Science of Cane Toad Invasion and Control. Proceedings of the Invasive Animals CRC/ CSIRO/Qld NRM&W Cane Toad Workshop

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Edited by Kerryn Molloy & Wendy Henderson

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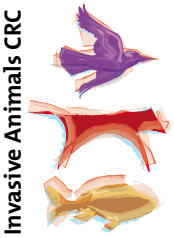

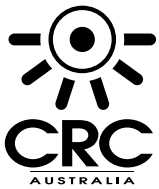

Contents

Introduction

Approximately 100 cane toads *(Bufo Marinus)* were introduced from Hawaii to Gordonvale, north Queensland in 1935 to control the greyback cane beetle. They are the only member of the family Bufonidae in Australia (we have no native toads of this group).

Toads soon established as a pest and have expanded their range across the north of Australia at a rate of 27-50 km/year. Larger male toads have been measured moving up to several kilometres in a single night. Toads are now estimated to occupy more than 500,000 km^2 of Australia and have reached densities of 2,000 toads per hectare in some newly-colonised areas of the Northern Territory.

Cane toads breed in summer – preferring it hot and wet. Females can lay 5,000 to 10,000 eggs per clutch. Tadpoles emerge from water bodies as toadlets (metamorphs) after 6-8 weeks. There is a high mortality rate at this stage.

The cane toad is poisonous in all its life stages, from egg to adult. Almost anything that eats the toad dies rapidly from heart failure. The poison is absorbed through body tissues such as those of the eyes, mouth and nose, so that even mouthing the toad may cause death. Cane toads have also been known to transmit diseases such as salmonella.

The cane toad has been nominated for listing as a key threatening process under the *Environment Protection and Biodiversity Conservation Act 1999*. The northern quoll *(Dasyurus hallucatus*), monitor lizards *(Varanus spp.)*, freshwater crocodile *(Crocodylus johnstoni)*, and some snake species have been severely impacted because of predation on toads.

At the moment the 'front line' is believed to be near the Victoria River in the Northern Territory. Large dominant male toads (warriors) have been observed to move ahead of the main group, and have been spotted up to 50 kilometres further west. The invasion front is therefore estimated to be between 250 and 300 kilometres east of the Western Australian border.

The Invasive Animals Cooperative Research Centre (IA CRC) has as one of its 13 operational targets to "deliver innovative, practical control measures against cane toads". The Terrestrial Products and Strategies Program of the IA CRC has several projects to develop short, mid and long-term solutions for cane toad control. These projects are being supported by funding of more than $500,000 over the next two years through the University of Sydney and University of Queensland.

The IA CRC's motto is "together create and apply solutions". This workshop is a further initiative of the IA CRC, and co-hosts CSIRO and the Queensland Department of Natural Resources, Mines & Water to address this goal, bringing people together from:

- CSIRO Australian Animal Health Laboratory, Entomology, Marine and Atmospheric Research);
- Australian Government Department of Environment and Heritage;
- New South Wales Department of Primary Industries;
- Queensland Department of Primary Industries;
- New South Wales Department of Environment and Conservation;
- Western Australian Department of Environment and Conservation (previously Conservation and Land Management);
- Queensland Department of Natural Resources, Mines & Water;
- South Australian Department of Water, Land & Biodiversity Conservation;
- James Cook University;
- University of Adelaide;

- University of New South Wales/Taronga Zoo;
- University of Canberra;
- University of Queensland;
- University of the South Pacific;
- University of Newcastle;
- University of Wollongong;
- Western Australian Institute for Medical Research;
- Animal Control Technologies;
- Cairns Frog Hospital;
- Kimberley Toadbusters;
- Stop the Toad Foundation;
- World Wildlife Fund.

Many of these organisations are members of the CRC. By bringing together active researchers, decision makers and research and development investors to share knowledge, identify mutual goals and potential areas of collaboration, it is hoped that a sense of national "team spirit" will develop to address this national problem. It is important that the route from research to development of applied solutions is expedited if we are to prevent the inexorable spread of the toad.

Workshop presentations cover discussion of the recommendations arising from CSIRO's previous work on cane toads, recommendations from the Vertebrate Pest Committee Cane Toad Task Force (2005), research into the biology of toads, current and prospective control measures (including biocontrols), and toxins and attractants.

SESSION 1: SETTING THE SCENE

Cane toad control research: The first decade

Prof. Michael J. Tyler, Environmental Biology, School of Earth and Environmental Sciences, University of Adelaide.

Abstract

March 1983 saw the first serious attempt to place cane toad control on the national agenda. In that month T.J. Bergin questioned whether Iridoviruses might provide an avenue for control. His suggestion was referred to CONCOM. Lobbying of the Federal Minister for the Environment eventually resulted in 1986, in funding and the appointment of a Research Management Committee based in Townsville. Initial studies were population dynamics and the search for endemic diseases, but progress was hampered by the failure of some states to honour financial commitments.

In July 1989 the Prime Minister's 'Statement on the Environment' made a commitment to further federal funding and announced an allocation before a review of progress had been completed.

After several years of research, the budget for the period 1994-1996 was increased to $3.48 million and the impact of the toad upon the native fauna was addressed.

Introduction

The history of the introduction of the cane toad into Australia has been documented adequately by Tyler (1976, 1989) and Lever (2001), and so I will confine my comments to the various research efforts and associated events of the first decade.

The first State to recognise the cane toad as a pest was Western Australia. On April 21st, 1950 it was gazetted as vermin under *The Vermin Act of 1918-1946*. The other States and Territories did not respond similarly.

The concept of biological control of the cane toad was first raised by T.J. Bergin, of the Australian Quarantine Service, in March 1983. His unpublished report was 'Potential Biological Control of the Cane Toad *Bufo marinus* (83/1665)'. He raised the issue of the use of a group of Iridoviruses referred to as the Frog Oedema Virus Complex. In August 1983 the Head of the Australian National Animal Health Laboratory forwarded the report to the Assistant Director-General of Animal Quarantine noting, "There would be greater problems associated with introducing biological control today than was the case with myxomatosis when it was released in the 1950's".

The matter was referred to CONCOM and its Standing Committee. Initially attention was focussed upon an assumed disease that was characterised by sick toads in eastern Queensland. It was reasoned that this endemic disease could eliminate the need to import another. (It was eventually demonstrated that the 'diseased' toads were actually exhibiting evidence of emaciation brought about by starvation. When provided with food *ad libitum*, in a controlled laboratory situation, all doubled or tripled their initial body weights in six weeks (Speare & Tyler unpublished data).

Throughout 1984 the Federal Minister for Home Affairs and Environment, Barry Cohen, fielded numerous parliamentary questions on the cane toad, and pressure to develop a biological control procedure was maintained. On November 23rd 1984 a meeting was held at the Queensland National Parks and Wildlife Service headquarters in Brisbane, in response to CONCOM's endorsement of research into biological control. A working plan was developed and a budget calculated at $480,000. Nevertheless, there was division on the source of the biological control agent.

Bergin expressed concern to the Standing Committee of CONCOM on September 24th 1985 that his report and recommendations had not been considered. Instead the focus had shifted to a recommendation by Bill Freeland to seek an agent in Australia. Bergin was concerned that Freeland had assumed that an agent existed in Australia and quotes him as writing "As the 'agent' is in Australia...", and Bergin highlighted his conviction that there was no justification for this assumption.

Despite the various concerns, the Terms of Reference for the research program were: "to define the factors presently affecting cane toad populations in Australia and to identify any agent which may be useful in their control".

CSIRO Wildlife was not keen to participate. It advised CONCOM in a telex: "our contribution to a possible future program is seen as expertise in an ecological approach to the study of vertebrate pests. Actual participation may follow a formal request: approval and goodwill of State fauna authorities: then proceed if adequate manpower and funds are available. As your meeting is likely to be 'disease' oriented our representation at this point appears unwarranted."

Under the Chairmanship of Professor Campbell a Research Management Committee was appointed to oversee the program and it held its first meeting at James Cook University (JCU) Graduate School of Veterinary Science on 22nd January 1986. Its half-yearly report was produced in July. Two activities described were Population Dynamics in the Northern Territory and a Control Program of looking for endemic diseases. This thrust was approved for the 1986-87 period.

During 1986 field sampling was conducted at fourteen sites and healthy and diseased individuals were sampled. One major disease outbreak was found at Mission Beach and identified as the bacterial pathogen *Aeromonas hydrophila.*

There were delays in starting the population studies at JCU, such that at 30th June 1986 the budget of $49,000 total expenditure was only $5.88. Nevertheless with appointment of postgraduate students and a technician, fieldwork started. The disease program report was similarly hampered by personnel shortages.

To judge from the tenor of CONCOM correspondence more positive results were anticipated. It simply was not appreciated by CONCOM that no one in the world had ever needed, or attempted to control a population of an amphibian and the task was one of considerable magnitude.

With the retirement of Professor Campbell, Professor Ladds was appointed Chairman of the Project Control Group and chaired the meeting held on 25th February 1988. There it was reported that the States were not honouring their financial commitment to the research program. The budget was not excessive and very little funding was available for travel in the wet season. Three people merit mention because of the extent of their involvement: W. J. Freeland of the Conservation Commission of the Northern Territory, who was leading field studies there, R. Alford leading the JCU field team and R. Speare heading the disease program.

In 1988 population studies were concentrated upon Calvert Hills and Townsville where populations had been marked. Despite good progress in all ventures CONCOM had misgivings. W.A. Thomas, Director of Conservation at the CCNT wrote to Philip Ladds on 10th May 1988 saying, in part: "Contributing agencies were reluctant to commit to future funding in the absence of clear criteria for success of the project. In particular, there appears to be little indication that a suitable control agent might be discovered within Australia." At that stage the work of JCU had been in progress for less than eighteen months.

The possibility of extending the cane toad studies overseas followed inquiries made by R. Speare when he attended a conference at Orlando, Florida, in January 1989. In July of that year, the 'Statement on the Environment', by the Prime Minister, included reference to a review of the work on the cane toad undertaken to date, and the encouraging words: "If necessary, the Commonwealth will undertake scientific investigations of a possible control agent, as well as studies of the Toad in its native South America."

By the end of 1989 the initial cane toad studies came to an end. A Committee was appointed under the Chairmanship of Jiro Kikkawa to review the project. Following a visit to JCU the committee submitted its report on 30th March 1990, making thirty-one recommendations including a commitment for funding for a further three years.

Curiously, whilst the review committee was deliberating the Prime Minister announced the allocation of $1.25 million to CSIRO for a three year research program on biological control.

At its July meeting the Standing Committee of CONCOM reached an agreement that the CSIRO and CONCOM research should be jointly managed through a Joint Management Committee. This Committee in collaboration with other scientists met at Gungahlin, under the Chairmanship of H. Tyndale-Biscoe, on 18-19 November 1990.The first matter of agreement was that "Studies to determine the impact of the cane toad on Australian native fauna should constitute a minor part of the research." This opinion was reached because of concerns expressed that to undertake such research thoroughly would make serious inroads into research funds.

Most projects funded started in April 1991: the JCU group studying toad movements in North Queensland and Sydney University examining dispersal movements in northern NSW and population studies in Manaus, Brazil and Caracas, Venezuela.

During 1990 – 1993 the Australian Government funded a three year project in association with Centro de Microbiologica y Biologia Celular, Instituto Venezolano de Investigaciones Cinetificas (IVIC) to identify disease-causing agents present in toads in their original habitat. Seven viruses were isolated and partially characterised. During a second funding period (1994 – 1996) these viruses were imported into Australia for further study, including animal infection trials, to evaluate their potential for use as biological control agents for cane toads in Australia. Because they were exotic viruses, the work was done under strict microbiological security controls at the Australian Animal Health Laboratory (AAHL), CSIRO, at Geelong. These viruses killed up to 100% of *Bufo marinus* tadpoles and one species of Australian frog used in a single transmission experiment designed to assess their specificity. The researchers recommended that the exotic South American ranaviruses should not be used as biocontrol agents for Australian cane toads.

A meeting and workshop was held at Gungahlin from 28th September to 2nd October 1992, with thirty-four participants, including six from South America. Substantial progress was reported by the various ecological and biological control projects. This triennial cycle of activity was maintained until the end of 1993 and numerous papers resulting from the research were presented at the Second World Congress of Herpetology held in Adelaide, December 1993 – January 1994.

Examining budget options for the period 1994-1996 the total needed was estimated at $3.48 million. In 1993, the CSIRO cane toad Research Advisory Committee (CTRAC) was established for the same term. The first research question to be asked was "Is the cane toad having a serious deleterious effect on the native fauna, particularly invertebrates, other Amphibia and predators?" Clearly this was a complete reversal of the November 1990 recommendations that this be a "minor part" of research, but conformed to the Kikawa Committee view that it was significant to have such information.

The first decade may be summarised as commencing with researchers persuading CONCOM to fund a control program. Efforts were initially focussed upon the false assumption of the existence of an endemic disease. With adequate funding research was conducted in Brazil and Venezuela as well as Australia.

Literature Cited:

Lever, C. 2001. The cane toad: The History of Ecology of a Successful Coloniser. Westbury Academic and Scientific Publishing, Otley, West Yorkshire. 230pp.

Tyler, M.J. 1976. Frogs. William Collins, Sydney and London. 256 pp.

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Outcomes from the CSIRO Cane Toad Workshop held in Queensland during February 2004

Alex Hyatt, Jackie Pallister, Veronica Olsen and Rhonda Voysey,
CSIRO, Livestock Industries, Australian Animal Health Laboratory,
5 Portarlington Road, Geelong Vic 3220
Private Bag 24, Australia (61) 0352275419, fax (61) 0352275555

Background to the workshop

In response to the real and perceived threats of *Bufo marinus* (cane toads) to Australia's biodiversity the Federal Government invested over a million dollars during 1990-1993 to investigate the ecology of cane toads in Venezuela, Brazil and Australia; and to isolate cane toad-specific pathogens from toads. The work resulted in the isolation of seven viruses all of which were identified belonging to the family Iridoviridae and 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. Infection trials at the Australian Animal Health Laboratory (AAHL) found that the Venezuelan viruses killed 80 to 100 per cent of cane toads; associated modelling (Hyatt *et al.*, 1998)) predicted that this high rate would be sufficient to control toad numbers in Australia. Whilst there was much excitement about this work, researchers discovered the viruses were not cane-toad specific and killed Australian frogs (Hyatt *et al.*, 1998); as such these viruses were discarded as potential primary biological control agents.

Following a brief absence of federally funded cane toad research, Senator Robert Hill, the then Minister for the Environment and Heritage, invited submissions for research initiatives into the biological control of cane toads ($1 million over two years). A successful submission was made by CSIRO Divisions of Animal Health (later to become Livestock Industries) and Sustainable Ecosystems. The proposal involved the development of a modified recombinant ranavirus for the potential control of Australian cane toads (refer to articles by Robinson *et al* and Pallister *et al*). The research was based on a concept developed at AAHL to use an Australian ranavirus and infect the tadpoles with a gene which was only expressed during or post metamorphis whereby the fitness of the animals was detrimentally compromised. By 2004 the initial phases of the research had been completed (i.e. 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 project was reviewed externally by the Australian Government Department of Environment and Heritage (DEH) and CSIRO and the following recommendations were made:

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 were kept informed of its progress. This led to the establishment of the "Biological Control of Cane Toads" Workshop held in Queensland during February 2004.

The recommendations were written into a final report and submitted to DEH where they can found at:
(http://www.deh.gov.au/biodiversity/invasive/publications/cane-toad-2004/index.html)

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.

Recommendations from the workshop

The following recommendations were generated from the workshop. We have commented on each recommendation in relation to what has happened since 2004.

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.

   Comments:

   No progress has been made in relation to the formation of such a group. Whilst no national group has been formed other initiatives such as modelling have been initiated via the DEH-CSIRO biological control project.

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) impact of cane toads on some animals, such as quolls, are significant. The long-term (greater than 10 years) impact 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 trade-off 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.

Comments:

Short and long-term impacts are still being investigated. No risk assessments have been conducted but *B. marinus* has been listed as a 'Key Threatening Process' (http://www.deh.gov.au/cgi-bin/sprat/public/publicgetkeythreats.pl): "The biological effects, including lethal toxic ingestion, caused by cane toads (*Bufo marinus*)".

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 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 (i) collect toads into plastic bags, (ii) 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.

Comments:

- Short term controls: To date the only funded short-term controls have been via State and Territory funded community groups who have designed and implemented the use of traps to reduce the rate of spread. For more information refer to papers in this publication by Sawyer, Boulter *et al* and Beros.
- Disposal of animals: The described disposal protocols are being used. Other protocols including burial and converting 'toads to fertilizer' can be obtained from G. Sawyer and S. Boulter.
- Impact: Research is proceeding into the impact of cane toads on Australia's biodiversity. For progress refer to associated papers within this publication by Shine *et al*, Doody *et al* and Grigg *et al*.

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.

Comments:

With the exception of the current workshop no progress has been made in relation to this recommendation.

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

(a) Short-term strategies:

Ecological, social and economic:-
Implement cane toad impact studies incorporating biodiversity, social and economic aspects of the impact of cane toads.

Biological:-
- Devise and assess effectiveness of **traps** or other methods to eradicate toads from specific geographical areas.
- Identify and assess **attractants**, such as pheromones, that would draw toads to traps.
- Research the feasibility of **physical barriers**, where locally appropriate, and assess effectiveness following construction.
- Relocate valued at-risk species to cane toad-free areas, where feasible.

