.

6.2.4 Is the use of self-disseminating GMOs a major/prohibitive issue?

Cost-benefit analysis of research initiatives needs to be performed. Such analysis must be cross referenced to both short and long-term risk assessment of the impact of cane toads. Note: any risk assessment studies should clearly define the definition of ‘impact’.

Identified Issues/Comments
- Are GMOs an issue in relation to cane toads? That is, does the public really care when it comes to using a GMO to control cane toads?
- Are GMOs a real long-term issue? Currently GMOs are being used and/or are being assessed in relation to human vaccines, veterinary use and control of rabies in Europe.
- Use of GMOs that could lead to significant declines in continental toad populations. This possible outcome might overcome public fears of the technology.
- Is public opinion now the same as it will be in 10 to 15 year’s time?
- The cost of R&D must be evaluated in terms of likelihood of success and the total (biodiversity, social, political and economic) impact of cane toads on Australia. (i.e. a cost-benefit analysis is required).
- Pathogen pollution; is there a quarantine and conservation issue in relation to species in their native countries? Will there be counter opinions from other countries?
- In relation to GMOs, will the same argument against the use of these organisms be directed to other strategies including those encompassing the idea of creating daughterless male genetically modified cane toads?
- GMO is not the problem – novelty and self-dissemination is.
Next Steps
(ii) Monitor any research on public acceptibility of GMOs in Australia.
(iii) Conduct cost-benefit analysis of current research (CSIRO) proposal.
(iv) The issues identified above relate, in the main to GMOs. Again it is evident that Government guidelines are required. Irrespective of this point, it is important that research initiatives into the control of cane toads be undertaken. If funding for cane toad research remains limited then research-funding priority should be determined in regards to feasibility of success, cost/benefit and what will be acceptable in Australia and overseas.

6.2.5 Should we target something other than metamorphosis?
Further research (as identified earlier in the workshop) should identify targets that encompass all life stages. The expressed reasons (noted during discussions) were (i) this would maximise exposure of cane toads to infection during their life and (ii) with multiple targets there would be greater chance of success.

Identified Issues/Comments
• There is a need to explore approaches that extend beyond the immunization of tadpoles. For example target juvenile/adult toad, pheromone/receptor sex determination genes, genes coding for key development hormones and those responsible for producing toxins.
• Can the viral delivery strategy be integrated with the strategy of producing single sex populations or infertile animals?
• Need to understand density dependence in each life history stage.
• Bidders Organ is found in cane toad but not in native frogs. Cane toads have the ability to change sex at a specific time of their life cycle; can this be exploited in producing (long term) a decreasing predominantly single sex population.

Next Steps
(i) Identify key gene expression for ‘survival’ of each life cycle stage
(ii) Determine effects of interference with different genes.

6.2.6 Evolutionary changes in the virus post release
As the viruses used in the CSIRO cane toad study are (i) DNA and (ii) have at least one gene deleted, it would be unlikely that the virus would revert to the wild type (i.e. become pathogenic). Having stated this, experiments would have to be performed to explore this possibility. The issue of competition must also be addressed.

Identified Issues/Comments
• Are the genetically modified viruses as competitive as the wild type viruses within and amongst hosts?
• Release of any form of infectious agent including GMOs is a national issue for all forms of biological control.
• Include multiple ‘fail-safe’ strategies (e.g. attenuation [cell passage] and deletions).
• Strategies for release and maintenance that reduce speed of adaptive responses.
• Is releasing genetically modified virus an issue if wild type virus is present in the environment?
• Is there an international issue associated with releasing infectious agents into the environment; i.e. are there quarantine issues for other countries?
• Will a non-disseminating virus overcome potential ‘quarantine’ issue?
DNA viruses are more stable than RNA viruses (such as influenza), therefore there is a higher likelihood of stability.

Next Steps
(i) Determine competitive nature of the genetically modified virus to the wild type virus.
(ii) Review literature: unless it can be shown that the competitive ability of a GMO could be as high as the wild type, then the GMO project is unlikely to work.

6.2.7 National toad study sites

Identified Issues/Comments
- National toad study sites are absolutely essential.
- Include sites at the invasion front.
- Benefit of National toad study sites: could streamline costs/effects (shared costs).
- Disadvantages: site-specific bias problems (applies to every study).
- Such sites would provide the opportunity to collect cane toad material to archive for later uses; e.g. measure changes in cane toads either over time, due to bio-control or even due to invasion (toads at the invasion front may be different to toads in endemic areas (?)).
- Link sites with national coordination and communication.
- National funding agencies (e.g. ARC) may be interested in funding research activities associated with such sites.
- What will be monitored? – Suggested areas: ‘impact’, serology, toad related infectious agents including wild type virus.
- Each State Government agency responsible for conservation should establish and monitor such sites in representative reserves as a matter of normal business using existing staff and recurrent funding.

Next Steps
(i) Establish monitoring sites. Toad study sites should be located throughout their range, as well as ahead of the invasion front, to assess changes and impacts on the environment.
(ii) National cane toad group (upon establishment thereof) to coordinate research and communication between sites.

6.2.8 National co-ordination

There was significant support for a National Working Group. Inherent with this suggestion is (i) a major funding initiative from the Australian Government and (ii) an integrative approach to the solution of ‘control’. The responsibilities of a National Working Group are outlined in section ‘Policy Makers’.

Identified Issues/Comments
- Dissemination of results, information and actions via websites.
- Formation of a dedicated CRC or integration of cane toad control into a CRC.
- Requirement for a National impacts workshop.
- Requirement for National toad distribution map and projections of future spread.
- Identification of places, especially islands, where toads can be excluded is critical first step.
- Provide assistance to community organisations e.g. Tiwi Island community who are establishing their own quarantine/exclusion/control programs.
- Small-scale maps of cane toad distribution.

Next Steps
(i) Form a National Working Group of managers/scientists/community/government, with a full-time coordinator.
6.2.9 **Compilation and synthesis of existing research data.**

**Identified Issues/Comments**
- Requirement for a Web page containing available reports and published data (all data must be verified; i.e. anecdotal information must be delineated from that in the published scientific arena).
- Moderated email list server (ProMed model).
- Requirement of a dedicated conference with refereed proceedings.

**Next Steps**
Fund a dedicated researcher to compile and synthesise existing research data, disseminate information and run a conference.

6.2.10 **Do nothing option**

The “Do Nothing” option is dependent upon a risk assessment. Although there is little long-term data available on the impact of toads, an assessment must be undertaken if any control measure(s) is/are to be implemented. If the risk is determined to be non-significant then a “Do Nothing” approach may be appropriate.

**Identified Issues/Comments**
- Communicate to general public that cane toads are here to stay at least in the medium term and that their threat needs to be addressed.
- Communicate potential risk of cane toads to vulnerable Western Australian species.
- Educate the public as to the possible barriers to release of biological control agents.
- What is the cost of ‘do nothing’ versus ‘do something’?
- Is it cheaper to deal with impacts, e.g. quoll relocations, and other species…or to change the impact balance, than it is to eradicate?
- ‘People power’ is available – tap into it.
- Animals in Queensland already adapting e.g. modification of behavior whereby some animals can successfully prey on toads.
- Anecdotal reports of cane toads in Queensland undergoing long-term population declines.
- Are the total impacts (compared with other environmental problems) sufficient to warrant action?
- Do we stop impact studies and long-term control research under this option?

**Next Steps**
The ‘do nothing’ approach may be relevant in endemic areas in Australia such as Queensland.
(i) Undertake cane toad population studies in areas of anecdotal declines; such a study may identify other processes that deleteriously affect cane toad populations.
(ii) Conduct risk assessments (short term and long term must be done on the ‘impact’ of toads - social, economic, biodiversity-).

6.2.11 **Field research studies on wild BIV and other viruses of Australian amphibians**

There is a requirement to understand the biology of BIV in cane toads. As such, nation-wide surveys are needed to identify the presence and distribution of current amphibian viral ‘fauna’. In addition, the current assays need to be established for frequent usage in one or more laboratories. The diagnostic assays also require a short turn-around time. Effort should be placed into
development of cutting-edge diagnostic assays such as PCR and the newer nucleic acid/antigen and antibody assays (e.g. microarrays and laser-based technologies).

**Identified Issues/Comments**
- Require easily accessible screening tests to identify and isolate viruses.
- Could (survey for infectious agents) be accomplished via integrating survey into the current Chytrid monitoring program.
- Receive/solicit specimens from frog/wildlife rescue groups and the community.
- Establish a repository of test results.
- Must know more about prevalence of any potential vector.
- Must know physical-chemical characteristics of cane toad environment in which virus is to be transmitted.
- Isolation and identification of other endemic viruses is important, as some may be useful.
- Wildtype BIV looks good as a vector. What is the best way of infecting toads for maximum impact?
- Require a quick and reliable virus/antibody assay.
- What density of toads is necessary for successful virus spread? Toads may become less abundant in years after toads arrive, which may affect transmission.

**Next Steps**
1. Need properly designed survey to collect tissues from Australian amphibians to determine the presence and distribution of ranaviruses and other viruses.
2. Identify laboratory or laboratories for pathogen isolation and characterization.

### 6.2.12 Alternative pathogen?
There is still considerable scope to identify infectious agents from other Bufo species. The past work in Venezuela concentrated on *B. marinus* and little work was done on other toad species. Based on past experience, a search for infectious agents of other toad species would require a major research effort. To negate problems with cultivation of viruses in cell cultures, consideration should be given to inoculation of homogenates into *B. marinus* and/or using molecular techniques such as ‘degenerate PCR’ assays which have the potential for the detection of viruses belonging to a specific genus.

**Identified Issues/Comments**
- Since the target is the tadpole stage, explore use of ‘spray’ application at breeding sites instead of self-disseminating virus (only good for very small areas).
- Specialists (pathologists, virologists, mycologists, bacteriologists and parasitologists) should examine the causes of ‘die offs’ of cane toads in Australia and overseas.
- An inherited modification dissemination strategy is more likely to be accepted by the community than virally disseminated factor.

**Next Steps**
So far, the search for pathogens has concentrated on one species (*B. marinus*) from one area, Venezuela. It is noted that earlier research looked for pathogens from other toads in Venezuela, but this was not successful. Future research should concentrate on other bufonid species other than *B. marinus*. The future research should be undertaken in Asia and/or Africa. That is the next step in this area should be:

With integrative collaboration between veterinary pathologists and virologists look for toad specific infectious agents within toad species other than *Bufo marinus*.
6.2.13  Humane dispatching of cane toads
It is important that a protocol for the collection and euthanasia of toads be accepted. This protocol must pass ‘animal ethics’ scrutiny and be agreed to by the relevant State agencies. The agreed recommendation is (a) collect toads into plastic bags/containers with an air hole. Note, if plastic bags are to be used (i) they should be wrapped in a towel as this prevents the animals from coming into direct contact with a frozen surface and (ii) the bags must have air holes. (b) cool animals to 4°C and then freeze in a -20°C freezer (conventional freezer) for at least three days. Euthanased cane toads can then be buried or incinerated.

Identified Issues/Comments
- This workshop to make a recommendation on a practical and humane method of killing cane toads The preferred protocol, as agreed at the conference was: Place the animal(s) in plastic bag, cool to 4°C then freeze (-20°C).
- In New South Wales, this recommendation should be taken back to the New South Wales Pest Animal Council for endorsement.
- Participants agreed that cruelty is not acceptable for any pest.
- When freezing the animal(s), they must be placed in ‘insulated’ container that prevents the toad(s) coming into direct contact with surfaces of the refrigerator at –20°C (i.e. metal wall)

Next Steps
Seek all workshop participants to endorse cooling then freezing as an acceptable and humane euthanasing technique. Endorsement was obtained at the workshop.

6.2.14  Will the virus (BIV) be self-disseminating?
The optimum strategy for biological control is to use a live virus. As it is not clear which strategy (self-disseminating versus non-disseminating) will be used, both strategies should be explored at this early stage.

Identified Issues/Comments
- These are two options that require different delivery design. Whether a disseminating or non-disseminating virus is used should be decided following the successful demonstration of proof-of concept.
- How would release/baiting work? For both disseminating and non-disseminating viruses, release strategies must be developed.
- Investigate transmission rates, routes of transmission of the virus in cane toads.
- Identification of possible carriers/reservoir hosts.
- Methods of dissemination must be modeled and tested.
- Self-disseminating viruses would be the most cost effective strategy for trans-continental control.

Next Steps
Continue working on the concept of interrupting metamorphosis and initiate scoping for non-disseminating strategies.

6.2.15  Potential for recombination (sharing of genetic material between the wild type virus and the modified virus)
This does not appear to be a major problem. What is clear is more basic biology on BIV is required. This type of work would involve strategies outlined in “Field research studies on wild BIV and other viruses of Australian amphibians.”
Identified Issues/Comments
- The virus must already be present in the environment for recombination with the modified BIV to occur.
- If the wild type virus exists this is not a problem as loss of the foreign gene would simply return the modified BIV to the wild type form.
- Being a DNA virus the genome should be comparatively (i.e. compared to a RNA virus) stable.
- What is the time frame for mutation/recombination to occur?
- Is risk of recombination/mutation to more virulent form of modified BIV any greater than for wild type virus?
- Will the modified virus re-acquire virulence?

Next Steps
Modified virus must be tested in the laboratory for reversion to virulence. Evidence of the stability of ranaviruses or other similar viruses must be presented (ie. scientific literature and detailed rationale) so that the scientific and lay community may be confident that the issue has been properly considered (from 2003 Review report by Hazell et al).

6.2.16 Testing of impact on non-target species
Non-target-testing is a critical area for all forms of biological control. It relates to the current CSIRO approach and any future strategies that incorporate infectious agents. Part of an integrative research approach should involve the participation of experts in amphibians, fish and reptiles. Considered thought must be given to the extent of testing and the methods used. For example can microarray technology be used to search for similar gene ‘targets’in other species?

Identified Issues/Comments
- Testing of biological agents applies to all forms of biological control.
- For BIV the testing is essential as the virus has been demonstrated (under laboratory conditions) to be pathogenic to some amphibian and fish species. Note: the current agent is being attenuated to remove the disease - causing characteristics of the virus.
- How extensive – Should the non-target testing be restricted to only Australian animals or include others from around the world?
- There is a need for a comprehensive testing plan that includes frogs, fish and reptiles. Team to include biologists, ecologists and virologists and land managers (including government representatives).
- Cost and feasibility of testing indicator species, particularly threatened species must be determined.
- Must include Bufo species worldwide, especially any that are endangered and threatened.
- It is not feasible to test impact on all non-target species; microarray technology could be an alternative?
- An alternative may be to test representative species that may be affected (determined from above dot point), or species of environmental (e.g. threatened species), agricultural and/or social importance.

Next Steps
Identification of experts (in amphibians, fish and reptiles ecology) who can generate a list of animals that should be included in non-target testing.

6.2.17 Risk of release/escape from Australia (quarantine)
The main issues relating to this section are the biology of ranaviruses, host specificity and quarantine. The first two issues have been covered in other sections. The issue of quarantine is a
major topic relating to all infectious organisms and will require significant discussion by the scientific-, public interest and policy-making groups.

Identified Issues/Comments
- National issue for all forms of biological control.
- If the current research is cane toad-specific we need to keep it out of South America. Note: (i) cane toads are not necessarily pests everywhere else and (ii) infectious agents must be *B. marinus* specific as some species of *Bufo* may be endangered.
- What is the exact status of cane toads in South America?
- Have ranaviruses been transported and colonised outside native ranges?
- Cannot stop escape from Australia, especially in tourism areas: What can be done? For example is it feasible to confiscate/treat shoes of all visitors before boarding outbound planes?
- Ranaviruses have some history of movement between countries: high environmental survival; low inoculation dose therefore there may be a threat for translocation.
- Can we test specificity against all *Bufo* species or all *Bufonids*?
- Have we surveyed ranaviruses inside and outside Australia to consider this question?
- Host range important – broad host range increases possible escape routes.

Next Steps
(i) The issue of quarantine (restricting infectious agents and transgenic animals (e.g. sterile animals and/or ‘daughterless’ animals) to Australia) is a major topic requiring significant discussion by the scientific community, public interest groups and policy-makers (State and Federal).
(ii) Examine quarantine issues where biological control agents have been released in Australia which have the potential to harm animals in other countries.
7.0 Additional/new research options

Following presentations and questions about the various options for cane toad control, participants were asked to consider the gaps in the current research effort and whether additional areas should be considered. The following suggestions are prioritised according to the importance identified by participants, with the higher priority issues first.