Comments:

The design and use of traps have advanced significantly (refer to comments associated with recommendation #3) since this workshop. The use of attractants has not progressed but is overviewed in this document (by Prof M. Tyler). Since the workshop in 2004 there have been no advances in the use of physical barriers.

(b) Long-term strategies:

Ecological, social and economic:–
- Implement cane toad impact studies incorporating biodiversity, social and economic aspects of their ecological impact over several decades.

Biological:–
- Research biological control via recombinant viruses targeting toad-specific 'vital' proteins
  - continue the exploration of the use of genetically modified organisms (GMOs) to determine if 'concept' is valid
  - encompass the use of non-disseminating agents in research
  - explore the use of other gene targets.
- Explore the concept of sterile males to reduce the number of fertile males.
- Explore the concept of "daughterless" technology for toads, (restricting all offspring to males).

- 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).

Comments:

Ecological Studies are on-going (refer to comments associated with recommendation #3). If the long-term impacts of toads are to be evaluated then these studies must continue for at least a decade together with associated ecological studies related to the associated food webs.

Biological research in continuing in the area of developing a genetically modified agent for control of cane toads (refer to Robinson *et al* and Pallister *et al*). The described concept is supported by experimental data. A literature search has been performed for the possible use of non-disseminating viruses and the conclusion drawn that to conduct such research will be lengthy, expensive and have limited chances of success. It would appear that the only way whereby cane toads can be controlled at a trans-continental level is via a self disseminating infectious agent. The search for primary pathogens has been initiated by A. Hyatt and R. Shine. Whether this initiative continues will depend upon funding. The use of sterile and daughterless technologies continue to be discussed (refer to Koopman; and Thresher and Bax).

6. Fund a major long-term coordinated program to address the above recommendations, prioritising short and long-term strategies from the above list.

Comments:

To date funding for the biocontrol of cane toads has come from both the Federal, State and Territory Governments. No coordinated National program exists.

Literature cited:

Hyatt, A. D.; Parkes, H, and Zupanovic, Z. Identification, characterisation and assessment of Venezuelan viruses for potential use as biological control agents against the cane toad (*Bufo marinus*) in Australia: a report from the Australian Animal Health Laboratory, CSIRO, Geelong, Australia. May 1998. Geelong, Vic.: Australian Animal Health Laboratory; 1998.

Recommendations arising from the VPC Cane Toad Task Force, June 2005

Dr Tony Robinson, CSIRO Entomology, Canberra.

Abstract

The National Cane Toad Taskforce was established in September 2004 as a subcommittee of the Vertebrate Pest Committee at the request of the Natural Resource Management Ministerial Council following a request from the Northern Territory Minister for the Environment. The terms of reference for the Task Force were to review the current threats posed by cane toads, review the states of research into developing tools to abate those threats, identify any gaps in current approaches and assess costs and benefits of options for priority joint national action. The report is divided into five chapters reflecting the terms of reference with an additional introductory chapter and reference list. The chapters deal with impacts, short-term or local threat abatement, long-term and/or widespread threat abatement, current management of cane toads in the States and Territory and overseas, and recommendations and costs for best practice management and priority research divided into impact, short-term and long-term.

Introduction

The National Cane Toad Taskforce was established in September 2004 as a subcommittee of the Vertebrate Pest Committee at the request of the Natural Resource Management Ministerial Council (NRMMC) following a request from the Northern Territory Minister for the Environment.

The terms of reference for the committee were:

1. Review the current threat posed by cane toads.
2. Review the states of research into developing tools to abate those threats.
3. Identify any gaps in current approaches.
4. Assess costs and benefits of options for priority joint national action.

The Task Force, Chaired by the Northern Territory, consisted of four scientific experts from CSIRO and universities, six government departmental staff representing New South Wales, Queensland, the Northern Territory and Western Australia, one representative from the Federal Government (Department of Environment and Heritage) and a community member.

A face-to-face meeting of the Task Force was held in Darwin on 21-22 October 2004 and after a number of telephone meetings and email exchanges the report entitled "A review of the impact and control of cane toads in Australia with recommendations for future research and management approaches" was released to the public in June 2005. http://www.feral.org.au/ref_docs_images/CaneToadReport2.pdf

As the report is publicly available there is no need to go into all the details here but the general thrust of the report is outlined.

The report is divided into five chapters reflecting the terms of reference with an additional introductory chapter and reference list. The chapters deal with impacts, short-term or local threat abatement, long-term and/or widespread threat abatement, current management of cane toads in the States and Territory and overseas and recommendations for best practice management and priority research.

Impacts

The impacts considered were environmental, social and economic. As toads are toxic to varying degrees depending on the life stage, there is a direct impact on animals that attempt to consume them from native predatory tadpoles to goannas and quolls. There are numerous reports of individual animals dying from the effects of the toxin but the effect on species or populations is not as clear. The difficulty and cost of mounting robustly designed impact studies has meant that impact at the level of populations relies on the observations of land managers and others who can detect changes in the populations of animals they are familiar with. The other issue is about short-term versus long-term impacts and whether or not individuals and populations adapt to the presence of toads. More indirect and even harder to measure is the competition for food and refuge sites and the flow-on effect at the ecosystem level of all these changes. The report provides a number of tables referencing these. There is also a table of current studies being undertaken on cane toad impacts.

At the social level the report cites a study done by the Sessional Committee on Environment and Sustainable Development 2003 on issues around the arrival of cane toads into the Northern Territory. The list includes impacts on people and pets, blockage of drains, fouling of swimming pools, visual impacts and potential substance abuse, in this case cane toad toxin. The impacts of loss of bush tucker to the Traditional owners is another consideration as is the potential for cane toads to transmit diseases such as Salmonellosis.

The economic impacts are potentially on tourism because of the decline in iconic species and the obvious presence of an exotic and, to many, repugnant invasive species. The other economic impact is on resource management where there is an imperative to control cane toads. This is already happening in the Northern Territory and places added demands on limited budgets.

Short-term or local threat abatement

The report defines "short-term" as a one to three year time frame. This chapter is divided into seven sections covering traps and attractants, exclosures, bonus or bounty schemes, community participation in control, protocols for controlling movement to offshore islands, identification of high priority sites for exclusion or other control methods and control via limiting resources. Attractants include light, olfactory and auditory stimuli and moisture. There are a range of traps available and the Report gives the results of trials on one of these. Trapping is currently the only extensively used method of cane toad control at the front in the Northern Territory and, although laudable with many community benefits, is considered by many to have little chance of reducing toad populations in anything but back-yard operations.

Exclosures offer an effective means of excluding toads from small areas but cost soon becomes excessive when large areas of exclusion are considered. It is also dependent on the resource one is trying to protect. The report provides tables on minimal habitable areas for a number of species considered at risk from cane toads as a means of estimating feasibility. They range from 3.3 km^2 for frilled necked lizards to 10,000 km^2 for the Australian bustard. The cost implications are obvious. There is also a table on the cost of fencing off the Coburg Peninsular and another on the comparison of annual costs for a number of exclusion scenarios for a range of vulnerable species. The numbers are frightening. Another problem with exclosures is that not only are toads excluded or contained, the natural home ranges of many other species are also affected.

Bonus or bounty schemes are often touted as the answer to the control of pest species. This section provides a comprehensive look at bounty schemes and their advantages and disadvantages. The conclusion for cane toads is that there would not be enough money available to make any significant impact on the toad population and that money would be better spent elsewhere.

The fourth section covers community participation. The important point is made that the first thing to decide if wanting to set up a community toad control activity is the objective of the exercise. If it is simply to provide the community with a sense of achievement (social) then the biodiversity conservation objective is irrelevant. An anecdotal survey of the effectiveness of community action is given and there are three reports given on toad harvesting campaigns.

The section on the mechanisms of island colonisation covers the status of many of the islands along the Queensland and Northern Territory coastline. It is an interesting account of how successful toads are at gaining access to the islands and, in one case at least, toads are considered to be responsible for the elimination of an island population of quolls. Toads get to the islands by a variety of means. Flooding rivers provide an easy means of transfer via the fresh water plumes that layer over the sea water many miles out from the river mouth. Toads can be washed directly in these plumes or be carried on logs and other debris to the island shore line. As toads are unable to control their water intake when immersed it would seem to me that transport on debris is a more likely mechanism of delivering live toads to islands than those that are continually immersed. The other main means of transport are boats, barges, and trucks on barges carrying building materials. Some of the offshore islands near Brisbane lacked toads until they became prime real estate and building began.

The last two sections in this chapter deal with identification of high priority sites for potential exclusion or control of cane toads and the ability to control toads via limiting their resources. The first does not list any sites but provides some useful guidelines as to how one would go about making those choices. The second provides an insight into how one might go about manipulating habitat to reduce toad numbers in specific areas mainly based on the toad's need to continually rehydrate. Toads lose water at about the same rate as a wet sponge and they need to replenish their water supplies regularly. They also need water to breed in. Not mentioned in the report but another observation has been that toads tend to avoid heavily vegetated areas. This could provide another means of habitat manipulation. However, one would need to consider the effects of such manipulations on other species in the ecosystem.

Long-term and/or widespread threat abatement

In this chapter the issue of how one might control toads over their current and potential range are considered. It is about the scale and cost of control as well as the time frame of the effect. With the exception of the section on toxins and non-disseminating genetically modified organisms (GMOs), it is recognised that unless some biocontrol for cane toads can be found similar to myxoma virus and rabbit haemorrhagic disease that were introduced for wild rabbits, we are limited to expensive local control. The four control methods discussed were natural pathogens, sterile male technology, cane toad specific toxins and disseminating and non-disseminating GMOs. Another biocontrol approach, "daughterless", that will be aired at this workshop was not covered in the report but can be considered broadly as having similar advantages and disadvantages to the sterile male approach. The final section in this chapter covered models that could be used to explore the feasibility of the four control approaches.

The first section deals with the history of the search for naturally occurring biocontrols of cane toads. Beginning in 1986 a survey was made of diseases and parasites of Australian anurans (frogs and toads) and a literature survey was conducted for all references to diseases of cane toads. This search did not reveal any pathogen that could be used as a biocontrol for cane toads. In 1990 the search was then moved to South America where again a number of

infectious agents were isolated from cane toads but after extensive testing none were considered suitable biocontrol agents. It was emphasised that the only successful biocontrol agents of pest vertebrates have been the two viruses used for rabbit control. It is further pointed out that these agents were discovered serendipitously rather than in a deliberate search and that we may have to wait for similar serendipitous events to discover a naturally occurring agent for cane toads. A strategy to maximise the chance of detecting such an event is given in the report as well as a pathway to determining the usefulness of any organism found.

A "sterile male" approach is the subject of the second section. An analogy for this approach is the use of sterile males to reduce populations and consequently the damage done to cattle by the screw worm fly. The characteristics of an organism that would be amenable to this approach are given and the cane toad has some of the required characteristics. The strategy is to produce tetraploid males that would mate with diploid females producing triploid offspring which are sterile. A detailed account is given as to how one would go about producing triploids by this and other means. The advantage of this approach is that it is completely species-specific effect and does not involve genetic engineering. The disadvantage is the difficulty of establishing such animals in the population.

The third section deals with cane toad-specific toxins. It is noted that toxins are currently one of the main means of controlling vertebrate pests and as such are already an accepted approach. Their advantage is that they can be controlled in their use but the disadvantage is that they can only be useful for small scale operations and can be costly to manufacture and deploy. Some examples are given of non-specific toxins that affect amphibians. Strategies for searching for suitable toxins are given and the ability to combine a toxin with attractants is mentioned.

The fourth section covers the current CSIRO approach which is to see if a disseminating or non-disseminating biocontrol agent can be constructed using modern molecular biological techniques. Progress toward this goal is given in the report and can be found elsewhere in these proceedings. The advantage of this approach is that provided it is species-specific, the agent could be deployed over wide areas and if disseminating it would not need to be continually re-released. The disadvantage is that it would be a genetically modified organism which would require a long lead time for approval for use.

The final section deals with models to explore the feasibility of the various approaches to biocontrol that are proposed. Four steps would be undertaken: problem identification; model construction; data collection and model parameterisation; and model analysis. Key questions are posed for each approach.

Current management of cane toads in Australia and overseas

The penultimate chapter deals with the current state of control in New South Wales, Queensland, the Northern Territory, Western Australia and internationally in Fiji and Florida. In the case of the Australian cane toad population the authors provide information on the current distribution of toads within their jurisdiction and the legislative frameworks under which pests can be suppressed or destroyed. Apart from the Northern Territory, the strategy is to provide information to the public about cane toads and to draw up strategies for future control. In the Northern Territory, as well as education programs and future strategies there is active government-funded on-ground control using trapping, relocation of northern quolls to off-shore islands, impact studies, and protocols for preventing the movement of toads to offshore islands. In Western Australia cane toads have not yet arrived but there is considerable government and community activity aimed at preventing toads crossing the WA/NT border.

Recommendations for best practice management and priority research

The objective of the Task Force was to review the current situation with regard to the distribution, impacts and current control methods for cane toads, identify gaps and make recommendations for future action. The report devotes the final chapter to the recommendations and it is important that we revisit these and add to or challenge them if necessary at this workshop to guide future work.

The first section in this chapter reminds us that management decisions and funding for research should, if possible, be guided by a cost-benefit analysis. Although ideal, the information to inform the analysis is often lacking and that is very much the case with cane toads. The main cost is environmental rather than to production industries and these costs are notoriously difficult to measure. A means though to overcome this is suggested. By estimating potential benefits of a particular management action, applying a probability factor of success and then calculating the cost of the action one can rank the different potential projects and choose those with the greatest chance of success. "Do nothing" becomes an option. Of course these approaches can all be confounded by social imperatives and political pressure. Cane toads fit into this category where the damage they do is poorly quantified but is probably high at the environmental level and there are strong social drivers and political pressures to do something about them. Their listing as a threatening process under the EPBC Act has provided additional justification for action. Nevertheless, there is still only a relatively small investment nationwide in cane toad control.

Best practice management is the next section. The highest priority identified for management action was to try to keep toads from moving into areas where they are currently absent. Recommendations for keeping toads off islands and maintaining their toad-free status are given. Two further recommendations with suggested strategies for implementation are education and community input and participation and this is followed by strategies for communication and coordination, the development of exclusion areas, population reduction and monitoring and biosecurity on the mainland.

The final section is devoted to prioritising and costing research projects. This is further subdivided into impact studies, short-term control and long-term control. The priorities for action in these three areas and the estimated cost for each are provided in table form in the report. These are summarised below in priority order but the report should be consulted for a more detailed description.

Impact

1. Quoll persistence and recovery project. $200,000 – 2 years.
2. Investigation of impact on threatened invertebrates. $350,000 – 3 years.
3. Status of impacted species in Queensland. $200,000 – 2 years.
4. Impact on bush tucker. $70,000.
5. Likelihood of impacts on other species. $100,000.
6. Unpublished reports to go on www.feral.org.au $15,000.

Short-term control

1. Island arks and biosecurity. $1.31M - 3 years
2. High priority places for protection. $3.4M - 3 years
2. Traps and attractants. $180,000
2. Community participation. $160,000
3. Limiting resources. $180,000 – 2 years
3. Exclosure fences $110,000 – 3 years

Long-term control

1. Modelling proposed biocontrol approaches. $95,000 – 5 years
2. Renewed search for naturally occurring pathogens. $37,000 – 1 year
3. GMOs – continuation of current DEH/CSIRO project. ~$550,000 – 7 years
4. Toxins – identification and testing. $350,000 per year.
5. Sterile male approach. $750,000 – 3 years.

(It was recognised that the registration costs for all biocontrol methods would be in the region of $1M)

The report has been submitted to the NRMMC and the Task Force has not met since.

SESSION 2: BIOLOGY AND IMPACTS

The biology, impact and control of cane toads: an overview of the University of Sydney's research program

R. Shine*, G. P. Brown, B. L. Phillips, J. K. Webb, and M. Hagman
School of Biological Sciences A08, University of Sydney, NSW 2006, Australia

* Corresponding author. E-mail: rics@bio.usyd.edu.au
Tel: +61-2-9351-3772, fax: +61-2-9351-5609

Abstract

Beginning in the early 1980's and continuing through to the present day, ecologists from the University of Sydney have conducted a major research program on reptiles and their prey species on the floodplain of the Adelaide River 60 km east of Darwin. That work has provided a strong empirical understanding of issues such as the ways in which year-to-year variation in wet-season rainfall influences the population demography (survival, growth, recruitment, dispersal) of tropical snakes. Cane toads *(Bufo marinus)* arrived in this area late in the 2005 wet-season, and we have expanded our studies to encompass the biology and impact of these toxic invaders. Although it is still too early for confident evaluation of ultimate impact, much has been learnt. For example, the toads themselves appear to have been modified by selection during the invasion process, such that their morphology (relative leg length) and behaviour (rate and directionality of movements) have changed from the attributes of toads in older (long-established) populations. Impacts of toad arrival on the native fauna have been relatively minor to date, except for widespread mortality of varanid lizards. Several of our initial results suggest that toad impacts may be less severe, more heterogeneous and of shorter duration than widely expected. Our results also challenge the effectiveness of current approaches for toad control, and suggest innovative ecologically-based approaches for reducing toad impact.