7.1 Gaps in the current research effort

1. Pheremonal/acoustic attraction of toads, especially in toad exclusion or quarantine zones.
2. Autecological studies (study of the ecology of individual species) in a range of systems to prioritise risk by ecosystem.
3. Pathogen delivery of different gene(s) to later life stage (not tadpoles), investigate a longer infection window. Include assessment of new molecular approaches using vectors targeting other critical processes.
4. Understanding the mechanism of impact – use to possibly ameliorate impacts.
5. Sex determining mechanisms of cane toads: use of genetically modified organisms for fertility control; daughterless males; sterility.
6. Models to assess feasibility of control options: including data to provide parameters for a cost/benefit analysis.
7. Identify pathogens already active in the Australian cane toad population.
8. Identify other cane toad-specific primary pathogens.
9. Studies on ecology/biology of target iridoviruses e.g. spatial and temporal distribution and abundance; temperature profiles of larval habitat.
10. Trapping and removal systems: methods of eliminating cane toads from small areas.
11. Continue and expand frog-monitoring studies. Include other taxa; perform studies temporally and develop an image survey.
13. Gather sufficient knowledge to enable cost/benefit analysis of any future control strategy.
14. Locate early invaders.
15. In biological control programs, establish species-specificity of control agent.
16. Impact studies especially for quolls and goannas where these animals persist with toads and where quoll and goanna declines are coincident with toads. (Note: this may be accomplished via extending radio-tracking studies.
17. Island “Ark”; analysis of project in Western Australia.
18. Quantitative impact on Northern quoll (flagship species).
19. Assess value of enclosures in high value conservation areas.
20. Coordinate investigations of serendipitous toad die-offs or apparent decline.
21. Dynamics of populations at the invasion front (opportunities to limit expansion).
22. Develop standardised cane toad monitoring techniques: manual survey; automated acoustic survey.
23. Compile and synthesise results of previous research and get into a form that is accessible and convenient e.g. updated website.
24. Control options involving non-disseminating agents.
7.2 Top Six Suggestions.
The top six suggestions were discussed. Participants were asked to address (a) what needs to be done? (b) by whom? and (c) other comments. During discussion it was decided to add another area – physical barriers. The following is a summary of responses.

7.2.1 Pheromonal/acoustic attraction of toads, especially in toad exclusion or quarantine zones

(a) What needs to be done?
- *Tadpoles*: Identify alarm pheromone to cause school dispersal. Could this be used in relation to cane toads in water?
- *Pheromones*: Conduct trials on crude extracts (note: use fractionation; characterise or develop large-scale extraction technology.
- *Trap design*: Use attractants together with traps.

(b) By whom?
- Leaders in pheromone research (amphibian) and those interested in trap design (refer to Appendix 3).
- Coordinated by a National Cane Toad Working Group.

7.2.2 Autecological studies to prioritise risk by ecosystem and understanding mechanisms of impacts

(a) What needs to be done?
- Factors influencing densities/presence of toads.
- Examine target (native) species – populations that persist in the presence of toads: how/why they persist; mechanisms.
- Prioritise risk by species and ecosystem.

(b) By whom?
- National Cane Toad Working Group: representatives to be decided, coordinated research across regions, series of national study sites.

(c) Other comments
- National workshop needed to identify priority species, areas, approaches and review previous work.

7.2.3 Pathogen delivery to later life stage and assessment of new approaches using vectors

(a) What needs to be done?
- Model kill rates at different life stages.
- How do we progress the biological control (e.g. information required on ‘quarantine’ – restricting agents/organisms to Australia; use of modified organisms within the environment)?
- Model current concept.
- Explore other targets: pheromones-mate recognition; sex determination; sexual maturation.
- Explore other life stages.
- Other methods of delivery: RNAi; multiple delivery in one virus
- Further investigations into what is unique about a toad?
Phylogeny of frogs and toads (e.g. generation of data detailing gene differences between toads and frogs).

(b) By whom?
- Integrated CSIRO and University research activities involving molecular biologists, virologists, pathologists, ecologists, herpetologists and modelers.
- Coordinated by a National Cane Toad Working Group.

7.2.4 Sex determining mechanisms of cane toads: GMO; fertility control; daughterless males; sterility

(a) What needs to be done?
- Need to establish sex-determining mechanism in *B. marinus*: other Bufonids known both ZZZW and XXXY present.
- Molecular biological methods: rapid resolution (12 months); primers for putative sex determining locus available (collaborators identified – Prof Michael Schmid).
- Opens possibility for a range of biological control options that disrupt fertility: straight sterility; daughterless males; viral vector target genes
- Associated with other non-disseminating methods: traps, round ups etc; establish ‘beach head’ for release

(b) By whom?
- Integrated CSIRO and University research activities.
- Dr Michael Mahoney, University of Newcastle.

7.2.5 Models to assess feasibility of control options

(a) What needs to be done?
**Problem identification**
- Identify the control options to be modelled.
- Identify questions models must answer.
- Identify criteria for evaluating control options.

**Model construction**
- Construct frameworks for each control option.
- Undertake sensitivity analysis and determine data requirements (iterations/refinement: discard inappropriate options).

**Data collection and model parameterisation**
- Data collation/collection.
- Data analysis and parameter estimation.

**Model analysis**
- Generation of recommendations: i) feasibility; ii) preconditions for success or failure.

(b) By whom?
- **Problem identification**: managers, modellers, field biologists, laboratory biologists.
- **Model construction**: modellers.
- **Data collection and model parameterisation**: modellers and field biologists.
- **Model analysis and generation of recommendations**: modellers, biologists, managers.
7.2.6 Physical barriers

(a) What needs to be done?
- First, prove that it works; series of control areas with appropriate research design.
- Foresight (mapping distribution) versus/and hindsight (removal of toads from an area – Queensland and Northern Territory).
- Identifying and ranking feasible landscapes.

(b) By whom?
- Sponsors to provide the capital expenditure for establishing the barrier and Governments (State and Federal) to do ongoing management expenditure.
- Universities; community groups.
- Community control: role for coordinator positions; mobile disposal facility (humane disposal of toads – as recommended); role for public relations and education.
8.0 Future Directions and Actions

The final stage of the workshop was to identify priority actions. Participants were divided into four working groups to consider the next steps: ecologists, virologists, policy-makers and ‘other’. The following is a summary of their suggestions.

8.1 Ecologists

It is clear there is a requirement for more impact studies, modeling and further surveys for the presence of pathogens in toads both in Australia and overseas. Inherent within this section is the uncertainty of the long-term impact of cane toads.

1) Conduct reality check modeling: evaluate validity of assumptions inherent in biological control options using models validated by field data.

2) In consultation with microbiologists, implement a properly designed, systematic survey of pathogens in toads and frogs in Australia and in cane toads overseas.

3) Identify the nature and scope of toad impacts on biodiversity.
   • Require urgent baseline data before toad arrival.
   • Require trial enclosures to assess and ameliorate impact.

Contacts: Rick Speare and Ross Alford, James Cook University; Rick Shine, University of Sydney, Alex Hyatt, AAHL; Mike Tyler, University of Adelaide, Hamish McCallum, University of Queensland.

8.2 Microbiologists

Again it is clear there is a need for (a) further surveys for the presence of pathogens in toads both in Australia and overseas and (b) the development of other strategies involving non-disseminating vectors and vectors with more ‘targets’ aimed at affecting different phases of the toad life cycle.

Each of the biological control options outlined below should be modeled for feasibility of success.

1) Identify gene targets specific to cane toads. Targets should encompass different phases of the whole life cycle.
   Contacts: Tony Robinson, Sustainable Ecosystems, CSIRO (consult with Mike Tyler, University of Adelaide; Mike Mahoney, University of Newcastle).

2) Evaluate feasibility of constructing a non-disseminating ranavirus.
   Contacts: Alex Hyatt and Jackie Pallister, AAHL, CSIRO.

3) Explore Bufo-specific pathogens more thoroughly.
   (a) Survey Australian cane toads for a new vector.
   (b) Assess which stages of life cycle are sensitive to new pathogens.
   (c) Determine whether pathogens are persistent virus (continual antigen presentation).
   (d) Survey toads other than B. marinus (overseas).
   (e) Trial new pathogens via inoculation with tissues homogenates from other Bufo species.
   Contacts: Alex Hyatt (exotic) AAHL, CSIRO; James Cook University (endemic).
4) In collaboration with ecologists, search for the presence of pathogens in toad and frog tissues both in Australia and overseas.

*Contacts: Rick Speare, James Cook University; Alex Hyatt, AAHL; Ross Alford, James Cook University; Mike Tyler, University of Adelaide.*

### 8.3 Policy-Makers

A National Working Group could be established to set-up the following functions. This approach would cover all levels of impact (short to long term). It would ensure that any research program would be comprehensive.

1. **Coordination.**
   The Working Group would be responsible for coordinating and advising on the national management approach to control cane toads. Such a group would have a dedicated coordinator position. The advice would encompass the acceptance of three levels of control, namely short term, medium term and long term.

2. **Short-term strategies for the control of cane toads (5 years).**
   The Working Group would explore the feasibility of short-term control options and make recommendations for their implementation. Such an approach would provide immediate strategies to eliminate/control/prevent some key areas from cane toad invasion.
   The Working Group would also be responsible for the standardisation of short-term measures such as (i) humane capture, euthanasia and disposal of cane toads and (ii) design and usage of traps.

3. **Medium-term strategies for the control of cane toads (5 to 10 years).**
   These types of activities could develop into long-term activities. The Working Group should explore the type of research opportunities that exist in this area. For example (i) assessment of Asian bufonids for potential pathogens (ii) undertake a range of autecological studies, particularly in relation to impacts and (iii) assess (and implement) whether techniques such as the daughterless cane toad male option is feasible via modelling.

4. **Long-term strategies for the control of cane toads (10 years).**
   The Working Group should also support studies into the long-term management of cane toads. Therefore the use of biological control (either self disseminating and/or non-disseminating) should be integrated into the full breadth of the ‘program’.

*Contact: Damian McRae, Department of Environment and Heritage; State and Territory agencies*

### 8.4 Other (conservationists and ‘custodians of the country’)

Both short-term and on-going (including long-term) strategies are required for the control of cane toads. The short-term strategies, including the dispatching of toads, need to be uniform throughout Australia.

**Short-term strategies (immediate actions):**

- Identify and/or establish toad-free islands and mainland barriers.
- For the above to be implemented the feasibility and conservation value (national significance) of the protective species/area must be assessed.
- Strategy protects species/area during the time lag that occurs while needed information is being gathered and decisions are being debated.
- States (Northern Territory and Western Australia) should implement common actions.
On-going actions:

Collection and disposal of toads.

- Conduct experiments to test and prove techniques (refer to comments for ‘Humane dispatching of cane toads’).
- The approach should be coordinated and involve non-Government organisations and the public using purpose-designed equipment (refer above) and a paid coordinator.
- Resources to come from Commonwealth/corporate sponsorship/non-Government organisations.

Future actions:

- Identify and isolate all active pathogens in Australia affecting frogs and toads.

Contact: Rick Speare, James Cook University

8.5 Common ‘threads’ from section 8.

From this section of the workshop, various groups identified some common areas. We have listed these areas below:

(a) There was a general consensus by ‘Ecologists’, ‘Microbiologists’, ‘Policy Makers’, and ‘Conservationists & Custodians of the Country’ that there is a need to look for other pathogens of toads and/or frogs.

(b) There was a general consensus by ‘Ecologists’, ‘Policy Makers’ and, ‘Conservationists & Custodians of the Country’ that:
   (i) there is a need for trial enclosures which would generate baseline data before and after impact.
   (ii) potential biological control strategies should be modeled to predict their chances of success.

9.0 Closing Remarks

The workshop was very productive. The outcomes from each of the areas identified in the Program were discussed and have endorsed by participants. From these outcomes a set of recommendations has been prepared*.

Workshop participants included people from many fields (policy-makers, ecologists, herpetologists, virologists, general biologists, molecular biologists, conservationists and public relations). The workshop atmosphere was positive with a collective dedication to constructive discussion of issues relating to the impact of toads and how their population numbers could be reduced. Many issues were discussed including ‘impact’ (are toads a serious risk to our environment), what control options are possible, what research has CSIRO been doing and what are the problems confronting the current research.

In addition to assessing current research on cane toads, participants identified a range of possible control strategies that could be used for short term and long-term control of cane toad populations. It was apparent that if the number of cane toads is to be significantly reduced in this country then a National Working Group should be formed whereby the recommendations of this report can be implemented.
This report will be submitted to the Australian Department of the Environment and Heritage where it is intended to serve as an initiative for future research on the control of cane toads.

10.0 Formulation of Recommendations

At the conclusion of the workshop, Ms Viv McWaters transcribed the workshop notes and submitted them to Dr Alex Hyatt and Dr Tony Robinson. Wherever invited presentations were given, these notes were checked by the invitees for accuracy. Dr Hyatt and Dr Robinson compiled the recommendations. Following the formulation of the recommendations, a draft of the report was sent to all participants and their returned comments were incorporated into the report.
11 Appendices
## Appendix 1: Workshop Participants

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<thead>
<tr>
<th>Name</th>
<th>Institution/Position</th>
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Appendix 2: Department of Environment and Heritage review of CSIRO research

Report on the project: The development of a cane toad biological control

Executive Summary
This document is a review of the federally funded project “The Development of a Cane Toad Biological Control” Commonwealth ID 29495. Terms of reference for the review are to assess: 1) methodology and effectiveness of the project in achieving objectives set to date; 2) the likelihood of the project achieving objectives proposed for the next twelve months and three years; 3) matters that may impact on the potential of the project to meet its overall aim of developing an effective, self-disseminating viral vector to disrupt the development of cane toads in a manner that will reduce the threat of cane toads on the survival of native Australian fauna; and 4) the administration of financial planning and project management.

Overall, objectives set to date have been achieved in a timely and efficient fashion with minimal disruptions. There have been some minor difficulties in establishing a captive colony of cane toads and this issue requires ongoing assessment. Excellent progress has been made with respect to identifying genes capable of blocking cane toad metamorphosis. The likelihood of achieving proposed one and three year objectives during the next stage of the project is considered high. However, a more comprehensive plan for testing native species is required to ensure the viral vector has been weakened successfully and that the genes selected to block cane toad metamorphosis are specific to this species. It is anticipated that several of the objectives will continue beyond the three-year timeframe.

Several issues impact on the potential for this project to meet its overall objective of producing an effective, self-disseminating viral vector to disrupt the development of cane toads. In particular, the paucity of information on the proposed viral vector ranavirus needs to be addressed. In addition, the risk of the released virus escaping to countries with native cane toads must be assessed. If the team is unable to address these issues, which are central to developing a successful biological control, this must be made clear. Environment Australia should assess the implications of these gaps in achieving the overall aim.

The generation of a fully tested biological control is a long-term solution to the cane toad problem. There is a need for Environment Australia to consider other avenues for addressing the problem in the short to medium term.

Recommendations
The panel contributing to this report recommends the following:

1. The project should proceed for three years subject to the considerations raised in this review.
2. Department of Environment and Heritage should investigate and execute complimentary research activities outlined in this review.
3. Department of Environment and Heritage should establish processes to consult with relevant stakeholders on the project’s progress, cane toad control issues, complimentary research, communication and research coordination. For example a biannual workshop coordinated by DEH to discuss cane toad research and future directions.
Background to the Project

Since their introduction in 1935, cane toads have spread rapidly across northern Australia. Current estimates (CSIRO, 2002) indicate the toads are spreading at around 27km/year and now extend from northern NSW into the Northern Territory. The toads now threaten many World Heritage areas including Kakadu National Park.

Cane Toads mainly affect native species through:
- Poisoning predators (e.g., Quolls, Goannas, Crocodiles) by secreting bufotoxin; and
- Competing for habitat and food resources with other reptiles and amphibians.

According to CSIRO (2002), cane toads may also spread diseases that could be responsible for declines in Australian frog populations.

During the 1990s, CSIRO further developed its significant expertise in the biological control of pest species in Australia. Their work on genetic manipulation of viruses to interrupt animal development was seen as a potential alternative traditional pest management to baiting, trapping and hunting.

At the same time, cultural pressures were pushing pest management from its traditional predation emphasis to more “humane” methods such as reproductive control. Apart from this cultural change, rapid advances in gene technology at the end of the 1990s provided major opportunities for biological controls using attenuated viruses.

This project builds on earlier (1990-1993) work where researchers embarked on a $1.25 million project to find cane toad pathogens in Venezuela. The project also included ecological studies in Venezuela and Australia.

From 1993-1997, a second, $2.0 million cane toad study completed the ecological work in Venezuela and continued a more intensive investigation of possible viruses useful as a biological control.

In early 2000, Senator Robert Hill, the then Minister for the Environment and Heritage, lent his support to biological pest controls for cane toads. This report reviews the success of that $1 million research project over 2 years.

Term of Reference 1

The methodology and effectiveness of the project research to:
- Establish and maintain a breeding colony of cane toads to ensure supply of all stages of life cycle for experimental work.

A breeding colony of cane toads has been successfully established at CSIRO Sustainable Ecosystems, Canberra. Breeding techniques were established with the help of Megan Bradley, University of Queensland. However, the first attempt at establishing a colony was unsuccessful due to a parasitic infection. The colony was re-established and had supplied material for the generation of a cDNA library and RNA samples for micro array screening. Recently, a fungal infection has affected the colony but it is hoped that this can be eliminated and that the colony can continue to breed and supply appropriate materials for molecular biology work.