Introduction

Invasive species are widely regarded as a major threat to biodiversity, with many authorities ranking them second behind habitat destruction in terms of their negative impacts (Lodge 1993, Vitousek *et al.* 1996). Accordingly, conservation biologists have allocated substantial effort, and wildlife authorities substantial funding, towards research that aims to ameliorate the problems caused by these exotic taxa. The relative allocation of resources has differed widely among taxa, however, with a few high-profile species receiving disproportionate attention. One such species is the cane toad (*Bufo marinus*), a large toxic anuran that has spread through much of tropical and subtropical Australia over the last 70 years (Lever 2001).

One unusual feature of the cane toad invasion is the repugnance that many members of the general public express towards these animals. The strongly negative perceptions of toads have undoubtedly done much to draw attention to the toads' potential impact on native ecosystems, resulting in the allocation of almost 10 million dollars in research funding from the Commonwealth Government alone over recent years (Table 1). Unfortunately, that massive expenditure appears to have achieved very little in terms of actual control (so far at least). The invasion front continues to spread rapidly (indeed, more rapidly than before: Phillips *et al.* 2006), with the toads apparently unaware of the concerted attempt to discourage them.

Table 1. Total Commonwealth Government funding for research related to the control of cane toads (pers. comm. Damian McRae, DEH as of May 2006). Note that financial support for the University of Sydney work has come primarily from other "basic science" sources (the Australian Research Council's Discovery program); this Table is focused on funds allocated specifically for "applied" research into toad control.

Item	**Commonwealth Funding**
Previous Government funding (1986–1996)	$3,330,000.00
Current Government funding (1997–current)	
National Heritage Trust (NHT)	
CSIRO Biological Control Project	$3,420,281.91
Other National NHT projects	$284,138.00
Regional/Envirofund NHT projects	$1,481,221.73
Parks Australia	$522,426.00
CSIRO (matching contribution to NHT)	$3,843,075.73
Total Current Government funding	**$9,551,143.37**

Even a cursory inspection of the funding directed to toad research (Table 1) makes it clear that the emphasis has been firmly on killing toads rather than attempting to understand them. The exceptions involve pioneering studies in northern Queensland, the Northern Territory and Venezuela that documented the ecology of toads and the kinds of impacts that they might have on Australian species (e.g., Freeland and Kerin 1988, 1991, Alford 1994, Alford *et al.* 1995, Lampo and Bayliss 1996*a,b*, Lampo and Medialdea 1996, Crossland 1998*a,b,c,* Lampo and De Leo 1998, Lampo *et al.* 1998, Crossland and Alford 1998, Crossland and Azevedo-Ramos 1999, Crossland 2000, 2001). Much of this work was very well done, and included detailed analysis of the population ecology of toads as well as their likely impacts on vertebrate and invertebrate predators and anuran competitors. Unfortunately, funding for this ecological component of the research program, as well as for other components, ran out as the toad invasion front moved into regions with low human populations and thus, where the invasion's profile dipped below the threshold for public (and thus political) interest as well as posing increasingly severe logistical obstacles for detailed study in these remote regions. The toad's subsequent arrival in better-known and more densely-populated areas, especially Kakadu National Park, stimulated a resurgence in public interest and accordingly, in research effort.

Areas around the city of Darwin, and east to Kakadu, have a well-developed infrastructure and several active teams of ecological researchers. Many of those groups recognized the opportunity to initiate research on the biology and impact of toads, at the same time as community groups recognized the opportunity to pressure governments into providing resources for community-based efforts at toad control. Thus, we are now in the midst of a renaissance in levels of interest in toads. In the present paper we will outline the background to one of those research teams, and how the University of Sydney has come to be involved in a detailed ecological study of these toxic amphibians. It is far too early to attempt a synthesis of our broad-ranging research programs – the toads arrived at our main study site little more than 12 months ago – so instead we will stress the approaches that we are taking, and some implications of our initial findings.

Background to the Fogg Dam project

The tropics contain most of the world's plant and animal species, and a very high proportion of its human population. Unsurprisingly, they also contain some of the world's most significant conservation problems, with many biodiversity "hotspots" under extreme threat. It is a matter of grave concern, therefore, that ecological research traditionally has centred on the cooler habitats of the temperate zone, reflecting the geographic distribution of major research institutions. We know much less about ecological functioning in tropical floodplains than we do about cool-temperate forests and grasslands, for example – and in particular, there are very few *long-term* ecological data sets for tropical habitats. Although such research efforts are logistically prohibitive to maintain, they provide the only means of genuinely grappling with ecological processes over a meaningful time scale. This is especially true for areas such as the wet-dry tropics, where stochastic variation in rainfall patterns from year to year generates massive corresponding variation in the biota (Shine and Brown 2006).

This situation stimulated one of us (Rick Shine) to initiate an ecological research program at Fogg Dam, 60 km east of Darwin, in the early 1980's. Fogg Dam was selected not only because of remarkably high population densities of snakes such as water pythons (*Liasis fuscus*), keelbacks (*Tropidonophis mairii*) and slatey-grey snakes (*Stegonotus cucullatus*), but also for its logistical convenience. In particular, the Northern Territory government maintained a research station at Middle Point, a facility that continues to offer broad-ranging logistical assistance and accommodation for the herpetological researchers. The early fieldwork was conducted mostly by Rick Shine and his research assistants (Rob Lambeck, Russell Hore, Peter Harlow), but a CSIRO-University of Sydney grant provided a salary for David Slip to begin more detailed radio telemetry work on water pythons. In 1989 a successful application to the Australian Research Council (ARC) by Shine provided funding for a fulltime postdoctoral fellow (Thomas Madsen) on a longer-term basis, and indeed Thomas still works on this system (albeit, now based at the University of Wollongong with collaborator Beata Ujvari). Further ARC support brought Greg Brown into the system in 1998, and Ben Phillips in 2004.

As well as these postdoctoral fellows, the site has been used for ecological research by many University of Sydney students over this 25-year period. One early result was to reveal unsuspected small-scale spatial variation in herpetofaunal assemblages on the Adelaide River floodplain; for example, death adders are abundant in the Beatrice Hill region (Webb *et al.* 2005), but are rarely encountered a few kilometres away on the Fogg Dam wall. Thus, the size of the study area expanded to incorporate this diversity, and we now work at multiple sites (Fogg Dam, Harrison Dam, and Beatrice Hill) within a much larger study area than was the case in the first few years. In consequence of this intense research effort over such a long period, an enormous amount of information has accumulated. The central thread of research has been to gather long-term data so that we can begin to understand how the erratic climatic conditions of the wet-dry tropics modify demographic processes within ectothermic predators. For this purpose, the twin techniques of capture-mark-recapture and radio telemetric monitoring have been the backbone of the work. In recent years, extensive egg-incubation studies have also clarified many facets of the early life-histories of the Fogg Dam colubrids (Brown and Shine 2004, 2005).

Appendix 1 lists 61 papers that have so far been published from the University of Sydney's ecological research at Fogg Dam and surrounding areas. In the present context, the most important consequence of this output is that Fogg Dam is far-and-away the most intensively studied area (at least with respect to population ecology of native predators) within the tropics of Australia. Indeed, it would rank very highly in this respect in any international comparison. The clear implication of this situation is that the existing database concerning the reptile fauna of Fogg Dam, and the insights that have been gained into the ecological functioning of this complex biotic assemblage, provide a unique opportunity to examine the ecological consequences of toad invasion.

That opportunity is particularly important because of shortcomings in previous attempts to assess toad impact. Contrary to widespread public perception, there is actually little unambiguous evidence that cane toads exert a markedly deleterious effect on the biota that they encounter. It is abundantly clear that some predators die as a result of attempting to consume toads, but there is little robust basis for extrapolating from the death of individual predators to system-wide effects. By analogy, there are undoubtedly many more cases where people have observed reptile mortality due to motor vehicles than due to cane toads, but nobody seriously suggests that road kills threaten the viability of most widespread reptile species. Why, then, do scattered observations of dead predators following toad invasion lead to the inference that cane toad arrival causes massive population declines? The most compelling evidence is the consistency of anecdotal reports of such declines; most notably in varanid lizards, some elapid snakes, and quolls (Breeden 1963, Pockley 1965, Rayward 1974, Burnett 1997, Phillips and Fitzgerald 2004). There seems little doubt that such declines do indeed occur, but the scarcity of quantitative data is frustrating.

Intuition suggests that any marked ecological impacts of cane toad invasion should be apparent from surveys of sites on either side of the invasion front. Several such surveys have been conducted, some with elegant experimental designs, and have generated the surprising conclusion that toads appear to have little impact on most native species (Freeland and Kerin 1988, Catling *et al.* 1999, H. McCallum, G. C. Grigg and A. Taylor, pers. comm.). No species is known to have become extinct as a result of the toad invasion (with the possible exception of a python parasite: Freeland 1994). Can we then conclude that toads are not an environmental problem? Clearly not, because the null results from such surveys may well reflect the power of the data-sets not the absence of an effect. As demonstrated in great detail by the Fogg Dam work on predator demography, the fauna of the wet-dry tropics exhibits massive levels of variation in a variety of traits at a range of spatial and temporal scales (e.g., Shine and Brown 2006). Hence, detecting the impact of any additional factor – such as the presence of an invasive species – is extraordinarily difficult because the signal is lost within huge environmentally-induced noise. The problem was well-summarised by Woinarski *et al.* (2004), who used power analyses on their Northern Territory survey data to conclude that the observed levels of variation meant that more than 1,000 sample sites would be needed to be 90% certain of detecting a 20% change in abundance in a given species. Such a sampling effort is well-nigh impossible in practical terms.

Thus, how can we best proceed? There is little compelling direct evidence that cane toads are a major environmental threat, but strong grounds for suspecting that they are, at least for selected species. The listing of cane toads as a Key Threatening Process under the *Environment Protection and Biodiversity Conservation Act 1999* essentially was based on that balance of probabilities. Understanding the nature and magnitude of toad impact is clearly an important goal, for without such an understanding we cannot effectively prioritise research on toads versus other threatening processes, nor target our conservation resources towards the most vulnerable components of Australian ecosystems. Faced with this challenge, it was immediately apparent that the long term data-base on the Fogg Dam system offered a unique opportunity to examine the consequences of toad arrival at replicate sites where the background information facilitated distinguishing toad impact from other kinds of environmentally-induced variation. That realisation stimulated a major extension of the reptile-ecology work at Fogg Dam into a new direction: the interaction between that well-studied fauna and the invasive cane toad.

This unusual historical basis for the "Team Bufo" research program inevitably has affected the way in which we have framed our questions, the native taxa that we have selected for detailed study, and the kinds of methods that we have adopted to study them. Most notably, (1) we have emphasised effects of toads on native reptiles and amphibians, because these are the organisms for which we have the most extensive prior data sets; and (2) we have framed our work in terms of general biology rather than specifically towards toad control, because the work has been funded primarily as "pure science" under the ARC's Discovery program (albeit

with recent support from DEH and the Invasive Animals CRC also). As the work began on toad impact (the original theme), however, it soon identified (1) attributes of toad ecology different from those documented in earlier work; and (2) possible opportunities for control of toad populations. Thus, the current and proposed research program now consists of three main components: toad biology, toad impact, and toad control. Below, we outline the major thrust and some preliminary results from each of these three subprojects.

The biology of cane toads at the invasion front

Few anurans have attracted as much study as *Bufo marinus*; indeed, it recently was the subject of a book devoted entirely to the invasion biology of this single taxon (Lever 2001). Nonetheless, there are some surprising gaps in our knowledge of this remarkable animal. Within an Australian context, studies on the biology of cane toads have focused on north-eastern Queensland, reflecting the expertise of researchers at James Cook University. That work has provided a robust empirical basis for understanding population demography of toads in tropical savannas as well as exploring potential interactions between toads and native fauna (Alford 1994, Alford *et al.* 1995, Crossland 1998*a,b,c,* 2000, 2001, Crossland and Alford 1998, Crossland and Azevedo-Ramos 1999). Importantly, these toad-impact studies adopted a directly experimental approach rather than relying upon field-based surveys. Hence, the work generated an understanding of mechanisms as well as consequences, and provided firm empirical tests of clearly-framed hypotheses about such interactions. At the same time, extensive radio telemetry of toads (Schwarzkopf and Alford 1996, 1999, 2002) explored the patterns and causes of movement by individual free-ranging toads.

Given this excellent background information, why repeat such studies at the invasion front? The answer is that such studies are needed because there is strong evidence that both the toads themselves, and their Australian victims, have undergone rapid evolutionary change during the course of the toad invasion. This work has documented substantial shifts in toad morphology (body size, relative size of the parotoid glands) as a function of time since colonisation of an area, hinting that the toads that arrived in our study site might well differ in important ways from those in the well-studied populations near Townsville (Phillips and Shine 2005, 2006*b*). Similarly, rapid adaptive shifts in the morphology, behaviour and physiology of anurophagous snakes coincident with toad arrival (Phillips *et al.* 2004, Phillips and Shine 2004, 2006*a*) suggested that interactions between toads and native predators might well be very different in newly-invaded areas than in regions where toads had been present for decades. It thus was clear that we needed to examine toad biology and impact at the invasion front itself, to complement the existing data from older populations.

Our snake-ecology work has involved regular nocturnal surveys by foot and motor vehicle virtually every night of the year for many years. Thus, we were well-placed to locate toads as soon as they arrived, especially since these animals utilise roads as corridors for movement (Brown *et al.* 2006). The first cane toad was encountered in December 2004, and a further 1,200 animals have turned up over the next 17 months. The 2004-05 wet-season finished relatively early, and with the cessation of rains the influx of toads ceased. We began attaching radio transmitters to toads as soon as they arrived at Beatrice Hill; they moved remarkably long distances throughout the wet-season, but were virtually sedentary during the dry-season (Brown *et al.* 2006). Continued telemetric monitoring over the much longer 2005-06 wet-season has revealed very much the same pattern of extensive and frequent movements. To date we have radio-tracked 47 toads, for a mean duration of 45.6 days each (range 2 to 255 days), yielding a total of 2,143 toad-days of data. The work is continuing.

The cane toad invasion front has consisted entirely of adult animals, and has been characterised by rapid and largely directional migration across the study area. Activity levels decline during the dry-season, when most of our radio-tracked toads remain in moist retreat-sites within anthropogenically disturbed areas. Our 245 recapture records from our 1,201 individually

marked toads give strong support to the radio-tracking results. Almost all of these toads moved northwest through our study area, with a mean recapture interval of only 32 days.

Our radio telemetric and mark-recapture studies on toads are continuing and it is too early to draw many firm conclusions. However, much of what we have already learnt runs contrary to established wisdom in respect of these animals. For example, their rapid migratory movements are nothing short of exceptional for any anuran, let alone previously-studied conspecifics in north-eastern Queensland (Phillips *et al.* 2006*a,b*). The puzzling tendency of toads to keep moving through the study area, rather than settling within it for long periods, means that as yet there is no resident toad population at Fogg Dam. Presumably, there will come a time when individual toads do remain in the local area and hence, we can talk of a "Fogg Dam population" of toads; it will be interesting to see how long this takes.

Another main thrust of our fieldwork on toad biology has been to explore breeding-site selection. Despite anecdotal reports that toads are non-selective breeders, we found that toads at the invasion front actively selected a highly non-random set of water bodies for egg deposition (Hagman and Shine 2006). Breeding toads avoided sites with thickly vegetated surrounds and steep banks, offering obvious opportunities for local control over toad reproductive activity (Hagman and Shine 2006). In other work (also funded by DEH), we are exploring the chemical communication systems of larval and metamorph cane toads. Preliminary experiments have identified some specific chemical cues to which young toads respond strongly whereas native frogs do not. In collaboration with University of Queensland researchers (Rob Capon's group), we are hoping that this work can rapidly generate useful chemically-based control approaches. Finally, the Invasive Animals CRC is funding a University of Sydney student (John Llewelyn) to explore toad-parasite interactions and test hypotheses about the effects of parasites on toad performance, invasion biology and population densities.

The ecological impact of cane toads

As noted above, there is little robust evidence that cane toads cause significant ecological effects apart from anecdotal (but consistent) reports of declines in populations of a few taxa (notably, varanid lizards and quolls). Although these species understandably have been the focus of considerable research, less attention has been focused on the fact that many taxa apparently remain unaffected. For example, despite the massive popularity of bird-watching among the general public, there are very few reports of serious concern about declines in bird populations following toad invasion. Mortalities certainly occur due to toad ingestion (e.g., Covacevich and Archer 1975) but apparently are sufficiently uncommon as not to raise public concern.

The effects of cane toads on native frogs and reptiles remain unclear, despite frequent assertions of ecological catastrophe. Logic suggests that the ecological similarities between toads and frogs would lead to significant competitive effects, but attempts to detect frog declines following toad invasion have generally reported no such effects (Freeland and Kerin 1988, Catling *et al.* 1999, H. McCallum, G. C. Grigg and A. Taylor, pers. comm.). Our own surveys of frog densities in sites behind and ahead of the invasion front similarly have detected no negative impact of toad arrival on frog numbers or biomass (Greenlees 2005). Our field-enclosure studies at Fogg Dam have provided the first robust experimental evidence that cane toads do indeed modify the invertebrate prey resource available for anurans, and can affect the foraging biology of native frogs (Greenlees *et al.* 2006). However, much remains to be done to establish whether or not such competition has any long-term consequences; invertebrate abundance during the tropical wet-season (the only time when many native anurans are active) may be so high that resource depletion is of no real significance. Another potential impact of toads on frogs is direct poisoning if frogs attempt to eat toads; our laboratory trials (and some field data also) confirm that this outcome does indeed occur, but that there is a complex diversity of responses among native frog species and hence, generalisations are impossible without further data.

What of the reptilian predators? Most Australian snakes that eat frogs have very limited ability to tolerate the cane toad's distinctive toxins and hence, a significant proportion of the Australian snake fauna are potentially at risk from toad invasion (Phillips *et al.* 2003). Consistent with that prediction, there are numerous anecdotal reports of population decline in predatory reptiles, especially varanid lizards and snakes. In an attempt to clarify this scenario, we have embarked on an ambitious series of studies to quantify the behavioural, physiological and ecological determinants of a reptile species' vulnerability to toads, and the pathways by which that vulnerability can be reduced. To date, we have collected prey preference data (the tendency to eat toads) from more than 300 individual snakes representing 10 species. During this process, it has become apparent that there is variation within and between snake species for the tendency to eat toads. Additionally, some species learn to avoid toads, others do not, and some species have complex behavioural adaptations that potentially reduce their likelihood of ingesting a toad. This work is ongoing in conjunction with a large radio-tracking program focusing on toad-vulnerable death adders (*Acanthophis praelongus*) to ascertain the determinants of interactions between snakes and toads. Our extensive radio-tracking dataset on death adders (53 individuals, >2,460 adder nights to date) has revealed that some individuals manage to survive despite regular interactions with toads.

As well as examining mechanisms in this way, we are also documenting overall changes in abundance of a very wide range of native taxa. Our extensive data-set from many years of nocturnal surveys using standardised methods over standardised routes is providing a quantitative picture of changes in faunal abundance coincident with toad arrival. So far, the only obvious casualties have been varanid lizards (*Varanus panoptes*) and death adders (*Acanthophis praelongus*), with several records of fatal encounters with toads. Presumably as a result, the abundance of goannas (as judged by daily sightings) has declined precipitously since the arrival of toads. However, there is no clear evidence as yet of declines in other taxa, even in species widely reported to be sensitive to toad presence. The advantage of our extensive pre-toad abundance dataset, however, is that we are very well placed to detect even small shifts in populations of many taxa.

As well as this focus on quantifying interactions between toads and native fauna at the time of their first encounter at the invasion front, we have also embarked on comparative studies to elucidate the potential for adaptive change in ways that facilitate coexistence with toads. The most extensive data come from detailed work on red-bellied black snakes (*Pseudechis porphyriacus*), a highly toad-vulnerable species that has apparently experienced severe population declines in areas invaded by the toads. Encouragingly, some black snake populations persist in such areas, and individuals from such populations display a series of "toad-smart" modifications of behaviour, morphology and physiology that render them less vulnerable to toads (Phillips and Shine 2004, 2006a). It is thus clear that Australian species can indeed evolve to deal with the toad's arrival, and the critical questions may involve which taxa are capable of mounting such a response, how rapidly they can do so, and to what degree populations can recover after an initial toad-induced decline. Importantly, much of our focus over the last few years has been on documenting variation in toad-relevant traits in naïve predators around Fogg Dam. These data will be used for comparison with both older toad-exposed predator populations as well as populations at our primary study site immediately after toads establish. In this way, we can examine the potential for susceptible native predators to mount rapid adaptive or plastic responses to the arrival of toads.

Black snakes are not the only species to persist in the presence of toads: highly toad-vulnerable species such as northern quolls (*Dasyurus hallucatus*) and varanid lizards (*Varanus panoptes*) also persist in areas of north-eastern Queensland where toads have been abundant for 70 years; indeed, there are many anecdotal reports that populations of some of these taxa have recovered to pre-toad levels (varanids) or are undergoing significant recovery (quolls). We have initiated a series of collaborative studies with scientists at James Cook University (Ross Alford, Lin Schwarzkopf) to examine these issues in greater detail, and clarify the mechanisms

that allow anurophagous native predators to persist in north-eastern Queensland despite the presence of toads. We also plan to extend our pre-invasion studies into the Kimberley region, to spatially replicate some of our major projects.

Implications for control

The central focus of the University of Sydney program on toad ecology is to understand the nature of the dynamic processes at work when complex tropical ecosystems are exposed to the novel challenges posed by toad arrival. The toad invasion provides a unique opportunity for research on topics such as rapid evolutionary change, because the situation generates novel and powerful selective forces. Thus, the toads are under intense selection as part of the invasion process, with dramatic shifts in morphology and behaviour related to selection at the invasion front (Phillips *et al.* 2006). Similarly, the toads constitute a massive selective force on some components of the native system, by adding competitive pressures and/or toxic prey to the environment (Phillips and Shine 2004, 2006*a*, Greenlees *et al.* 2006). Our data already reveal rapid responses via learning or adaptation, sometimes in organisms in which we did not expect to see such responses (and in many cases, in those with close relatives that show no such responses). The toad invasion also allows us to test many of our ideas about ecological functioning within the Fogg Dam ecosystem, by recording how the component species react to this new arrival.

Although these kinds of "pure science" issues were the initial stimulus for the expansion of the Fogg Dam work into invasion biology, it rapidly became apparent that many of our results might be relevant to local control of toad populations: either by clarifying the usefulness of current methods for control, or by suggesting opportunities for innovative methods. For example:

1. Most current approaches (such as trapping or hand-collecting) implicitly assume that toads are relatively philopatric, such that there is a local toad population. If this is true, then removing a certain proportion of those animals may reduce toad densities, at least in the short-term (the extraordinary fecundity of toads means that such efforts will have no long term impact, unless ALL adults are removed). One of the most surprising results from our mark-recapture and radio telemetry work on toads at Fogg Dam is that, at least for the first two wet-seasons of the invasion process, the animals are simply moving through. That is, there is no resident toad population, so that the animals that are captured at a specific site would not have remained there for long anyway, even if they had not been captured and removed. Hence, any effects of toad control will be manifested on a much wider spatial scale than would have been anticipated.

2. Public perceptions of the exact location of the toad front are likely to be inaccurate. Our data from Fogg Dam suggest that the first toads to arrive are extraordinarily mobile animals that move large distances in a sustained direction. They are hidden by day, so that their presence is likely to go unnoticed until densities build up sufficiently for occasional animals to be discovered – both by local humans and by local predators. Certainly, local residents in the Fogg Dam area were unaware of the toad invasion for many months after the first arrivals; it was only our nightly intensive surveys that detected these animals. Hence, the westernmost toads are likely to be much closer to the Kimberley region than is currently believed.

3. Vagaries of toad behaviour also provide deceptive cues about the location of the invasion front. One as-yet-unexplained phenomenon involves the dearth of wet-season calling by males, followed by a sudden aggregation of calling male toads at a single water body. In the course of the 2005-06 wet-season, we rarely heard calling males even when we knew that there were many hundreds of (individually-marked) adult toads in the area, and local native frogs were breeding vigorously. Late in the wet-season, groups of these hitherto-silent male toads would suddenly begin calling in a chorus one night at a specific water body – in some cases, a site where we were confident that there had been no previous calling whatsoever.

Unsurprisingly, local residents inferred that "the toad invasion front has suddenly arrived" and that the front is dominated by male animals. The reality was that hundreds of toads had been passing beside the water body for months, but had only just begun calling. Unrealistic public expectations of the efficiency of "toad traps" also contributed to this belief, in that residents often assumed that the traps would capture any toads to arrive. In practice, two traps set and zealously maintained within our study area failed to capture any of the first few hundred toads that passed beside them. The first toad captured in one of these traps was in September 2005, seven months after the toad invasion front had passed through this site.

4. Evaluating the effectiveness of local control measures requires careful experimental design. The immense spatial and temporal variation in toad activity levels (and thus, in apparent abundance) means that simply counting the number of toads in an area (or even worse, the number removed by control operations) gives no useful insight into actual densities. For example, our repeated nightly counts of toads along standardised sections of road frequently reveal sudden "disappearances" – easily misinterpreted as evidence of successful control by some local method such as a trap. In fact, such abrupt shifts in overt toad numbers are common, and can tell us nothing about the efficacy of control. In order to evaluate whether or not control methods are effective, there is simply no substitute for a properly-organised mark-recapture program to provide independent evidence of the numbers of toads actually present in an area before and after the application of the control method; and of course, such data need to include sufficient replicate areas as well as suitable control areas where no control methods have been applied. Absolutely nothing of value can be concluded from data taken at a single site, or at single "toad removal" versus "control" sites: replication is indispensable. There is a large and well-developed literature on methodologies for such analyses (Quinn and Keough 2003), but surprisingly little evidence of any attempts to genuinely evaluate the effectiveness of toad control initiatives.

On a more positive note, our work is revealing unexpected opportunities for local control of toad populations. For example, the toads have proved to be highly selective in their choice of spawning sites (preferring disturbed areas, shallow ponds with little surrounding vegetation, etc.) and thus there is an obvious potential for simple habitat manipulations to modify ponds in ways that make them less attractive to breeding toads (Hagman and Shine 2006). Our field surveys suggest strong sexual segregation during much of the wet season (presumably reflecting avoidance by females of males, who will often attempt to amplect unwary females). If we could understand and manipulate the cues driving this process, we could potentially limit toad breeding by spatially segregating populations or concentrating one sex in specific locations for control purposes. Similarly, our work with behavioural responses of toad larvae and metamorphs to chemical cues suggests that we may be able to manipulate the behaviour of these animals in ways that facilitate control.

More generally, our early results provide grounds for cautious optimism. First, the toads have not been as dramatically successful at exploiting the Adelaide River floodplain as we had anticipated. Numbers of adult toads are high but there has been relatively little successful breeding within our study area; for example, we recorded only a single metamorph from the 2004-05 wet-season. The toads have fared better in 2005-06, but still have bred later and at much lower levels than we had anticipated. These observations suggest that ecological conditions at Fogg Dam may somehow impede toad recruitment, and an understanding of such a process has obvious implications for control.

The magnitude and duration of the ecological effects of toad invasion also may prove to be less dramatic than is generally predicted. Although there is little doubt that a few native species are badly affected, the majority of taxa that we have examined seem to be more resilient to toad arrival than would have been predicted from the doomsday scenarios often proposed in the public media. Clearly, we may yet see more dramatic negative effects of toad arrival

– but so far, the effects of toad invasion have been surprisingly subtle (especially given the massive abundance and very obvious presence of these animals). Perhaps more importantly, the comparative evidence from northern Queensland suggests that native species are capable of adapting to the challenges posed by toad invasion, so that even if toads do induce significant declines, populations of many taxa are likely to recover to some extent. Thus, the preliminary results from the University of Sydney's research suggest that the ecological impact of toads is likely to be more narrowly concentrated (temporally, spatially and taxonomically) than is generally thought to be the case.

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Appendix 1. Scientific publications from the University of Sydney's ecological research program at Fogg Dam and the Adelaide River floodplain.

Please note that some of these papers were also supported by other institutions and/or are co-authored by workers from other institutions, notably Charles Darwin University and the University of Wollongong.

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Initial impacts of invasive cane toads (*Bufo marinus*) on predatory lizards and crocodiles

J. Sean Doody, Brian Green, Rachel Sims, and David Rhind.
University of Canberra, Invasive Animals Cooperative Research Centre

Abstract

The marine toad or cane toad, *Bufo marinus*, has spread westward across the Australian continent in the last 70 years since its introduction there. Because the Australian fauna has not co-evolved with true toads, which contain lethal skin toxins, cane toads reach high densities in Australia and there are numerous reports of mortality of frog-eating predators after mouthing or ingesting the toads. Notable examples include northern quolls (*Dasyurus hallucatus*), monitor lizards (*Varanus* spp.), freshwater crocodiles (*Crocodylus johnstoni*), and some snake species. Yet, quantitative data revealing the extent of impacts on such predators are lacking, despite the importance of such data for understanding toad impacts on species and communities, and thus, for decisions regarding cane toad control. We documented the relative abundance of three species of monitor lizards (*Varanus mertensi, V. mitchelli*, and *V. panoptes*) and freshwater crocodiles before and after the arrival of cane toads at two sites along the Daly River, Northern Territory, Australia, from 2001-2005. Herein we report on initial impacts of the toads on relative adundance of the monitor species. Declines in counts of all three monitor species at both sites were generally synchronous with the arrival of cane toads, but to differing extents. The sharpest decline in counts was found for *V. panoptes*, with 77-90% decreases in counts, followed by 86-92% declines in *V. mertensi* counts, and 28-40% declines in *V. mitchelli* counts. No measurable decline occurred in freshwater crocodiles, despite the occurrence of dead individuals with cane toads in their stomachs. A delayed impact may have occurred in the more aquatic lizard species (*V. mertensi, V. mitchelli)*, highlighting the need for continued monitoring in the determination of short- and long-term impacts of cane toads.

Key words: exotic species; relative abundance; conservation; cane toad; *Bufo marinus*; *Varanidae*; *Varanus mertensi*; *Varanus mitchelli*; *Varanus panoptes*.

Introduction

The South American marine or cane toad, *Bufo marinus*, has invaded over 50 countries (Easteal 1981, Lever 2001) including Australia, where the toads were introduced in 1935 in north Queensland as a bio-control agent (Freeland and Martin 1985). Since then cane toads have spread south to New South Wales and west to the Northern Territory (NT), and are moving rapidly toward Western Australia (reviewed in Phillips *et al.* 2003).

Several features of the cane toad render it likely to impact heavily on the Australian frog-eating fauna: (1) its success as a colonist allows it to obtain a wide distribution within Australia over time (Sutherst *et al.* 1995, Lever 2001); (2) it reaches population densities far beyond those within its native range (Covacevich and Archer 1975, Lampo and Bayliss 1996, Evans *et al.* 1998); and (3) its toxins, typical of the family Bufonidae, are not present in any native frog species, and as such the majority of Australia's fauna has not evolved any resistance to (or avoidance of) its effects (Tyler 1987).

Quantitative evidence of such impacts is surprisingly rare (reviewed in Phillips *et al.* 2003; but see Catling *et al.* 1999), and is attributable to lack of study rather than the lack of impacts (Lever 2001). Nevertheless, anecdotes and local reports indicate that many predators succumb to toads following ingestion (reviewed in Lever 2001; White, 2003). Population declines resulting from toad ingestion are suspected for northern quolls (*Dasyurus hallucatus*), monitor lizards

(*Varanus* spp.), dingos (*Canis lupus*), and possibly snakes (reviewed in Lever 2001; White, 2003), and studies of impacts of cane toads on selected predators are currently underway in the Top End of the Northern Territory (e.g., current study; B. Phillips, pers. comm.).

Determining the impacts of cane toads on native predators is fundamental to an assessment of the need for toad control and the financial and scientific investments required for development of suitable control techniques. In addition, because control should be aimed at reversing impacts rather than the elusive idea of eradication, knowledge of the effectiveness of control measures need to be underpinned by quantitative, baseline data. Our study provides such a baseline for any future control measure.

Our main objectives were:

1. To determine the relative abundance of three species of monitor lizards (*Varanus mertensi*, *V. mitchelli*, and *V. panoptes*) and freshwater crocodiles (*Crocodylus johnstoni*), at two locations along the Daly River, Northern Territory, prior to cane toad (*Bufo marinus*) invasion.
2. To document the timing of arrival of cane toads into the two study areas.
3. To repeat the above assessments during and immediately after the cane toad invasion, to assess any immediate impacts on relative abundance; and
4. To repeat the assessments in subsequent years to detect any recovery of lizard densities over the medium- and longer-term.

Herein, we report our results for the first five years of the study, which includes three pre- toad years and two post-toad years at one site, and two pre-toad years and one post-toad year at a second site.

Materials and methods

Study area, study period, and study animals

We studied predator-prey relations during the dry season at two sites along the Daly River (14º 04'40" S; 131º15'00" E) in the western Top End, Northern Territory, Australia. The first site is the 'upper Daly' site (UDS) between Oolloo Crossing and the Douglas River junction, and the second 'lower Daly' site (LDS) is near the Daly River Township. Navigation of the Daly River by boat provided a means in which to observe and count considerable numbers of monitors during a day-long survey. We anticipated the arrival of the toads by initiating the surveys in 2001. During this time the toads were only just reaching the Top End Region from the east (B. Phillips, pers. comm.). Monitor surveys were conducted along two 30 km stretches of river that were approximately 30 km apart (UDS and LDS). The UDS spanned from approximately 15 km downstream of Oolloo Crossing to the junction of the Douglas River, while the Lower Daly LDS spanned from the Daly River Township 30 km in the upstream direction. Site designation was based on our prediction that cane toads would invade the UDS at least one year prior to invading the LDS. In this way the LDS was used as a control site during the first year of toad invasion into the UDS.

Surveys for monitors and cane toads were conducted for three years prior to the arrival of toads (2001-2003) and two years post-toads (2004-2005) at UDS, and four years prior to the arrival of toads (2001-2004) and one year post-toads (2005) at LDS.

We chose *Varanus mertensi, V. mitchelli*, and *V. panoptes*, because they are frog-eating predators, and because we anticipated potential impacts of cane toads on those species - all three species were listed as 'high risk' by Burnett (1997). Similarly, freshwater crocodiles eat frogs, and there are unpublished reports of individuals dying from cane toad ingestion. Our search image for *Varanus panoptes* was that of a large (1-1.5 m) stationary lizard, and these

animals seldom moved quickly upon noticing us. During the dry season, this species tended to bask in the early morning hours, forage during late morning, either become inactive or forage in more shaded areas above the riverbanks during midday, become active again late in the day, and roost in self-excavated holes before dusk (pers. obs.). Our search image for *V. mertensi* was similar, as individuals seldom retreated upon discovery. *Varanus mitchelli*, on the other hand, was often seen scurrying up the bank as we moved past, although many individuals remained stationary. Individuals of this species were most often observed on logs, trees, and pandanus bushes, although some were seen on the bank, especially when fleeing. This species was often stationary and basking near retreat sites in early morning, moving during late-morning, inactive or in shade during the hotter parts of the day, and moving again in the evening. Some individuals were seen entering their retreat sites in late evening. Most crocodiles we counted were basking out of water, but a few were basking in water.