The fact that a there have been a number of difficulties in establishing an ongoing colony of cane toads raises several issues including the following:

a) Are improved facilities or more expertise required?

b) Will this require more resources?
c) Is it feasible to obtain appropriate animals from the wild on a needs basis? This was raised with the researchers and we were informed that such an exercise would be costly and would lead to a lower guarantee of animal supply when required. However, this issue needs to be regularly assessed.

d) AAHL (Australian Animal Health Laboratories) in Geelong will require a supply of cane toads in the future for virus testing. The possibility that one colony may be sufficient to provide materials for both labs should be investigated.

- **Demonstrate that ranaviruses can be attenuated i.e. do not cause disease in indicator species.**
- **Demonstrate ability to create recombinant ranaviruses.**

Attempts to attenuate the ranavirus have been carried out in two ways:

1. Passage of the virus up to 100 times in cell culture; and
2. “Rational” attenuation where a neomycin resistance gene has been inserted into the selected gene of the virus.

This latter work required considerable background work, searching for “non-essential genes” in the ranavirus using comparisons with other large DNA viruses, cloning these genes from the ranavirus and using recombination techniques to insert a foreign gene into the virus. To date a neomycin resistance gene has been inserted into the selected gene but experiments are underway to insert the neomycin resistance gene into 3 other areas of the virus genome. It was also necessary to find a viral promoter to “drive” the expression of the reporter gene.

This has been successfully achieved. The insertion of foreign DNA into the ranavirus is a “world first” for ranaviruses. Clearly, the ability to create a recombinant ranavirus has been demonstrated.

Both of the viruses generated above have been tested for attenuation in Litoria infrafrenata. This experiment involved bathing frogs in $10^5$ TCID$_{50}$/ml/frog. All the frogs infected with wild type virus died whereas none of the frogs infected with the “attenuated” viruses died. This is a very promising result and needs to be repeated. It may be that the “attenuated” viruses did not infect the animals as initial screens for virus using PCR were negative. This will need to be further tested. From this initial experiment it appears that the ranavirus may be able to be attenuated but more experiments will need to be carried out to confirm this.

This part of the project is progressing very well and if the initial result is repeated and the modified virus is in fact still infective then this can move to the next phase of research as outlined in plans for the next year and three years.

- **Identify genes critical to distinct developmental stages and assess their potential to disrupt development.**

Micro array technology was used to identify genes involved in toad development. This is a very feasible approach and has already yielded some interesting and potentially useful results. Details of the technology are given in the Major Review Document for the project (November 2002). The technology has now been well established by the researchers.

To date, a number of genes specifically expressed in adult frogs and not in tadpoles have been identified. Some of these genes may be useful candidates for insertion into the viral vector to block metamorphosis. However, more needs to be done to screen for expression across different developmental stages to ensure that potential genes are expressed at limited stages of development.
In addition, subtractive cDNA libraries need to be made in order to screen for genes that are less highly expressed. Overall, there has been excellent progress in this part of the project.

A previous publication has shown that injection of the adult beta-globin protein into Bullfrog tadpoles blocked development (Maniatis et al 1969, Science, 165, 67-69). The adult beta-globin gene was cloned from the cane toad, protein generated in vitro and injected into cane toad tadpoles as a test of the principal involved. The survival rate for those animals injected with beta-globin or mock-injected was identical. The reason for this was investigated and it was found that while the adult cane toad was no longer producing adult beta-globin, it maintained expression of the tadpole globin, thus allowing survival. Thus, while the globin gene may not be a good target for blocking metamorphosis in cane toads, this experiment shows that injecting an adult gene into a tadpole can alter gene expression. Whether this is through an immune-related mechanism or another mechanism is unknown.

The focus now needs to be on the identification the most suitable gene(s) that can potentially inhibit metamorphosis.

Findings from Term of Reference 1

1. While a cane toad colony has been established there have been a number of problems with maintenance. The panel recommends that the feasibility of maintaining a breeding colony be continuously monitored. The possible sources of infections need to be rigorously investigated and the need for higher containment levels or more expert advice also investigated.
2. Good progress has been made with respect to creating recombinant ranaviruses. Current activities should continue.
3. The development of an attenuated virus is ongoing with some success evident. Current efforts should be continued with the development of a more substantial plan for screening of native species (see Term of Reference 2).
4. The technology to identify gene critical in cane toad metamorphosis has been established and some progress made towards identifying such genes. It is recommended to continue these activities.

Term of Reference 2

The likelihood of the project achieving the proposed 1 and 3-year objectives, which have a time frame beyond the current approved funding.

One-year objectives

1. Continuation of passaging in cell culture for attenuation.
   • On the basis of progress thus far this aim is considered achievable.
   • This aim is ongoing for three years.

2. Continuation of targeted attenuation by insertion/deletion in up to four non-essential genes of the ranavirus.
   • This is achievable.
   • This aim is ongoing for three years.

3. Construction of prototype viruses containing either adult globin or tadpole globin (control construct).
   • This is achievable.
4. Development of technology for producing a recombinant that does not contain antibiotic resistance genes.
   • This is achievable.

5. Testing in tadpoles of genes found in the current micro-array screening.
   • This is achievable.

6. Creation of a subtracted library to search for further differentially expressed genes.
   • This is achievable.
   • This should be a priority.

7. Produce a gene subtracted library on micro arrays to look for species-specific genes as well as more potential target genes.
   • This is achievable.
   • This should be a priority.

8. Investigate the RNAi concept.
   • This is achievable.
   • This should be a priority.

Three-year objectives
1. Attenuation of virus by passaging in cell culture as well as deletion/interruption of one or more non-essential genes.
   • This is achievable.

2. Construction of a prototype virus containing adult globin, tadpole globin or other potential targets identified in Canberra.
   • This is achievable.
   • It should be possible by this stage to focus on other potential targets.
   • Construction of prototype virus with either adult or tadpole globin should be completed within one year.

3. Testing of potential recombinant/attenuated viruses in a number of indicator species as well as cane toad tadpoles.
   • A more substantial plan for comprehensive testing needs to be developed.
   • This is not a trivial task (see Term of Reference 3) and should precede the proposed aim as currently stated.

4. Creation of a recombinant virus lacking antibiotic resistance genes.
   • This is achievable.
   • The researchers have a reasonable plan to achieve this.

5. Assessment of level of expression of foreign genes using different promoters.
   • This is achievable.
   • Once this task has been undertaken in tissue culture it will need to be tested in toads or tadpoles since promoters can behave very differently in an in vivo situation compared to cell culture conditions.

6. Development of the ranavirus as a vector for delivery of RNAi.
   • This is achievable.
7. Continuation of identification of metamorphosis genes using micro array analysis.
   - This is achievable.

8. Investigation and characterisation of the beta globin switching phenomena.
   - This objective seems peripheral and unnecessary for achieving the overall project aim.

9. Development of the RNAi approach
   - This is achievable.

10. Development of the use of micro arrays to identify cane toad specific genes.
    - This is achievable.

11. Use of subtracted libraries to search for further differentially expressed genes expressed at low levels.
    - This is achievable.

12. Refinement of micro array analysis of gene expression across development using thyroid hormone.
    - This aim may take longer than 3 years to complete but is achievable given the expertise of the researchers.

Findings from Term of Reference 2

1. The likelihood of achieving both the one-year and three-year objectives is high.
2. Refinement of the micro array analysis for all the studies outlined in the three-year objectives is likely to continue past the three-year timeframe.
3. A comprehensive plan for testing of native species, both for the specificity of the genes isolated as well as for virus attenuation, needs to be established and some of this testing commenced.
4. Complete testing will take considerably longer than three years.

Term of Reference 3

Any matters that may impact on the potential for this project to meet the overall objective of developing an effective self-disseminating viral vector to disrupt the development of cane toads in a manner that will reduce the threat of cane toads on the survival of native Australian fauna.

The Likelihood Of Obtaining A Gene That Will Disrupt Cane Toad But Not Frog Metamorphosis

Discussion with stakeholders and the project team strongly suggests that this is possible as there is sufficient developmental difference between cane toads and native frogs. The micro array technology is now in place and should allow the identification of potential genes. The ability to clone these genes, generate protein and/or clone them into the virus, and deliver the protein or the virus to cane toads is also in place. This part of the project is feasible.

Potential genes should be obtained and some tested within the three-year timeframe. It is important, however, that screening for species-specific genes is guided by a detailed plan to ensure that native species are comprehensively tested. It is unlikely to be feasible (and is probably unnecessary) to screen all of the 200 plus species of Australian frogs. Determination of species to be screened and rationale behind their selection needs to be presented. This would require consideration of phylogenetics, which is not well established for Australian frogs.
The Feasibility Of Generating An Attenuated Virus
Preliminary experiments suggest that the team have already obtained an attenuated virus but this needs to be verified and the infectivity of these viruses also verified. The focus on “rational” attenuation is very appropriate.

Comprehensive testing is required to ensure that Australia’s native species are not at risk through the release of the attenuated, recombinant ranavirus. This is a major task that will require substantial expertise from outside the project team. Considerable planning needs to go into determining which species are to be tested.

Ecology (eg. habitat preferences and behaviour in the environment) may differ substantially between species that are closely related taxonomically. Inter-specific variability in time spent basking, for example, can influence susceptibility to pathogens.

The costs and feasibility of comprehensively testing indicator species should be considered carefully as well as associated ethical issues. For example, there are threatened frog species with very few remaining populations like the Southern Corroboree Frog. Questions remain as to how their susceptibility will be determined.

Species, such as the Gastric Brooding Frogs *Rheobatrachus silus* and *R. vitellinus*, are now considered extinct in the wild. Is this assumed to be the case or would the susceptibility of such species (that have unique physiology) be considered?

Ranaviruses are also known to infect other major animal groups, namely reptiles and fish. These animal groups would also require testing. Susceptibility to the virus may vary across different life stages of an amphibian species. Effects of exposure during the egg, tadpole, metamorph and adult stage should all be considered.

Susceptibility to disease from the attenuated ranavirus is likely to differ with environmental conditions and the health of the exposed individual. These should be considerations for testing.

The objective of testing the recombinant/attenuated virus in a number of indicator species should be preceded by a more specific objective of developing a detailed strategy for comprehensively testing Australian frogs, fish and reptiles for successful attenuation. Such a strategy should demonstrate that expertise has been sought from frog, fish and reptile biologists, ecologists and virologists.

Selection Of A Viral Vector That Will Be Effective And Self-Disseminating
The ecology of ranaviruses remains virtually unknown (eg. its survival, mobility and pathogenicity in the wild). The capacity for a ranavirus to provide an appropriate viral vector will be influenced by these characteristics. Research is required addressing these issues to ensure that the considerable effort being invested into the attenuation and recombination of the ranavirus is not wasted.

Specifically, further information is required on the following issues:
- Is the ranavirus present in the wild cane toad population?
- Ranavuses have been isolated only once in Australia from a native frog species - *Limnodynastes ornatus*. This is useful foundational information. However, the prevalence of the ranavirus in wild cane toad populations needs to be determined, particularly during the tadpole stage (as this is the proposed stage of the life cycle to be targeted). It is understood that there is a ranavirus present throughout the range of the wild adult cane toad population (shown in data presented by the research team during review process).
The prevalence of ranaviruses specifically has not yet been determined. Ranaviruses are considered extremely robust in nature and readily transported through the environment. For this reason no specific host for transmitting the virus around the environment has been considered necessary.

Given the high densities at which cane toad populations occur (and the robust nature of ranaviruses) it seems logical that if ranaviruses are present in the wild population, the effects should have been considerable. Either ranaviruses are not present, or they are not as robust, virulent or transportable in the natural environment as thought. This issue must be resolved. If ranaviruses are found to be present in the wild cane toad population but prevalence is low then another means of delivery for the virus may be required.

- How does environmental variability influence the prevalence of ranaviruses?

Factors driving prevalence in the natural environment will need to be explored and understood if a ranavirus is to be used as the viral vector. This may relate to variability in mobility or capacity to infect under natural conditions. This is a major challenge, requiring substantial field work and monitoring of cane toad populations (if ranaviruses are present in wild populations). It is important to note that a ranavirus in its wild form may not reflect the nature of the attenuated virus. Similarity between behaviour of wild type ranaviruses and the attenuated recombinant ranavirus will need to be demonstrated.

Concern Regarding The General Issue Of Releasing A Recombinant Form Of Ranavirus

The potential for virus mutation is naturally a major issue of concern in releasing a form of ranavirus into the natural environment. Ranaviruses are considered a stable virus and the probability of it reverting back to a pathogenic form is considered by the project team to be close to zero. However, the broader scientific community does not share this confidence.

Evidence of the stability of ranaviruses or other similar viruses must be presented (ie. scientific literature and detailed rationale) so that the scientific and lay community may be confident that the issue has been properly considered. Personal assurance is unlikely to be sufficient. Confidence from the broader community will be essential if the virus is eventually to be released.

Containment Of The Recombinant Virus Within Australia

Ranaviruses can survive in the environment (ie. not in a host) in a moist or desiccated state for extended periods. For example, data presented by the project team showed that in its desiccated form (at 27 degrees Celsius) the virus could survive up to eight weeks.

It is therefore conceivable that someone could transport the virus unknowingly (eg. on their boots or fishing gear) to another country. The implications of such an event are two fold. Firstly, the virus could be transferred to a country where native species of amphibians, fish or reptiles were susceptible (ie. the virus was not sufficiently attenuated for these species) resulting in mortality and or sickness. Secondly, the virus could be transferred to a country where cane toads were native, resulting in the disruption of cane toad metamorphosis and subsequent loss of the species from their natural ecosystem.

Risk of disease transfer between countries is clearly an issue for quarantine authorities. However, a recombinant form of a ranavirus that can disrupt cane toad metamorphosis should not be released unless it is feasible that a ranavirus could be contained within Australia. The outcomes of this feasibility exercise will influence the future directions and continuation of this project.

It should not be the responsibility of the project team to undertake this feasibility exercise. However, it would be appropriate for the project team to consider how the virus may be manipulated to reduce risk of spreading to other countries. For example, it may be possible to
manipulate the virus and reduce its survival time in a desiccated state, without reducing its effectiveness as a biological control. If the adult cane toad is able to carry the virus then survival of the virus in a desiccated state will be less crucial for virus mobility.

Demonstration Of Proof Of Concept To The Scientific And Broader Community
The project team has clearly been proactive through the media in providing the general community with information on the research. However, it is just as important that the broader scientific community is informed of the progress of the research, through forums such as scientific conferences, workshops and journals. The project team is no doubt engaged in such activities, however it is important that evidence is presented in future progress reports, along with media coverage.

This project is clearly of interest to a diverse range of scientists and practitioners from amateur herpetologists to ecologists and virologists. Scientists and other practitioners are likely to be able to assist the project in some way if they are kept informed of its progress. Keeping the entire community informed of project progress is in the best interests of the project, as concern will only build over time.

Capacity Of The Project Team To Address Overall Objective
If the overall objective of this project is to develop a biological control then the issues raised through this review should be incorporated into a comprehensive plan. For example:
- Developing a comprehensive approach to testing attenuation;
- Developing an understanding of the pathogenecity and prevalence of the ranavirus in the wild; and
- Exploring the feasibility of containing the virus within Australia.

The project team would probably incorporate these issues into the program after the next three years. Understanding how the virus behaves in the wild is likely to require a long-term study and should therefore receive attention within the next three to five years. If the project team does not intend to address these issues, which are pertinent to the overall objective, then this must be made clear and EA should assess the implications of these gaps in achieving the overall aim.

Generation of a fully tested biological control is a long-term solution and should not be the only method for addressing the cane toad problem.
This is a long-term research project that is unlikely to yield results (ie. a reduction in cane toad numbers in the wild) within the next decade. Meanwhile, the cane toad problem continues to spread geographically. This issue is not a criticism or limitation of the project in question, but an outcome of the review panel examining the overarching issue of the need for cane toad control.

Research to date has identified the potential to reduce cane toad numbers in targeted areas. Specifically, cane toad preference for breeding in open areas offers opportunities for decreasing habitat quality through revegetation of riparian zones and wetlands. In addition, cane toads differ from native amphibians in that they have a well-developed sense of smell that is used to locate food. This trait could potentially be used in baiting to attract cane toads to certain areas, or to deter them.

While such approaches will not reduce the numbers of cane toads overall, they are likely to provide benefits to specific areas where cane toads are likely to have their greatest impacts. For example, areas such as Kakadu, or localised environments inhabited by rare or threatened species, could be targeted especially in the short to medium term.
There are areas within Australia that have not yet been invaded by cane toads. Currently, there are no obvious barriers to cane toad spread on the Australian mainland. However, islands may be effectively protected against cane toads through the development of a strategy and support framework for local communities managing these areas. This should be a high priority for the conservation of island biodiversity.