Monitor and crocodile surveys

Five monitor surveys were conducted by boat at each site each year between 20 May and 9 June. Surveys were only conducted on days with sunny weather. Each survey consisted of visual searches along one bank for the 30 km, and a return search along the opposite bank of the same 30 km stretch. Because we were interested in consistent counts rather than unbiased activity patterns (e.g., associated with aspect and sunlit banks), we started each survey at the same location, and surveyed in the same direction. The duration of each survey was approximately 9 hours. Surveys started at 0830 hours and ended at approximately 1750 hours. Two 15-minute breaks were taken at approximately 1015 hours and 1445 hours, and a 45-minute break was taken at approximately 1330 hours.

Surveys involved 4 persons (two observers, one driver/observer, and one recorder that did not observe). We visually searched the entire riverbank. Boat speed was kept constant at approximately 10 km/hr, and the distance of the boat from the bank was approximately 7-10 meters. Air and water temperature was recorded before and after each survey, and during each break.

Toad surveys

We surveyed for toads by vehicle along road transects. One road transect was near the upstream end of the UDS, and a second road transect was near the downstream end of the LDS. Both transects were 8 km in length. We conducted three surveys at each site each year between 20 May and 9 June (we did not conduct toad surveys in 2001-2002 because cane toads were > 50 km from the sites). Surveys began at approximately 1930 hours. Each survey consisted of driving 40 km/hour along a dirt track with high beam headlights on, and counting toads on the road and shoulder. For each survey there were two observers and a recorder. Surveys were only conducted on nights following sunny days. Air temperature and weather were recorded at the beginning of each survey.

Results

Toad Surveys

Our survey data indicated that cane toads moved through the UDS during the 2003-2004 wet season. Toads were not present at the UDS during the dry season survey of 2003, but were common throughout that site during the dry season surveys of 2004. This conclusion was supported by reports of toads arriving at the nearby Douglas Daly Research Farm and Douglas Daly Tourist Park during the wet season of 2003-2004 (P. O'Brien, pers. comm.).

We did not survey for toads in 2001-2002, because no sightings were reported from the area, and because unpublished information indicated that the toad 'front' had not yet reached the sites (> 50 km from the sites).

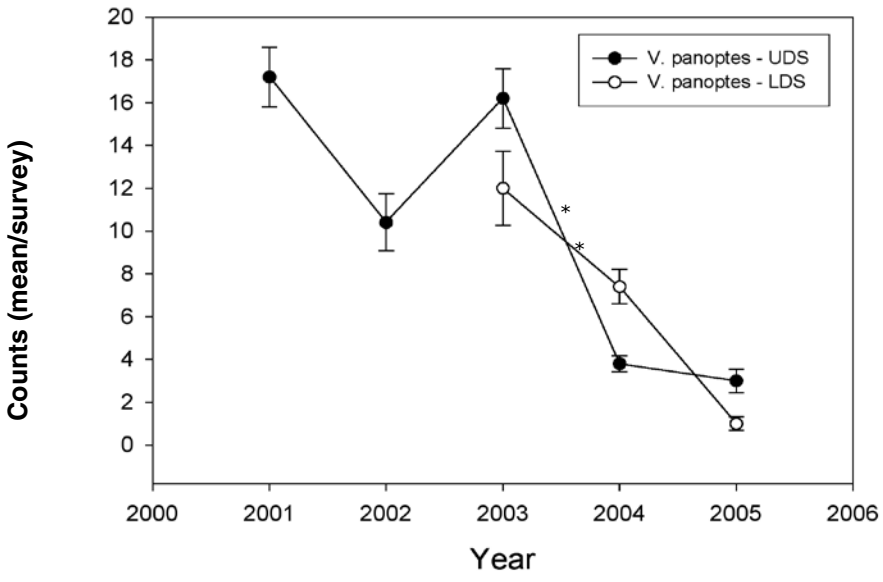
We found no toads during surveys of the LDS in 2004. However, because these surveys were conducted at the end of the LDS farthest from the direction the toads were approaching from, we needed to confirm that the toads had not reached the LDS by other means (there are no roads near the rest of the LDS). To achieve this we attempted to find the cane toad 'front' along the river during a single-night call survey through the 30 km LDS. On this survey we heard no toads calling from 10 billabongs along this river stretch. However, we did hear toads calling from a billabong just 200 m upstream of the LDS (the end closest to the approaching toads). Closer inspection revealed 25 calling males around a small semi-permanent billabong, about 150 m from the river. Subsequent searches on foot at the edge of the LDS revealed one toad in 3 hours of searching. This contrasted with casual observations of several toads active each night near our camp at the UDS. However, because toads were at the edge of the LDS during the 2004 surveys they may have begun impacting lizards in part of this river stretch.

Monitor and crocodile surveys

Due to the unbalanced design (different number of data years at each site) we analysed monitor counts separately for each site using repeated measures MANOVA. We found significant differences in mean *V. panoptes* counts among years at both the UDS ($F_{1,4} = 256.90$, $p = 0.047$) and the LDS ($F_{1,3} = 22.30$, $p = 0.016$). At the UDS, mean *V. panoptes* counts after cane toad arrival (2004-2005) were significantly lower than counts before the arrival of cane toads (2001-2003) (Fig. 1; Bonferroni-adjusted multiple comparisons, all $p < 0.05$).

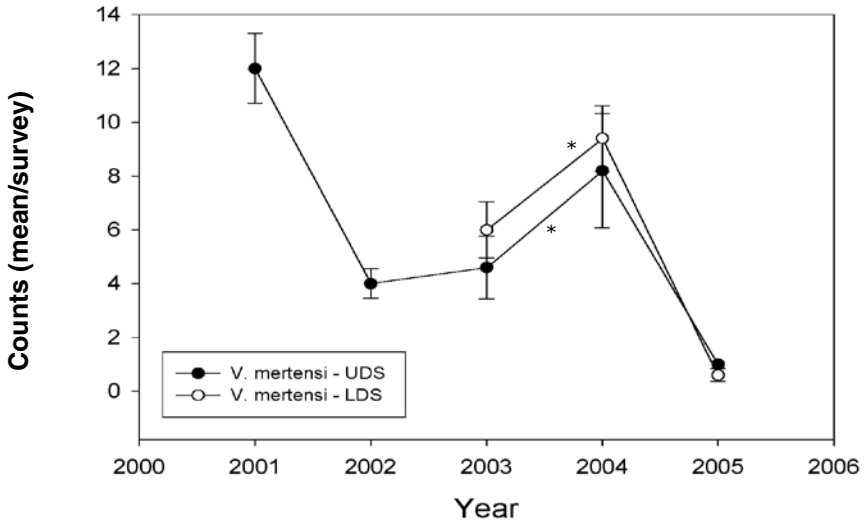
In contrast, among-year differences before toad arrival and among-year differences after toad arrival were not significantly different (Fig. 1; all $p > 0.05$). At the LDS, the mean 2005 *V. panoptes* count was significantly lower than the other two mean counts (2003-2004) (Fig. 1; Bonferroni-adjusted multiple comparisons, all $p < 0.01$), whereas there were no significant differences in mean counts between 2003 and 2004 (Fig. 1; $p = 0.190$).

Fig. 1. Relative abundance of* Varanus panoptes *at two sites before and after the arrival of cane toads (*Bufo marinus*). Counts are based on five surveys annually.
Error bars are ± 1 SE. ✶ indicates the approximate arrival of toads to each site.



For *V. mertensi*, we also found significant differences in mean counts among years at both the UDS ($F_{1,4}$ = 4347.39, p = 0.011) and the LDS ($F_{1,3}$ = 26.32, p = 0.013). At the UDS, mean *V. mertensi* counts in the final year (2005) were significantly lower than in the other four years (2001-2004) (Bonferroni-adjusted multiple comparisons, all p < 0.05; Fig. 2). The 2001 count was also significantly lower than the 2002 count (p = 0.009). There were no other differences among years at the UDS (Fig. 2; all p > 0.05). At the LDS, the mean 2005 *V. mertensi* count (post-toads) was significantly lower than the other two mean counts (2003-2004) (Fig. 2; Bonferroni-adjusted multiple comparisons, 2003 vs. 2002: p = 0.006; 2003 vs. 2001: p = 0.037), whereas there were no significant differences in mean counts between 2003 and 2004 (Fig. 2; p = 0.385).

Fig. 2. Relative abundance of* Varanus mertensi *at two sites before and after the arrival of cane toads (*Bufo marinus*). Counts are based on five surveys annually. Error bars are ± 1 SE. ✳ indicates the approximate arrival of toads to each site.



Mean *V. mitchelli* counts were not significantly different among years at the UDS ($F_{1,4}$ = 24.75, p = 0.149), but were significantly lower in 2005 than in 2001 Bonferroni-adjusted multiple comparisons; p = 0.009). Mean *V. mitchelli* counts were significantly different at the LDS (Fig. 3; $F_{1,3}$ = 32.89, p = 0.009). At the LDS *V. mitchelli* counts were significantly lower in 2005 (post-toads) than in the pre-toad years (Bonferroni-adjusted multiple comparisons; 2003: p = 0.011; 2004: p = 0.038; Fig. 3), while there was no difference in counts among the two pre-toad years (p = 1.00).

Mean crocodile counts showed no obvious decline (Fig. 4). However, because our crocodile counts were largely based on basking individuals, temperature (water, air, and the interplay between them) needs to be accounted for in the analyses to determine the influence of year on counts. These data are currently being analysed.

Fig. 3. Relative abundance of* Varanus mitchelli *at two sites before and after the arrival of cane toads (*Bufo marinus*). Counts are based on five surveys annually.
Error bars are ± 1 SE. ✳ indicates the approximate arrival of toads to each site.

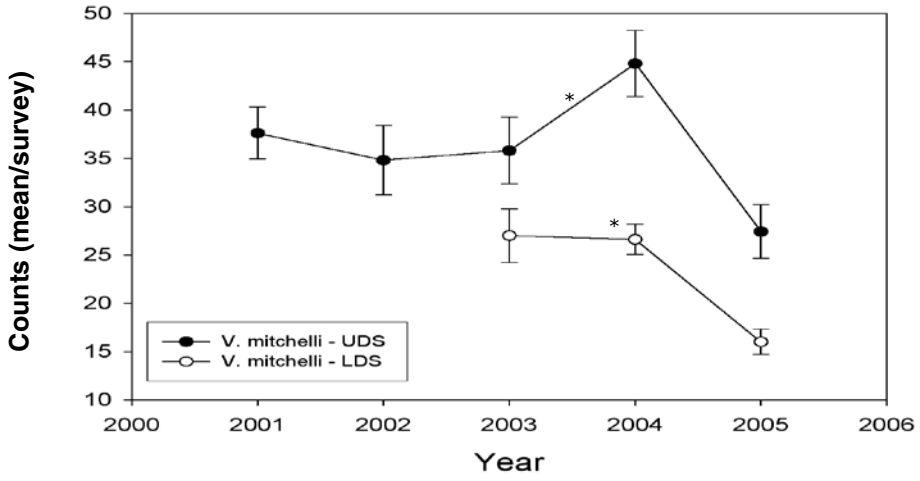
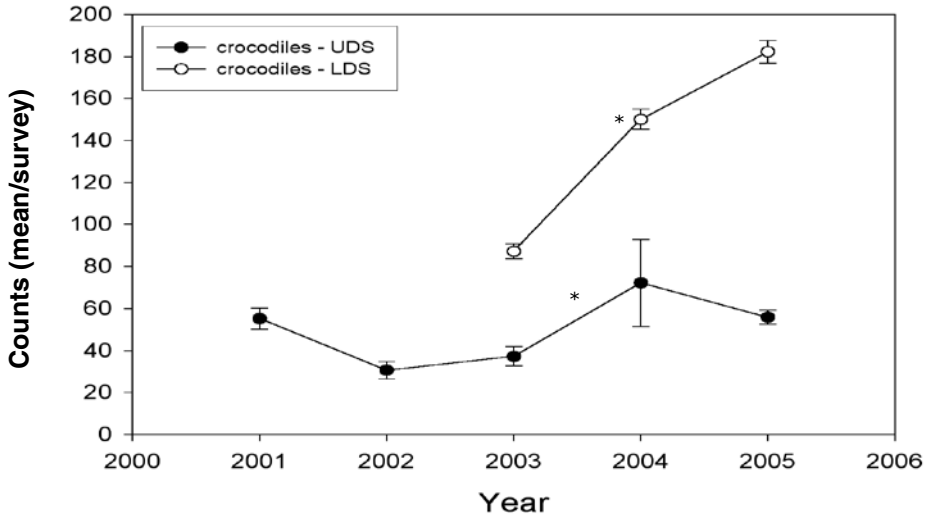


Fig. 4. Relative abundance of freshwater crocodiles (*Crocodylus johnstoni*) at two sites before and after the arrival of cane toads (*Bufo marinus*). Counts are based on five surveys annually.
Error bars are ± 1 SE. ✳ indicates the approximate arrival of toads to each site.



Observations of mortality

We observed several dead monitors during the last two years during and after arrival of the toads. We found three dead individual *V. panoptes* in 2004 and one dead in 2005 at the UDS (Fig. 5). We found one dead *V. mitchelli* at the LDS in 2005. Only one of the monitors (the 2005 *V. panoptes*) contained a cane toad in its stomach. We also found 11 dead freshwater crocodiles at the site in 2004-2005, all of which contained cane toads in the stomach (Fig. 6).

Fig. 5. A* V. panoptes carcase *found at the Upper Daly site in 2005. A cane toad was found in the stomach.

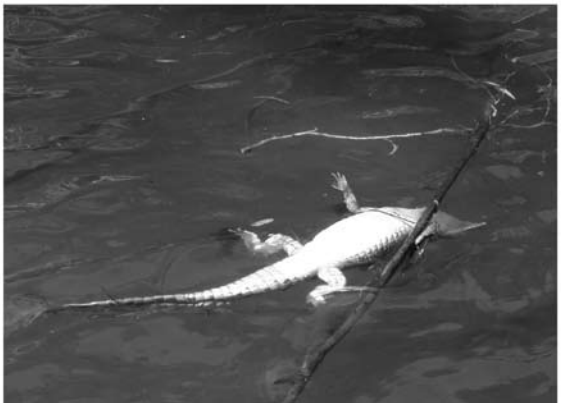


Fig. 6. A dead freshwater crocodile (*Crocodylus johnstoni*) found floating in the upper Daly River site in 2005. A cane toad was found in the stomach of the carcass.

Discussion

Collectively, our data and observations indicated that: (1) cane toads arrived at the UDS during the wet season of 2003-2004, and arrived at the LDS during the following wet season (2004-2005); (2) dramatic declines in counts of all three monitor species at both sites were generally synchronous with the arrival of cane toads, but to differing extents; (3) no measurable declines occurred in freshwater crocodiles; and (4) a delayed impact may have occurred in the more aquatic or riparian species of lizard (*V. mertensi, V. mitchelli*).

Impacts

The sharpest decline in counts was found for *V. panoptes*, with 77% and 90% decreases in counts between pre- and post-cane toads years (at the UDS and LDS, respectively; Fig. 1). Only 1-3 individual *V. panoptes* were observed, on average, in each 60 km survey, following the arrival of cane toads. Prior to arrival of toads these numbers ranged from an average of 10.4-17.2 *V. panoptes* per survey (Fig. 1). This decline in counts was also the fastest, with no apparent lag between toad arrival and declines in monitor counts.

In contrast to *V. panoptes*, the declines in counts of the other two species lagged behind the arrival of cane toads. For example, *V. mertensi* counts declined by similar amounts (86% and 92% at UDS and LDS, respectively), but only when comparing the last post-toad year with the other years (Fig. 2). Finally, declines in *V. mitchelli* counts, which also lagged behind the arrival of toads by one year, were much less severe at 28.4% and 40% (UDS and LDS, respectively; Fig. 3). However, the trend indicates that this decline is still occurring (Fig. 3), and another year of surveys is needed.

We conclude from these results that both *V. panoptes* and *V. mertensi* have likely experienced severe declines since the arrival of cane toads at the sites (Fig. 1; Fig. 2), while *V. mitchelli* either has experienced a lesser decline, or is still declining (Fig. 3). Both *V. panoptes* and *V. mertensi* are known to die after ingesting or mouthing cane toads (Burnett, 1997, Lever 2001). There are no published reports of *V. mitchelli* dying after attempting to feed on cane toads, other than our observation of one individual in 2005. All three species we surveyed are listed as 'high risk', based on how syntopic the species is with cane toads, whether or not the species eat frogs, and the proportion of the species range that lies within the potential distribution of the cane toad (Burnett, 1997).