Findings from Terms Of Reference 3

1. Identifying a gene that will be specific for toad development/metamorphosis is achievable. A plan for screening for specificity needs to be developed.
2. It would appear feasible to generate an attenuated virus. Once again a comprehensive plan for the testing phase, as detailed above, needs to be established. The strategy has to take into account issue raised above.
3. It is unclear if the ranavirus will self-disseminate to the extent necessary for a successful biological control. This issue requires research on the ecology, survival, mobility and pathogenicity of the ranavirus as a long-term goal. Specifically the team needs to develop an assay to isolate the ranavirus or to detect the ranavirus antibodies in cane toads. It appears some of this work has been undertaken (see Whittington O'Rourke, Hyatt and Chisholm 2002 MOLECULAR AND CELLULAR PROBES 16 (2): 137-151). EA and the research team need to investigate sources of sick toads that could have the ranavirus. This could be facilitated through existing networks (eg The Northern Territory Frog Database - http://www.frogwatch.org.au/website; Double Helix) or through the development of a new network. Resource constraints may limit the research team’s ability to fully achieve this (see point 7 below).
4. From a scientific perspective it is reasonable to assume that mutation(s) to alter the attenuated virus to a more virulent form are very unlikely to occur if the virus is released. However, this needs to be communicated to the public so that concerns are covered. Stakeholder interviews implied that some in the scientific community also held concerns about the release of a virus. If the scientists are not in full agreement then this is likely to lead to conflict in the wider community.
5. EA and AQIS need to plan for the possibility of a released virus escaping from Australia. Such issues as illegal export of live frogs and physical transfer of the virus (eg. on fishing equipment) need to be investigated.
6. Notwithstanding intellectual property issues, good communication with the scientific community as well as media needs to be undertaken. A communication strategy that takes into account scientific conferences, scientific publications, and media releases needs to be put in place by the research team.
7. The project team is already fully committed and does not have the capacity to engage in additional activities without significantly more resources. EA needs to address this issue.
8. While the project is achieving its stated objectives, cane toads continue to spread. EA needs to investigate short to medium-term strategies to manage this issue. EA needs to engage a wider range of stakeholders and expertise to develop and execute this strategy.

Term of Reference 4

The administration of financial planning and project management with particular reference to the effectiveness of current arrangements in ensuring the best possible use of available financial and staffing arrangements.

Current Project Management Arrangements

Proponent reports and panel interviews indicate the administration of financial planning and project management is effective. Available financial, human and physical resources have been applied with
due diligence. The co-location of facilities and staff does not appear to have hindered the project. This indicates a high level of integration within the project team and its management.

Panel interviews and progress reports indicate:
- Established reporting procedures are consistent with the contract requirements.
- The project team is focused and not burdened by administrative tasks, multiple priorities and dependent tasks stalling future progress.
- The approach to project management demonstrates a flexibility that has enabled the project team to move forward despite occasional barriers.
- Project logistics, employment, permit procedures, and financial administration has been executed to high standard.

Current Financial Administration Arrangements
The strategic and timely application of human resources, lab facilities, and new technology indicates rigorous financial administration within the project. This has been demonstrated through:
- The budget discipline detailed in progress reports;
- Adherence to contracted financial arrangements;
- Appropriate cost sharing;
- Acquittal of in-kind and cash inputs; and
- The achievement of “cutting edge’ scientific results without additional costs.

Possible Future Project Management Barriers Influenced By Current Arrangements
The project achieved all its milestones within the contracted deadlines except for testing the attenuated virus in indicator species. This projected four-month task was executed over 12 months resulting in an eight-month delay. The task was not critical to subsequent activities.

According the proponent’s Major Project Review (November 2002), the delay was caused by:
- Unspecified problems with the supply of *Litoria infrafrenata*; and
- Unspecified health problems with the supplied frogs.

The first batch of experimental cane toads supplied from the wild suffered health problems in the laboratory at CSIRO, Canberra. According to the panel interviews a subsequent breeding colony of cane toads was established on time (January 2002) but is currently stressed by a fungus. This is the second batch of toads to encounter health problems. Stakeholder interviews, panel discussions and progress reports indicate that establishing and especially maintaining a toad colony in the lab remains a difficult task.

All these factors point to a problem with the supply and propagation of test species. If maintaining a cane toad colony and individuals of *Litoria infrafrenata* proves to be difficult then presumably the maintenance of other frog species may also be problematic. Possible problems associated with the external supply of testing frogs indicate that these issues may delay future testing milestones. According to the proponent’s Major Project Review (Nov 2002, p12), “It is essential that the cane toad-breeding colony is maintained in Canberra to supply material for the discovery and testing of potential biocontrol targets.”

This is especially significant in the medium-term (three years) where the proponent’s planning has a milestone delivering the testing of recombinant/attenuated viruses in a number of indicator species as well as cane toad tadpoles. Assuming the project achieved its three-year target, the project infrastructure for raising and maintaining test animals will be substantial and may need additional resources. The panel did not inspect facilities at AAHL so a definitive assessment is not possible at this time.
As stated in TOR 1 this may be achieved with a single cane toad breeding facility that consolidates the team’s infrastructure and resources. More importantly, discussions with stakeholders indicate that a rigorous testing program will be required for fish, amphibians and reptiles should the ranavirus be used as the delivery mechanism for the subject gene.

Those discussions also indicate that lab testing is profoundly easier than testing under wild or natural conditions. In the long-term (greater than three years), this consideration may be one of the more difficult project management obstacles to overcome. Notwithstanding the project logistics, the permit requirements associated with the release of a genetically modified virus will be problematic and complex. Many of these problems will relate to the security, quality and diversity (i.e. extent of testing among different species) of any future testing program.

Looking at the proponent’s plans and considering the panel’s discussions with stakeholders there appears to be several critical areas requiring complimentary research for this project. These include the following:

1. Complimenting biological controls with ecological controls to establish a high kill-rate. According to stakeholder interviews, cane toads are density-dependent. A 100 per cent kill-rate may be required for a biological control to achieve its objectives.

2. Virus ecology (e.g. are ranaviruses environmentally mediated?)

3. Cane toad ecology, extent and population dynamics

4. Other possible management techniques that stress local cane toad populations (e.g. trapping through “olfactory” baits or lures)

5. Cane toad impact studies to establishing baseline and success monitoring for any future controls (e.g. how do cane toads affect native species and how do they recover with cane toad control?).

6. The integration into the project of capacity building and monitoring programs to: establish a reporting network on the extent of existing cane toad populations, possible effects of any controls and the collection and delivery of sick toads.

Findings from Term of Reference 4

1. Project management and financial administration is effective.
2. Testing is likely to be a significant management obstacle.
3. Complimentary research activities need to be coordinated with the project.
4. The establishment of these complimentary projects cannot be effectively achieved within a one-year but could be executed within three years.
## Attachment 1: Stakeholder Interview Record

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<th>Name</th>
<th>Organisation</th>
<th>Phone Number</th>
<th>Interview Time/Date</th>
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<tr>
<td>A/Professor Michael Tyler</td>
<td>Uni of Adelaide</td>
<td>08 83035977</td>
<td>22/1/03 11.08am</td>
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<tr>
<td>A/Professor Ross Alford</td>
<td>James Cook Uni</td>
<td>07 47741659</td>
<td>21/1/03 10.00am</td>
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<td>A/Professor Richard Speare</td>
<td>James Cook Uni</td>
<td>07 47225777</td>
<td>24/1/03 11.10am</td>
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<td>Dr Jean-Marc Hero</td>
<td>Griffith Uni</td>
<td>07 55528661</td>
<td>22/1/03 9.00am</td>
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<td>Dr Gerry Marantelli</td>
<td></td>
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<td>22/1/03 9.40am</td>
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<td>Deborah Pergolotti</td>
<td>Cairns Frog Hospital</td>
<td>07 40530367</td>
<td>22/1/03 2.00pm</td>
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Appendix 3: Traps (Northern Territory)

Northern Territory Government

Cane toad management

Development of lure and trap method

Commercial-in-confidence

An expression of interest to Ms Delia Lawrie, MLA Chair Cane Toad Enquiry Sessional Committee on Environment and Sustainable Development

Prepared by FDF Pty Ltd for Auszeal Plastics Pty Ltd in association with M.K. Bird Deterrents Michael J. Tyler, A.O., D.Sc. and FDF Pty Ltd

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Preamble
The Legislative Assembly of the Northern Territory Sessional Committee on Environment and Sustainable Development (SCESD) reported in October 2003 on the Issues associated with the progressive entry into the Northern Territory of cane toads.
In the course of this investigation, Dr Michael Tyler AO, has assessed that an astonishing one million square kilometres of Australia’s subtropical and tropical zones has been colonised by cane toads (Bufo marinus).
The SCESD has reported that the rate of spread of cane toads is some 30 kilometres per year. A significant number of species of the Northern Territory’s native fauna is threatened by this encroachment of the cane toad. And there is a commensurate concern for the socio-economic impacts that cane toad infestation will have upon the Northern Territory’s indigenous food sources; water resources; tourism, agricultural and other economic sectors.
The SCESD also reported that:
• there is no short-term solution in managing the impact of the cane toad;
• there has been no effective biological or chemical control method found yet for the cane toad; and
• there is a need for a comprehensive management approach towards the control and possible eradication of cane toads in Australia.
It is within this context that this document has been prepared to introduce a proposal to develop a lure and trap method (L & TM) for cane toad management. The method employs only electro-mechanical components. However, it is possible that the efficacy of the aural lure could be abetted by complementary pheromone and or olfactory (food) emitters.
This expression of interest calls for a statement of the support which the Government of the Northern Territory might provide for the development of a lure and trap method for cane toad management.