The reason for delays in the declines of *V. mertensi* and *V. mitchelli*, particularly at the UDS, is unknown, but may be related to different encounter rates and/or the sizes of toads preyed upon by each species. While *V. mitchelli* are not large enough to prey on adult or large juvenile cane toads, they are capable of ingesting metamorphs and small juvenile toads. In addition, while adult and juvenile toads are nocturnal, and therefore not contemporaneous with diurnal monitors, the toxic metamorphs are diurnal and congregate at the waters edge, the natural foraging area of *V. mitchelli*. Thus the potential for *V. mitchelli* to encounter and prey on metamorphs of cane toads is high. A recent study of the diet and feeding responses of death adders to cane toads predicted similar size-dependent mortality rates (Webb *et al.* 2005).

In contrast to lizard impacts, no measurable declines were found in freshwater crocodiles (Fig. 4). Although statistics are in progress to account for the potentially-confounding factor of temperature (water, air, and the interplay between the two), it is highly unlikely that measurable declines have occurred (Fig. 4), despite our finding of dead individuals and unpublished reports of others. In support of this, two of four captured individuals (dissected for a study of *C. johnstoni* internal parasites from the site) contained cane toads in their stomachs (S. Snyder, unpubl. data).

Cane toads have been implicated in declines of Australian frog-eating predators (reviewed in Lever 2001), most notably the northern quoll (*Dasyurus hallicatus*) and monitor lizards, although quantitative evidence is lacking. Declines may also be occurring in some frog-eating snakes (Phillips *et al.* 2003) and dingoes (Catling *et al.* 1999). Our study supports these claims for two species of monitors (*V. panoptes, V. mertensi*), possibly a third. Our study is continuing, and it will be interesting to see (1) if *V. mitchelli* numbers follow the extreme downward trend exhibited by *V. panoptes* and *V. mertensi*; (2) if there is any future recovery in the relative densities of each of the three species under study; and if so, (3) the rate and extent of any such recovery. Anecdotal data from Queensland (Burnett 1997, White 2003) suggest that declines in monitor numbers will be marked and sustained.

Importantly, our study revealed that dead individuals does not equate to measurable declines. Our observations of dead *V. panoptes* translated into dramatic declines in the species, while similar observations of dead crocodiles did not. This highlights the need for quantitative data in determining impacts of invasive species on native species.

Recommendations

The strong impacts indicated by our data suggest three future recommendations. First, while declines in *V. panoptes* and *V. mertensi* have apparently 'levelled off', the apparent decline in *V. mitchelli* has not, underscoring the importance of at least one more year of survey data for that species. Second, given the serious declines, all three species should be monitored in subsequent years for any recovery. This could be accomplished on a yearly basis, or bi- or tri-annually if recovery seems slow and/or resources are constrained. Third, we do not know the generality of our findings for sites other than the Daly River. It seems likely that similar impacts are occurring across the Northern Territory and Western Australia, but currently no quantitative data are available for comparison. We recommend monitoring a second site ahead of the cane toad front in Western Australia to assess the generality of our findings.

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Characteristics of *Bufo marinus* in old and recently established populations

Ross A. Alford[1], Lin Schwarzkopf[1], Greg Brown[2], Ben Phillips[2] and Richard Shine[2]
[1]School of Tropical Biology, James Cook University, Townsville, Queensland 4811
[2]Biological Sciences A08, University of Sydney, Sydney, NSW 2006

Introduction

Approximately 100 individual cane toads were imported to Australia in 1935. In 1936 and 1937 their offspring were dispersed at numerous locations on the Queensland, coast, and their range has rapidly expanded to the north and west and more slowly to the south. Our best present estimate of the total Australian population is approximately 2 X 10^8. Efforts to control cane toads were first initiated in the mid nineteen eighties, when it was realized that their expanding range would eventually cause them to occupy the Top End of the Northern Territory and potentially northern Western Australia, including many environmentally sensitive areas such as Kakadu National Park (Freeland 1985). One of the areas of focus of initial control efforts was comparisons of old, established and new, expanding populations of cane toads. This was done because many reports suggested that toad numbers were declining in areas where populations had long been established, suggesting that biological agents might exist in these areas that could be moved to cut populations at the front, reducing or stopping their spread (Freeland 1985). This impression was aided by the idea prevalent among the general public that nothing controls or preys on cane toads.

There are several other general ideas regarding differences between came toad populations that have been established for extended periods and newly established ones. It is often thought that the first cane toads that arrived in an area have unusually large body sizes. It has also been suggested that the first arrivals are more prone to dispersal than toads in older, more established populations, and that they may move greater distances per unit time (Freeland 1986; Freeland *et al.* 1986). Recent analyses (Phillips and Shine 2005, 2006; Phillips *et al.* 2006) have suggested that differences between old and new populations may be the result of differential selective pressures acting on animals in dispersing and established populations.

In this paper we report on tests of a series of hypotheses about the biology of cane toads, using data collected on populations near the expansion front at three times and collected on long established populations in the late 1980s and early 1990s. We also used data collected at that time on the reproductive rates of cane toads, combined with data on their abundance, to produce a rough estimate of survival rates since toads were first introduced to Australia.

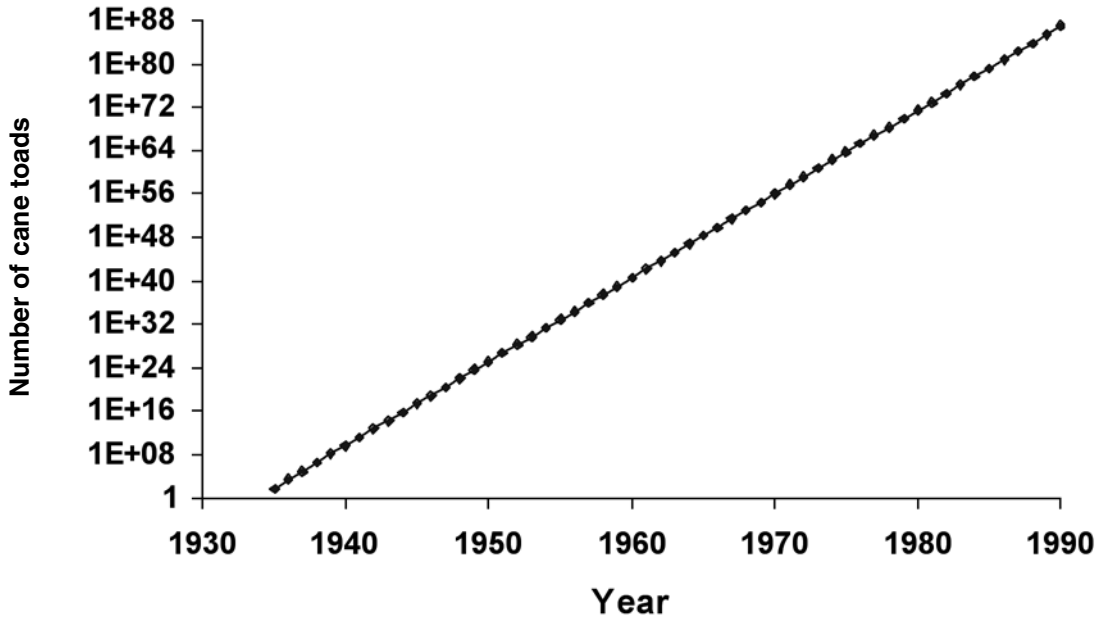
Methods, results, and discussion

Average survival rates since introduction

Alford *et al.* (1995) estimated the minimum egg production of female cane toads as 7000 eggs per reproductive episode. They suggested that female toads may often reproduce more than once per year. They also showed that when toads are reared under good conditions in favourable environments, they can survive to metamorphosis at high rates. Cohen and Alford (1993) demonstrated relatively high survival from immediate post-metamorphic size to 25mm juvenile size. Taken together these studies indicate that it would be entirely possible for 1% or more of cane toads to survive from eggs to reproductive maturity if, in fact, they were free from diseases and predators in the wild. To examine the implications of this, we used extremely conservative estimates of fecundity. We assumed that each female toad reproduced only once,

laying 7000 eggs. We also ignored the fact that in the first years while a program was in place to propagate toads, survival rates were undoubtedly artificially high. We assumed that random environmental sources of mortality might account for a mortality rate of 99% between egg and adult, so that only 1% of eggs survive to reproductive size. We use these figures to estimate the total population size of toads in Australia from 1935 to 1990, assuming these conditions applied during the period since introduction (Figure 1).

Figure 1: Number of cane toads that would have existed in Australia in each year from 1936 to 1990, if 1% of eggs survived to reproduction, and females reproduced only once, laying 7,000 eggs.



These calculations emphasize the fact that survival rates of cane toads from egg to adult are extremely low. If 1% had survived per year after introduction, the population in Australia would have reached 10^{80} in 1986. This is an absurdly large number, equal to cosomologists' present estimates of the number of atoms in the universe.

This indicates that the true survival rates of toads to reproductive maturity must be substantially below 1%. We used calculated a rough estimate of the true mean survival rates from egg to maturity in Australia over the period 1935-2005.

We made these assumptions:

1. 100 toads were introduced in 1935, 50 males and 50 females.
2. Approximately 2 X 10^8 toads were present on the continent in 2005; with a sex ratio of 1:1.
3. Toad populations have grown exponentially, with a constant survival rate, since 1935.
4. The sex ratio of toad populations is approximately 1:1 in the field.

These assumptions mean that we can estimate the exponential rate of population growth per year, by solving the equation

$$ae^{kt} = 2 \times 10^8$$

Where *a* is the initial population size (100), *k* is the exponential rate of increase of the population, and *t* is the time since the population was founded (50 time periods).

Solving this gives an exponential rate of increase, *k*, of 0.290173155, which gives a year-to-year increase of e^k = 1.336658917, implying that for the cane toad population to have increased as it has, on average each cane toad must produce 1.337 toads in the next year.

If the population sex ratio is 1:1, so that half of toads are males, and if we make the extreme simplifying assumption that females reproduce only once and die (which is conservative in that allowing iteroparous reproduction would lead to greater estimated egg production per female and would thus require lower survival rates), this implies that 2 X 1.336658917 ≈ 2.6733 eggs laid by each female must survive to maturity. If the average female lays 7000 eggs, then 6997.327 or 99.9618% must fail to reach maturity, which suggests that an upper bound on average survival rates from egg to maturity must be about 0.03819%.

This indicates that, far from the situation often suggested in the popular media that "nothing kills cane toads," they actually experience extremely high mortality rates, which only need to be increased slightly to halt their spread or even reduce their populations.

These calculations also emphasize the precarious nature of toad control efforts; if toads become established in a new area and experience even slightly reduced mortality rates for a few years, populations can explode extremely rapidly.

Densities of old and newly established populations

We compared the densities of populations in the Calvert Hills region of the Northern Territory, which was colonized in 1986, and the Townsville area of Queensland, colonized in the 1940s, over a 6 year period from 1986-1992, using data from Cohen (1995). In the Calvert Hills region, toad populations took less than one year to reach densities around water bodies that had similar medians and ranges to those in the Townsville region. Populations in the two regions fluctuated over successive years, but there was no indication of systematic differences between the two regions; instead, densities appeared to be determined largely by recent weather conditions, with poor wet seasons leading to decreases, and good wet seasons followed by increases.

Body sizes of toads in old and newly established populations

We used data from Cohen (1995) to examine distributions of body sizes in newly established populations at Calvert Hills over the period 1986-1992 and old populations in the Townsville region over the same period. The body sizes of both males and females were larger at Calvert Hills in the first year of colonization; males were a median of 117mm SVL and females were a median size of 130mm. In the second year after colonization, as individuals recruited into the new population entered the pool of adults, median body sizes at Calvert Hills decreased sharply, to 10mm for males and 96mm for females. They increased over the succeeding years, until by 1992 median sizes were 104mm for males and 108mm for females. Over the same period in the Townsville region, median body sizes remained relatively constant at approximately 104mm for males and 106mm for females. These data indicate that the first toads to arrive at Calvert Hills actually did have larger bodies, and that these individuals either rapidly died or continued moving out of the area, so that after the first year the Calvert Hills populations were dominated by toads that recruited locally. It supports the idea that the first immigrants to an area are large, fast-moving "dispersers".

Movement behaviour

We used data collected using radio telemetry in 1992 and 1993 by Schwarzkopf and Alford (2002) on toad movement behaviour in established populations in the Townsville region and in newly immigrated populations at Heathlands Ranger base, where toads first arrived in 1991, and data collected using similar techniques by Brown, Phillips and Shine near Fogg Dam in the Northern Territory, where toads first arrived in 2005. To enable comparisons of the data, we used only information on the locations of diurnal retreat sites of toads, which were usually determined once for each individual on each day.

We compared mean distance moved per day, the probability of changing shelter sites between days, the mean daily displacement of toads, and the straightness of their movement patterns over the periods in which they were tracked.

We found that distributions of mean distance moved per day by individual roads were similar for all three populations and times. However, toads in the newly established populations at Heathlands and Fogg Dam showed greater rates of moving to new shelter sites. Toads in both newly-established populations also moved along straighter paths. This caused total displacement per day to be greater in the Northern Territory populations than in either of the Queensland populations; toads in the Fogg Dam region increased their displacement from their original location at a median rates in the 10s of metres per day, with some individuals increasing displacement at rates of hundreds of metres per day, while toads in the Queensland populations moved off at median and maximum rates only about 10% as great.

We also examined the net movements of individual toads and the total populations of tracked toads in each region using circular statistics. In the Townsville region, no toads showed a significant directional bias in their movements. Only 11% of toads at Heathlands were significantly biased, while most toads in the Fogg Dam region showed overall directional biases. At the population level, there was no net bias in either the Townsville of Heathlands regions, while in the Fogg Dam region, there was a significant overall bias towards the north-northwest.

Conclusions

Cane toad populations have expanded relatively rapidly in Australia, but it is clear that they have grown at only a very small fraction of their potential rate. Mortality through accident cannot account for the fact that at most, about 0.03819% of toads have survived from egg to maturity each year since their introduction; there are many natural enemies, predators and diseases that limit their rate of increase.

There are real differences between toads at the front of the expanding range and in older established populations. The first arrivals are larger, and apparently continue to move on. In the Calvert Hills region of the Northern Territory, toad populations in the years immediately following their arrival appeared to be largely composed of individuals that had recruited locally, and their densities reached and stabilised at densities close to those of long established populations within one year of first colonization. This suggests that the first generation of recruits probably does experience reduced mortality, and supports the suggestions made by Alford *et al.* (1995) and others that a substantial fraction of mortality in the egg, hatchling, and metamorph stages may result from intraspecific competition and predation, so that cane toad populations are strongly intraspecifically density-dependent.

There are also real differences in movement behaviour that are consistent with the idea that individuals at the invasion front have different movement behaviour from those in established popoulations Schwarzkopf and Alford 2002. Animals at the front both in the Heathlands region of Queensland and the Fogg Dam area of the Northern Territory moved in straighter lines and tended to change shelter sites more often than those in established populations near Townsville.

These effects were particularly strong for toads in the Fogg Dam region; this may reflect the fact that they have been exposed to selection for properties favouring dispersal for a longer period. Individuals in the Fogg Dam region also showed strong directional biases in their movement; this could be an effect of the local topography, or could be the cumulative result of selection, in which toads that showed a northwesterly bias in their net movements would have remained at or near the invasion front in that region for an extended period.

Our results have implications for control efforts. The fact that on average toad populations have grown relatively slowly at the continental level, while they can grow extremely rapidly in local areas following initial establishment, reinforces the suggestion that intraspecific density dependence is likely to be a major regulator of toad numbers, raising the possibility that reductions effected through control measures may quickly be negated by increases in population size due to relaxation of intraspecific density dependence. The larger size, straighter movements, and biased directions of movement of toads at the invasion front mean that efforts to slow or stop invasions will require great vigilance, and the near-instant increase experienced at Calvert Hills following initial invasion suggests that any failure of this vigilance can lead to rapid population explosions in newly-colonised areas.

Acknowledgements

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Monitoring the impact of cane toads (*Bufo marinus*) on Northern Territory frogs – a progress report

Gordon Grigg[1], Andrew Taylor[2], Hamish McCallum[1] and Les Fletcher[1]
[1] School of Integrative Biology, University of Queensland, Australia 4072
[2] School of Computer Science & Engineering, University of New South Wales, Australia 3052.

Abstract

We have been monitoring the calling activity of native frogs nightly throughout the wet seasons for several years at wetland sites along the Roper Valley Highway (ten sites) and in Kakadu National Park (six sites), before and since the arrival of cane toads. At the first site toads invaded before we had a good baseline, but the data suggest a drop in the number of species calling, followed by a return to original numbers. We cannot be sure whether or not this was an effect of the toads. It is worth noting that, 6-7 years post-toad, all frog species there at the beginning of the study are still present. Toads have arrived only recently in Kakadu National Park, giving us a longer baseline. We now await the results of data post-toad in this area. A valuable outcome from the research has been the development of software and hardware which allows automated identification and monitoring of frog calls night after night for up to more than a year unattended.

Introduction

Although there is much anecdotal evidence or opinion about the negative effects of cane toads, *Bufo marinus*, on native fauna, there was very little hard data when we commenced this study in 1996 and, although more is known now, there is still a dearth of solid information. A useful and interesting desktop study by van Dam *et al.* (2000) reviews much of the relevant literature on the possible effects of cane toads on the fauna in Kakadu National Park, making predictions which are of course relevant more broadly in similar habitats across Australia's north. Most concern has been expressed about the direct effects of cane toad toxin on potential predators and, although attempted toad predation leads to the death of many goannas, snakes and quolls, at least, the long term impacts are much less certain (Freeland 1990). Work by Meri Oakwood within Kakadu National Park has, unfortunately, backed up the anecdotal information and predictions about quolls, for her radio tagged individuals all died with the arrival of toads at her study area in Kakadu National Park (Oakwood 2003a,b). Whether some will survive and become the founders of a toad-proof population, as has been suggested for goannas (Freeland 1990), is unknown at this stage.

Our interest has been broader, thinking about the possible indirect as well as direct effects that toads might precipitate within the ecosystem at large, including the effects of competition with other insect predators such as frogs.

Measurement of possible impact by toads has tended to be regarded as lower priority than research that might yield a control method. In the first tranche of funding to CSIRO for research into cane toad control, resulting from a Bob Hawke 1983 election promise, research into cane toad impacts was given a lower priority by the Hugh Tyndale-Biscoe committee than research into cane toad ecology, pathology and parasitology. Studies on these topics were conducted at various sites within Australia (notably Townsville) and in Brazil. However, whereas a lot of the negativity towards toads is aesthetic, and that alone may be considered to be sufficient justification for expenditure into a control method, if toads really do cause long-term negative impacts on our biodiversity or ecosystem processes, that might strengthen the case for funding research into control methods.

In the 1995 tranche of funding to CSIRO, this reasoning was accepted by and the new Cane Toad Research Advisory Committee, chaired by Tony Robinson, and expressions of interest were called for publicly for research to assess cane toad impacts. We put together an application to assess their impact on native frogs as they invaded the NT and (Gordon Grigg having left the room during the committee's deliberations) were awarded a grant of about $90,000 to put it into effect. Cane toads were then somewhere between Borroloola and Roper Bar, and heading west. Our plan was to assess the frog community to the west of Roper Bar and follow changes as the toads established themselves in the new area.

Methods

Why frogs?

There were several reasons for choosing frogs. Firstly, there is a rich and diverse fauna in the Northern Territory's Top End, more than 20 species, and frogs are sometimes regarded as indicators of ecosystem 'health' (Welsh and Ollivier 1998). Secondly, because toads may share similar habitats and have similar diets to frogs both as adults and as larvae, there is a potential for competition, which could be deleterious. An additional, compelling reason to choose frogs was that male frogs sing, with advertisement calls which are characteristic for their species, so they offer the opportunity to monitor them acoustically. They are also fairly sedentary and can be expected to vocalise reliably in their mating season, particularly in association with rainfall.

Automatic, long-term monitoring of frog advertisement calls.

Recording advertisement calls is a long standing practice for herpetologists (Heyer *et al.* 1994). However, the remote nature of the proposed study site (somewhere north west of Borroloola) coupled with the notorious variability of Australia's 'wet-dry' tropics, dictated a need for an automated monitoring system that could maintain a frog vigil at numerous sites throughout the wet season and over a period of at least several years. At the start of our study, no existing technology was available to us which would meet the requirements. The solution was provided by Andrew Taylor, who had been exploring software to recognize the flight calls of migrating birds. In a remarkably short time he developed the software and hardware for a computerised monitoring system which could monitor frog calling activity year round. Our system is solar powered and, having been 'taught' to recognise the sounds of all the frogs in the area, could be turned on each evening and write to flash memory the identifications of any vocalisations that were recognised during sequential five minute intervals (Taylor *et al.* 1996). We could visit once a year to download the data to a laptop computer. A library of frog advertisement calls was provided by Graeme Watson, then at the University of Melbourne, and added to by Graeme and Andrew in 1996. Recognising the need for tight experimental design and the potential problems of managing a huge amount of data, Hamish McCallum was recruited to the team. A lot depended upon suitable ways to mount the recording equipment in a 'bullet-proof' way, so Les Fletcher joined us as well. An account of the beginnings of the study can be found in the (now sadly defunct) Nature Australia (Grigg 2000, http://eprint.uq.edu.au/archive/00001461/). We now have sixteen such systems operating every night, taking data continuously and which we download once a year. We have ten between Roper Bar and Mataranka and six in Kakadu National Park.

Experimental design

Our experimental design followed the general principles of a BACI (Before-After-Control-Impact) design (Stewart-Oaten and Bence 2001). Five pairs of recording stations were arranged east of Mataranka along the Roper Valley Highway, which runs roughly parallel to the Roper River, about 25 km apart. We hoped that pairs of 'toadpoles' would be overrun successively by toads

each year, so that we would have before toad-arrival and after toad-arrival data for most pairs, and data in most years from sites with both toads present and toads absent. This design should permit detection of the impact of cane toad arrival, allowing for overall differences between sites and between wet seasons in frog calling frequency.

Sites were selected on a preliminary field trip early in the wet season of 1996-97. We chose sites with good frog choruses at the time, both in terms of numbers of frogs and diversity of species calling. All sites were in open woodland, with natural or artificial depressions within 20m that could be expected to hold water for most of the wet season. Given our collaboration between biologists and a computer scientist, we named the sites after prominent biologists or computer scientists. At each recording station, we erected a hollow steel pole (recycled lighting pole) approximately 5m high. A solar panel, microphone and rain gauge were mounted on the top of the pole, which contained a computer and rechargeable batteries. This equipment hangs on a rope over a pulley so it can be pulled up high into the pole to get it safely above water level during the wet season. Throughout the wet, each station turns itself on after dusk, listens for and identifies any frogs calling and logs that and environmental covariates (rainfall and temperature) at intervals of approximately 10 minutes. The software is capable of identifying the calls of 22 species of anurans (including *B .marinus*) on an intensity scale of 0-3.

Extension of study to Kakadu National Park

As the original stimulus for Bob Hawke's injection of funds was to prevent cane toads reaching Kakadu, it seemed logical to extend the study area by placing additional monitoring systems there. With funding from Parks Australia North, this was done progressively from 1997. In Kakadu we now have six sites, two in rocky stream habitat (with an interest in monitoring the Kakadu endemic *Litoria meireana*), two in floodplain habitat and two in savannah woodland. The two sites in rocky stream habitat have different configuration, being on four-legged aluminium stands which support the solar panels, batteries, rain gauge and computer. These sites are less likely to flood and, being distant from trafficked areas, less likely to suffer vandalism, hence there is less need for robust and uninteresting looking installations.

System reliability

The Roper Valley Highway sites are subject to bushfires almost annually but the fires apparently sweep through rapidly and, so far, no damage has resulted. Lightning strike is another potential hazard and one or more unexplained failures might have been caused by nearby lightning strikes. As the monitoring systems are mostly within steel poles and NT's Top End receives world record rates of lightning strike, it is surprising that no poles have been struck. Even apart from these potentially catastrophic hazards, it is quite a challenge to ensure continuous long-term operation of computer systems in such a harsh environment. Days with shade temperatures exceeding 40°C are common at the end of the dry season and the wet season brings torrential storms, extensive flooding, prolonged high humidity and, on several occasions, cyclones. Only limited protection can be provided to microphones without impairing their function. Our dynamic microphones have fared surprisingly well in the harsh conditions, with insect attack the worst problem. Our failure rate improved with experience. While we always expected some failures, early gear failures left significant gaps in our data collection. Even a simple problem, such as a dry connection at the voltage regulator can stop months of data being collected. As well as making pragmatic improvements, all of the original computer hardware has been replaced during the study with Pleb single board computers, custom designed and fabricated at UNSW. We now have a high degree of reliability. In the last two years we had 15 out of the 16 systems running correctly when we visited for the annual data download.

Ground-truthing

Matt Webb kindly volunteered to visit some of the sites on wet nights and list species he heard calling. Matt's species lists agree quite well with the automatic system. For example, over eight nights at Rifle Range, one of the savannah woodland sites, Matt listed 13 species, only one of which was not listed by the system. The system listed the same 12 species, plus three others which were heard uncommonly and could reasonably easily have been missed by Matt.

Data stream and data management and analysis

A sample of the output is shown in Figure 1.

Figure 1. An example of the output (daily summary) from a monitoring system over two weeks early in the wet season.

South Alligator

Date	Internal Temp. (C)	Rain (mm)	Monitoring Time (min)	Species 10min intervals
Dec 15	28-47		193	
Dec 16	28-48	2	221	australis(19) inermis(1)
Dec 17	29-46		198	
Dec 18	29-47	1	199	australis(16)
Dec 19	29-48	1	204	australis(13)
Dec 20	29-48		196	australis(6) inermis(1)
Dec 21	30-47	1	192	
Dec 22	30-47	21	190	australis(16)
Dec 23	27-48	7	188	convexiusculus(6)
Dec 24	27-45	9	199	australis(17)
Dec 25	28-47	1	202	australis(3) longipes(1) caerulea(1)
Dec 26	26-49	78	198	australis(2)
Dec 27	28-41	2	189	inundata(2) australis(2) inermis(15) pallida(1) rothii(2) watjulumensis(1)
Dec 28	28-44	2	191	inundata(2) australis(2) longipes(1) convexiusculus(1) inermis(7) pallida(6) watjulumensis(4)
Dec 29	28-43	2	202	inundata(3) australis(3) inermis(15) pallida(11) watjulumensis(2)

Results (provisional)

The study has a long way to go in both areas, but it is appropriate to provide this progress report.

Roper Valley Highway

The rapid movement of the expanding toad front through the area was unexpected. Perhaps the line of the front was oblique to the line of the highway, meaning that the movement down the road was very swift. The data set is also compromised by the early gear failures.

Table 1 Number of frog species calling (excluding* Bufo marinus*) per station, per wet season, Roper River Valley.

Site	1997-98	1998-99	1999-00	2000-01	2001-02	2002-03	2003-04	2004-05
Darwin	14	9	10	9	13	9	15	16
Huxley	16	11	1	-	15	5	9	12
Babbage	14	12	7	9	14	8	10	16
Lovelace	18	13	-	11	11	10	13	12
Dumeril	15	-	12	-	11	12	9	11
Bibron	13	6	-	-	3	1	4	6
Turing	11	7	10	7	9	10	11	12
Church	13	7	0	0	1	-	5	6
Andrewartha	12	6	8	2	9	8	9	9
Birch	8	-	-	-	-	-	-	-

Stations are ordered from Mataranka eastwards. The shading indicates wet seasons when cane toads were known to have reached each site, based on independent information, such as toads observed on roads. Dashes indicate wet seasons when the station concerned failed to record data. The number of recording days is shown in Table 2.

Table 2 Number of days on which at least one frog species (excluding* Bufo*) was recorded. Roper River valley.

	Wet season							
Site	1997-98	1998-99	1999-00	2000-01	2001-02	2002-03	2003-04	2004-05
Darwin	160	85	58	98	92	36	219	276
Huxley	142	100	1	-	147	53	44	40
Babbage	104	105	43	74	147	204	254	181
Lovelace	109	72	-	98	123	69	174	164
Dumeril	108	-	71	-	126	152	117	129
Bibron	44	25	-	-	4	2	12	11
Turing	104	73	30	55	42	38	7	80
Church	80	84	-	-	1	-	32	14
Andrewartha	48	17	21	16	99	26	107	50
Birch	28	17	15	-	-	1	-	1

Nevertheless, some tentative conclusions can be drawn. Table 1 shows the number of species of frogs recorded per wet season. Overall, the numbers of species recorded in 2003-04 and 2004-05 are similar to the numbers recorded at the start of the project, before toads arrived. In the middle of the project there appeared to be a substantial decline, but this may well have been due to fewer recording days (see Table 3). It may conceivably also have been a transitory impact of toad arrival. It will be interesting to see if a similar effect appears in Kakadu National Park.

Table 3: Days per station, per wet season, on which at least one frog species was recorded. Kakadu National Park. (Note: data from the 2005-2006 wet season will not be downloaded until late in 2006.)

Site	1997	1998	1999	2000	2001	2002	2003	2004
floodplain	n/a	70	-	114	145	169	176	241
Koongara	n/a	n/a	n/a	n/a	6	83	108	7
Mamukala	n/a	n/a	n/a	n/a	5	49	148	155
Nourlangie.camp	n/a	-	-	118	39	-	85	111
Nourlangie.rock	n/a	-	12	97	38	11	14	29
Rifle.range	119	111	62	72	104	149	214	188

Kakadu National Park

Toads started arriving in the park in the 2004-2005 wet season, but none had arrived at any of our monitoring sites by then so we have a much better pre-toad baseline for Kakadu than for the Roper Valley Highway.

A good indication of the number of days for which we have useful data is the number of days on which at least one frog species was recorded. This is shown in Table 3. We have eight wet seasons of good data from our initial recording station at the rifle range, five successive wet seasons from the South Alligator floodplain site and at least four years data from all other sites, with the exception of the Koongara Saddle site.

Table 4 shows the number of frog species recorded per wet season so far. Taking into account the likely gear failures at Mamukala in 2001-02 and Koongara in 2001-02 and 2004-05, the overall impression is of a high degree of stability in the frog communities at each site. No *Bufo marinus* calls have been recorded at any of our sites to late in 2005, but we expect we will find some records of toads from the 2005-2006 wet season when we visit the sites later in 2006.

Table 4: Number of frog species recorded per station per wet season. Kakadu National Park. (Note: data from the 2005-2006 wet season will not be downloaded until late in 2006.)

Site	1997	1998	1999	2000	2001	2002	2003	2004
floodplain	n/a	10	-	12	11	12	12	13
Koongara	n/a	n/a	n/a	n/a	4	6	8	1
Mamukala	n/a	n/a	n/a	n/a	3	13	12	12
Nourlangie.camp	n/a	-	-	5	9	-	9	11
Nourlangie.rock	n/a	-	5	5	8	6	5	7
Rifle.range	13	13	7	10	10	12	10	12

Discussion

Substantial and detailed analysis of the Roper Valley data is not yet complete. However, even cursory examination shows that approximately the same number of species of frogs is still present at most stations since the toads have arrived. It is worth bearing in mind that frogs have a lifespan of several years, so that populations of adults could survive for some time even while a population is facing severe decline from reduced recruitment.

Nevertheless, it is encouraging that Queensland still has a rich anuran fauna (Cogger 1992, Barker *et al.* 1995) despite living with cane toads for more than 60 years in some areas. Admittedly a complete survey of Queensland frog fauna had not been undertaken prior to their arrival, so we cannot be sure that no species were eliminated. Nor will we ever know whether or not the non-toad amphibian community became altered in any permanent way.

We anticipate publishing the results from our study in the Roper Valley area once the data from the 2005-2006 wet season are downloaded and incorporated.

In Kakadu, we have a longer and less compromised pre-toad baseline, and should be able to draw conclusions about the impact of toads, if any, with greater confidence.

Both data sets offer the opportunity for extensive analysis in the area of frog community ecology, reproductive seasonality, temporal variation in calling frequency and the effects of environmental conditions.

One of the interesting outcomes from the project has been the development of the automatic monitoring system, which provides the capacity for reliable long-term monitoring of any sounds for which it has been programmed. It has the capacity to detect the component species in complex choruses and store data from nightly observations for more than a year. The units tolerate the harsh and variable environment of the NT and so far they have survived bushfires and cyclones. In the event of several days of rain, after which they lose power, they re-boot and resume operation without losing their time base.

Their limitations, however, are that they need to be pre-trained to recognise the required sounds, from an existing or especially collected library of recorded sounds. This is time consuming and requires above average computer programming skills.

A second generation monitoring system which addresses these limitations is now in prospect. The proposed new system will be able to be deployed without pre-training into any acoustic environment. Its software will categorise sounds and store short samples for *post hoc* identification. These identifications will then be 'known', so an individual system will gradually acquire its own sound library, relevant to the environment in which it is located. Beyond this, there could be the capacity to share and compare its categorised sound library with a 'wiki' bioacoustic library either off line or via a wireless broadband or satellite internet connection. Individual units could, therefore, become part of a 'shared knowledge system', monitoring birds, bats, insects as well as frogs, quite a large proportion of the fauna. They could be portable, e.g. for 'snapshot' assessments of vocalising fauna as might be undertaken on a walked or driven transect, or be installed at fixed sites, returning data over months and years. We think that systems such as this have the potential to revolutionise biodiversity monitoring.

Acknowledgements

We are grateful to CSIRO and Parks Australia North for funding the research. Graeme Watson made his library of recording available to us and helped expand that on a couple of the early field trips during which we selected the sites along the Roper Valley Highway. Frank Seebacher, Peter Kind, Tim Jessop, Tim Schulz, Maurizio and Ida Bigazzi and Jan Grigg helped with installation of the 'toadpoles' and other support in the setting up phase. Numerous staff at Kakadu National Parks have assisted as well, and we are grateful to them all. Matthew Webb made independent observations at our monitoring in Kakadu to enable ground truthing.

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SESSION 3: CURRENT CONTROL

Frogwatch report on the Community Cane Toad Control project

Graeme Sawyer, FrogWatch, graemes@entity1.com.
Web http://www.frogwatch.org.au

Abstract

FrogWatch has been instrumental in motivating the population around Darwin to tackle the invasion of cane toads head-on rather than allow them to establish in the region. The project has been very successful to date, with cane toads failing to penetrate into Darwin in numbers expected in the 2005-06 wet season.

This project has shown that it is feasible to generate very significant levels of public action in relation to environmental problems like that posed by the invasion of the cane toad into the region. It also shows that community government partnerships can be used to develop significant action in response to such problems.

FrogWatch background

FrogWatch was started in the Northern Territory (NT) in 1991 by Ian Morris and Graeme Sawyer in response to frog decline issues in other parts of Australia.