Part 1
Proposed lure and trap method
The lure and trap system entails:
• a device which emits the mating call of the male cane toad to attract egg-laden females. This device incorporates heat, moisture and light sensors to trigger the selection of an apposite mating call, from a library of digitally formatted cane toad calls. The sound power level of the mating call can be varied and the repetition of the call will replicate that of toads at large;
• a solar cell and storage battery system to provide electrical power for the operation of the calling device and a light at the trap which acts as an insect lure and feed source for the toad, thereby complementing the mating call lure; and
• an essentially cylindrical, moulded plastic vessel which contains the trapped toads and supports the calling device and associated solar power system. The vessel of some 1.800mm diameter and height of about 400mm, which sits upon and is anchored to flat terrain, includes regularly spaced tapered inlet tunnels with a non-return wire flap. The vessel is stackable in the interest of minimising (volumetric) freight costs. A flap is also incorporated to facilitate the collection of the captured toads prior to their controlled extermination.
A Provisional Patent Application 2004900197 has been granted to Mark Anderson for a cane toad lure and trap described essentially as above.

Part 2
Proponents and roles
The principal for the cane toad lure and trap is Auszeal Plastics in association with M.K. Bird Deterrents, Michael Tyler and Associates and FDF Pty Ltd.
Auszeal Plastics
This enterprise, owned by Mark Anderson, is a manufacturer, using rotational moulding technologies, of vessels and other items used in agricultural production. Mark Anderson completed a fitter and turner apprenticeship before graduating in mechanical engineering from the University of Queensland in 1966. Mark’s career has also included teaching, product research and development (with Weapons Research Establishment (now DSTO), James Hardie, ACI Electronics, J Furphy and Sons). Auszeal Plastics will manufacture and supply the cane toad lure and trap product described in Part One.

M.K. Bird Deterrents
This company manufactures and distributes electronic bird scaring devices used as a bird control by the viticultural and stone-fruit industries. The product has been developed by M.K. Bird Deterrents. It uses a library of the alarm calls of the principal species which destroy high unit value fruit crops. The alarm calls are replicated by electronic circuitry, amplifier and speaker which are powered by a solar system.

M.K. Bird Deterrents will manufacture and assemble the electronic and acoustic systems which will be embodied in the cane toad lure and trap. These systems will adapt the technologies developed and commercialised for their bird scarers.

Michael Tyler and Associates
The principal, Michael J. Tyler A.O., M.Sc., D.Sc., is a renown environmental biologist with expert knowledge of frogs. Michael provided advice to the Northern Territory Sessional Committee on Environment and Sustainable Development which addressed issues associated with the progressive entry into the Northern Territory of cane toads.

Michael Tyler first proposed cane toad control in 1963. He was seconded to the Department of Agriculture in Papua New Guinea in 1967 to examine the impact of toads on various plantations. Michael has published extensively on native frogs and cane toads (more than 350 scientific papers and 14 books) and was a member of the former national Cane Toad Control Committee.

Dr Tyler will provide advice on all behavioural and biological characteristics of the toad which need to be reflected in the design and fabrication of the lure and trap system.

FDF Pty Ltd
FDF is a services company engaged in project and environmental management. Trevor Fuller, the founding principal, holds degrees in engineering and management and has more than 30 years’ experience in project evaluation and implementation. He will manage and administer the program of research and development for the cane toad lure and trap product.

Nigel Rockliffe, an associate of FDF, is an economist, expert in benefit cost analysis. He is a graduate of Oxford University and London School of Economics. Nigel will undertake the economic evaluation of the lure and trap product proposal. This will entail estimation of the value of benefits and costs, ecological and otherwise, which will accrue to Northern Territory communities, tourism, agricultural and other economic sectors.

Other
Auszeal Plastics and its associates as proponents of this research and development program for a lure and trap envisage collaboration with a broad group of stakeholders including the Parks and Wildlife Commission of the Northern Territory and CSIRO. An immediate prospect for collaboration is the matter of creating a suitably rich library of toad mating calls which will be reproduced by the aural lure.

We will also seek close engagement with those stakeholders who might assume responsibility for deployment and maintenance of the lure and trap product in the course of field trials and subsequent field use.
Part 3
Outline of research and development program

Chart 3.1 provides an overview of the process foreshadowed for the research and development program. This work is directed at realising a product which will maximise the unit economic welfare benefit derived from the lure and trap.

The research and development will complement and build upon the extant knowledge we have from Auzseal and M.K. Bird Deterrent products.

Scope
Our work will focus initially on the development of an effective aural lure and of a trap which will secure the enticed toads.

Some of the matters of efficacy of the product which have already been addressed at concept level, and which will be examined in the product pilot development phase are listed here under several categories.

A. Biological (cane toad behaviour and physiology)
- characterisation of mating call, its variation with heat, humidity, light and other environmental conditions; toad size etc;
- toad call fundamental frequency (about 0.5kHz);
- olfactory and feed complements to aural lure;
- pheromone complement to aural lure;
• seasonality of toad mating;
• toad mass and body dimensional characterisation; leap and ambulatory characterisation;
• reproduction of call (lure);
• species specificity (singularity) to avoid collateral frog and toad catches;
• call range catchment (geographic area of influence of functioning unit);
• catch degradation (repelling toads?);
• catch disposal;
• efficacy of lure across toad size range;
• efficacy over time given learned responses of toads; and
• ecosystem (wetland, agricultural, urban/residential) performance dependencies/influences.

B. Trap mechanics
• volumetric size and form;
• toad access systems (tunnel/funnel entrance form, or tilting platform);
• toad containment;
• anchorage/security;
• robustness/durability; and
• transportability (without damage);
• system for toad catch removal;
• integration and mounting of aural lure and power systems with trap vessel; and
• trap vessel durability.

C. Electronic and electrical system
• aural performance of speakers; reliability;
• solar power system; and
• sound reproduction system and programming of mating call library for heat, moisture and
  light parameters.

Method
The critical phases of the lure and trap product development are:
(i) proving up the efficacy of the aural lure. This requires a library of mating calls of
  appropriate sound quality and integrity to be established. With this material the prototype
  electronics can be manufactured and integrated with speakers to permit field trials
  independent of the mechanical trap. Using this prototype we will establish an efficacy
  profile for the lure with and without complementary food and or olfactory lures and thus
  finesse the design.
(ii) test alternative trap formats. There are matters of the tunnel entrance funnel geometry to be
  optimised and of the non-return system. These aspects will need to be responsive to the size
  distribution of the female toad population.
(iii) optimise the lure and trap elements to minimise unit production costs in the context of the
  estimated scale of field application.
Timeframe
In broad terms we envisage the following time line (of broadly sequential activities) to establish a toad lure and trap product:

(i) field recordings of toad mating calls: 1 month
(ii) sound recording cleaning and preparation: 1 month
(iii) electronic reproduction and amplification system and solar power unit design and manufacture of pilot unit: 6 months
(iv) field test aural lure system (subject to seasonal conditions): 3 to 6 months
(v) construct alternative trap prototypes (activity in parallel with (i) to (iv)): Nil
(vi) integrated aural (and other) lure and trap pilot test phase and prototype development: Up to 12 months elapsed time subject to seasonal conditions.

Part 4
Costs and funding
It is proposed that the research and development program proceed on a staged basis. The first will entail the development and evaluation of the aural lure. We have estimated that the cost of services for establishing the library of mating calls, designing and manufacturing the aural lure system, field testing and its technical, economic and financial evaluation will cost in the range of $100,000.00 to $150,000.00.

Equipment, materials, travel, accommodation, sustenance and communication costs are likely to be a further $50,000.00.