Initially we were unable to get cane toads on the "political" agenda and could not get the government agencies to consider the issue.

The core aims of the FrogWatch (Nth) project are to:

1. Provide a focus point for information about the frogs of North Australia.
2. Provide a specific focus for cane toad related information in Northern Australia.
3. Facilitate community knowledge and awareness about frogs and related issues.
4. Educate people about frogs and frog calls in an attempt to increase knowledge about species and their distribution.
5. Facilitate collaboration by researchers and other professionals working in this area.
6. Facilitate knowledge transfer, in both directions, between the general community, including indigenous communities, and the researchers.
7. Raise the profile of frog related issues in the tropical north through education.

We were able to establish a significant public and media profile which led to the eventual success in getting the cane toad issue on the agenda.

NT government funding

FrogWatch was able to pay workers and spend money on some aspects of the project after the NT Govt committed to funding approximately $400,000.00 over 16 months for the project to engage the community in efforts to minimise the damage from cane toads.

The media coverage and the hours of community work have been very significant given the government investment.

Strategy

The basics of our strategy are to:

1. Minimise the number of toads breeding through the wet season; and
2. to reduce their numbers to a very low level, even local extinction, during the dry season to minimise breeding the following year.
3. Protect and manage areas of habitat for native species, especially native frogs.

We are promoting a 'ToadBuster' campaign to boost public participation and will organise 'ToadMusters' where toads become established. Wet season and dry season strategies are a little different due to difference in toad behaviour during these periods.

Traps

We are encouraging people to build or buy a trap for their yard and we are placing permanent traps in major wetlands. Traps can make a significant impact as well and provide a focus for groups as well play a key role in removing toads.

ToadMusters

We are encouraging people to pick up and remove toads from their yard and local areas. We are also organising major events such as NIMBY week (Not In My Back Yard).

Wet season

We may not have the resources to 'stop' toads during the wet season but we can manage their impacts to a degree through trapping and musters.

Interestingly because females stay away from the males near water bodies, many female toads are found in people's yards. Over 65% of toads put in the detention centre are females.

Dry season

The plan is to remove toads from areas, especially after they congregate as the Dry season sets in. At this time traps work at their peak as shown by the Ringwood research project.

At this time we also do more ToadMusters as they become easier and more effective as toads congregate and water recedes, leaving low grass areas where toads congregate to feed.

Community education role

We took the attitude that even if we could not stop toads we would develop a lot of environmental awareness from the issue.

We see this education function as a core way to increase awareness and we have conducted a range of activities across a wide spectrum of the community. This has included community events, specific public meetings as well as specific meetings with Land Care groups, indigenous groups as well as school visits and media events.

There have been an enormous number of media articles on radio, television and in the print media and the public awareness of the issue is extremely high. There were over 150 media publishing events about FrogWatch and cane toads.

Market stall and information displays at public events

We have conducted many activities of this nature with significant exposure for the toad issue being achieved and many people being educated about what to look for in relation to toads and also what to do to minimise their impact.

It is obvious that people at these displays had a real interest in the issue and the questions were more focused and detailed than at many of the other information displays. It should be targeted as a major focus each year.

Schools

The NT Museum and annual CSIRO Science Fair, provided opportunities to deliver key messages to thousands of school students.

We have also made specific visits to many schools throughout the area. A part of the focus for this is to get students active in their school and the community in relation to cane toads and native frogs.

Howard Springs frog pond launch

Many schools have developed a frog pond with design and concept assistance from FrogWatch. These are set up as areas native frogs can utilise, but cane toads cannot.

The holiday care programme had a visit from FrogWatch including a presentation about toads and native frogs and a chance to see a cane toad. Some interesting feedback was received at this activity that indicates the focus on schools is working. One mother commented about the fact they had caught a "toad" and were about to place it in a freezer when their school age daughter was able to positively identify it as a marbled frog.

University students

Presentations were made to CDU Cert IV Conservation and Land Management students for lecturer Prue Adamson. The group was taken on an excursion to the research site at Ringwood where the students cleared the traps and discussed toad issues. The group is progressing plans for toad control on the lake at the Palmerston campus and we have continued an email dialogue in relation to this.

Landcare groups

We have had meetings with the landcare groups in relation to the use of traps and musters to minimise the numbers of toads in wetlands. These groups form the basis of the control strategy for the major wetlands around Darwin and will manage the traps placed in these locations.

A test trap of the new secure SuperTrap design was tested along the Margaret river and caught 109 toads in the first week.

Toad audit

FrogWatch nominated August 18th as the day for a media campaign to check toads around the greater Darwin region. The date was chosen as it was the 70th anniversary of the release of toads in Australia.

The exercise attracted very significant media attention and was well supported by people across Darwin, Palmerston and the rural area. ABC radio made several live crosses to people out looking for toads on the night and made the exercise a focus for the night's broadcast. One school in the rural area had approximately 150 families check their backyards on the night and return a slip to the school. Many people emailed, telephoned on the night of the Toad Audit and many others commented afterwards that they had checked their yards, local public land, on the night, although they hadn't rung in or emailed because they had not found toads.

The audit will become a major annual event for FrogWatch and will be the launch point of the major dry season assault on cane toads.

Toad musters

The first ToadMuster was called to make sure the Darwin Botanic Gardens area was clear of toads as we move into the wet season breeding peak. FrogWatch is aware of 25 toads removed and the incursion was completely removed as no further toads have been sighted in the area. Undoubtedly there would have been breeding in the area if these toads had not been removed as there were a number of mature females among them.

The muster had a very good turn out with over 125 people coming out on a Wednesday night to assist.

Other support

We have been successful in attracting sponsorship from Telstra in the form of the supply of second hand panels. These will mean we can extend the number of SuperTraps placed in the wetlands from the planned 37 to approximately 50.

Additional funding has been sourced though Federal Envirofund funding providing $40,000.00 to put SuperTraps into key wetlands across the region with the support of Landcare groups. This is to ensure there are no major toad populations near to the population centres at the start of the next wet season.

Campaigns

A toad awareness campaign has been started to focus on the industrial areas of Darwin. These areas are a significant risk factor because they are the areas where there are buildings and equipment and few people at night, during the wet season they are areas where there is water. The area is also close to bushland providing access to the areas for toads.

Posters

New frill neck poster, with a "Can we save them?" caption has been printed and distribution commenced. This campaign is aimed at using the frill neck lizard as an icon species to further motivate people to do something about toads.

Toad detention centre and mass disposal

The first cane toad Detention Centre, a place where members of the public can dispose of live toads was installed in February and the publicity generated by the event was extensive with TV coverage by ABC and Ch 9 news, a live radio cross on ABC and followed up by radio stations around the country from NSW, QLD and WA.

The community response has been enthusiastic, as many more people are prepared to join the ToadBusting and trapping effort with the knowledge that they don't have to kill and dispose of toads themselves. Over 2,000 toads were placed in the Centre in the first 4 months and only 4 native frogs (these were released unharmed).

The collection effort through such centres leads directly to the strategy for mass toad disposal – in cooperation with Darwin fertiliser manufacturer, Moeco. FrogWatch is part funding, in conjunction with Moeco, analysis of the 'Toad Juice' product, a first batch of which was processed in February. The product will be further tested by Greening Australia before being launched through local distributors and through FrogWatch displays and market stalls later in the year. The Moeco experiment potentially provides both a means of disposing of large numbers of toads and an additional source of income for FrogWatch to reinvest in research and other activities.

Community awareness through the media

FrogWatch has generated a number of stories in the local, national and even international media by being aware of the stories that interest the public and thus the media. Over 110 radio mentions and 130 newspaper articles have been identified.

Partly as a result of media attention there is a high level of interest and public knowledge of the toad and the threat it poses to biodiversity and lifestyle.

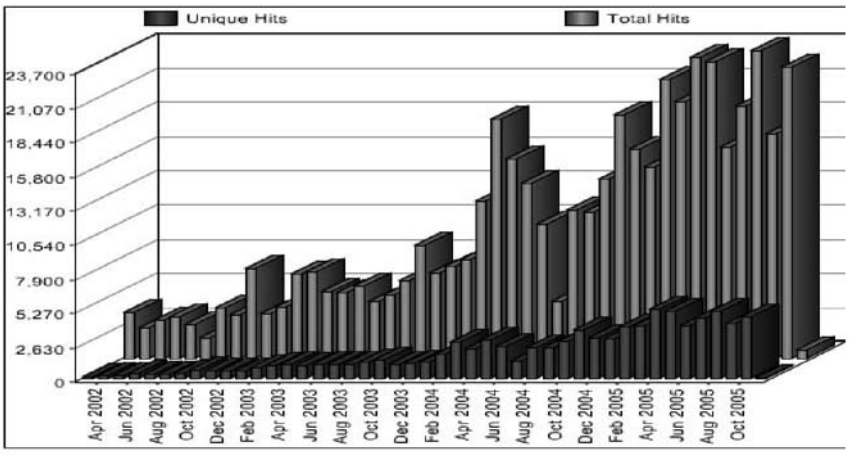
The free 1800 Toad Report phone now receives an average eight calls a week from city people reporting toad sightings and three times that number from people seeking basic information about protecting their land, backyard pools and the like. Toad disposals and toad sightings are listed on the FrogWatch website. A number of other calls are taken directly on mobiles, in excess of 20 per week.

Website

The website continues as our major information source for people across the NT and it continues to deliver information on a large scale. Registered users on the site continue to grow(Figure 1): Oct 2005 had 811 registered users, Feb 2006 had 1003 registered users, May 2006 had 1114 registered users. Interestingly in early 2006 the most common search on Google from Darwin was for the term cane toad.

The site provides over 20,000 information requests a month from over 4,000 unique visitors.

Figure 1: Graph of website visits



Newsletters

Regular newsletters have been sent out along with specific notices relating to events, These have been sent to the addresses in the website system using the email capability of the web system. Newsletters can be downloaded from the website.

Research

We have continued to monitor the toad population on the control site a Ringwood to look at the toad numbers in an area where nothing is being done about them.

The increase of toads in this location has been quite large during the course of the first year of the toad invasion. This work has shown strong indications of the movement of toads to water during the dry season which may prove to be a key factor in long-term management of toads. We will continue to monitor this site into the second year of the invasion in that area and we are seeking funds to extend the research.

Following is a summary of the research done in that area with volunteers from FrogWatch doing the trap checks and providing the labour. Results from the placement of the traps on the shoreline of the dam on Ringwood station is that traps can limit the build up of toads. Compared to the nearby control site the numbers of cane toads at the trapping location have been reduced significantly, approximately 70%. The total capture to date is 1846 toads.

Frogwatch trapping report

Graeme Sawyer, FrogWatch**,** graemes@entity1.com.
Web http://www.frogwatch.org.au

Background

FrogWatch is a not for profit organization focused of raising environmental awareness, especially issues relating to frogs. FrogWatch has been active in the Northern Territory (NT) for since 1991 and has developed a very strong profile across the local community.

FrogWatch is dependent upon the voluntary efforts of members at all levels of our community. In 2004 we recognised the need to get some paid resources working for FrogWatch in order to organise action against cane toads in the NT. We are actively seeking the support of governments, businesses and individuals to provide us with the resources needed to galvanise public action against cane toads. We have received invaluable support from the Northern Territory Government for a cane toad control initiative in the populated areas near Darwin.

Whilst our primary focus is on native frogs we have played a very significant role in raising community awareness about cane toads and their impact on native ecosystems. For a full report on the Community Control Initiative see the FrogWatch website http://www.frogwatch.org.au.

FrogWatch has developed a number of traps that catch toads (Figures 1 and 2) and have commenced trials of various traps using the one-way gate mechanism developed by Graeme Sawyer and Dave Wilson.

Figure 1: Clear-fingered one-way trap gate

FrogWatch has trialled traps in a number of settings, including remote bush locations and areas around dwellings as a part of their research into ways to minimise the impact of the cane toad invasion of the top end of the Northern Territory.

Figure 2: : SuperTrap at Ringwood station trial site

The trials showed that, during the dry season, traps can catch all the toads in an area around a house or block in a few weeks and that once the toads have been removed any new toads moving into the area seem to be quickly caught in the traps. Test sites have been kept relatively toad free by a single cage trap. Landowners have commented that they never see toads around the house anymore, except for those in the trap.

The larger cage traps would appear to be able to play a significant role in capturing toads on a broader scale. The Bonrook trial trap, in Dec 2004 - Jan 2005, captured 224 in the first week and 543 in total over 6 weeks (Table 1). This was a single Supertrap (3 door) placed at the homestead area of a cattle station near Pine Creek in the Northern Territory.

Table 1: Weekly Capture rates from the Bonrook trial trap

Week	Captures
1	224
2	130
3	80
4	42
5	39
6	24

The captures and observations of the station managers indicated a very significant and rapid decline in toad numbers around the area. This gave us hope that broader scale control was feasible. To test this we set up trap trials in a bush location at Mt Ringwood station.

The FrogWatch 'Supertrap' trial at Ringwood station, 130kms south of Darwin, has shown that a large capacity, solar powered and automated trap systems can continually capture toads around a wetland and reduce the toad numbers in the area. The traps are capturing toads during the wet season as well as the dry season, which is a boost to our confidence that the traps will help to reduce cane toad numbers in areas where traps are used.

Somewhat surprisingly there has been no by-catch in the cage trap trials with in excess of 250 nights of trapping catching nothing but cane toads. It appears that we have an effective, manageable and species-specific cane toad trap.

These results invoke the possibility that the traps can be used as a broad scale control mechanism and play a key role in cane toad management and threat abatement plans.

This would particularly be the case if toad behaviour in the wet dry tropics makes them susceptible to control, especially in the dry season. Their need for water and ability to move indicates they will congregate on remnant water. Preliminary field observations and research support this.

Keeping toad numbers suppressed in areas where the blocks of land are small and there is a reasonable density of people would seem to be achievable.

Broader scale control or minimisation strategies also seems to be feasible leading to regional control strategies or larger scale eradication programs or threat abatement strategies, especially in significant wetlands such as those listed under RAMSAR or JAMBA agreements or in national parks. There is still further testing needed to verify the extent to which toads in an area will congregate on remnant water in the late dry season and as they become more hungry, their susceptibility to traps.

Methods

FrogWatch has set up traps on a man made dam on Mt Ringwood station 125 km south of Darwin. The initial trap was set up on January 1 2005. A second trap was added on the eastern side of the dam on April 19 2005. A third trap was added on 26th May 2005. The plan is to just use traps to see what impact they can have on the cane toad population at the site. The population of cane toads at a second dam about 2.5 kms from the trapping site is being used as a comparison. Spotlight surveys of the entire perimeter of each dam, taken on the same night, are used as comparisons samples of the population at each location.

It was our expectation that the trapping site would have significantly less toads by the end of the dry season compared to the control site, and the subsequent build up of toads will be slower on the trapping site than at the control site in the following wet season.

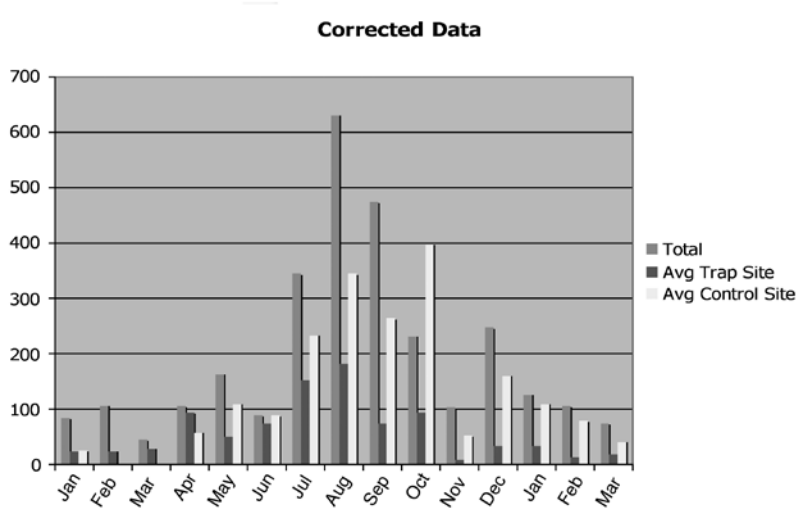
Traps were set with lights to attract the toads. A range of fluorescent lights have been trailed, including black light UV and white light tubes. All types of lights caught toads but the UV lights appear to work better. We are currently testing black light insect and black light blue wavelengths of light.

Research outcomes

Results from the placement of the traps on the shoreline of the dam on Ringwood station is that traps can limit the build up of toads. Compared to the nearby control site the numbers of cane toads at the trapping location have been reduced significantly, approximately 74%. The total capture to date (Apr 2006) is 2990 toads.

Results have shown a build up of numbers as the surface water decreased at the end of the Wet season (Late April 2005). This build up continued during July and August. The trials have also shown an increase in the effectiveness of the traps in terms of the capture rate and the impact on the population at the trapping site compared to the control site.

Figure 3: Results from the trial site. The mid grey bars show the total monthly captures, the dark bars show the average of the monthly counts on Dam 1 (trapping site) and the light bars show the average counts for Dam 2 (the control site). Note there were no counts on the control site in Feb and March due to access issues during the wet season.



The toad population would appear to have been significantly reduced by the trapping to date, approximately 70%. We have used the traps alone and no other mechanisms and are confident that we could accelerate the process by using more traps and some manual control measures such as toad musters.

The low capture rate in March is probably due to a combination of long grass making the lights harder to see and the fact that insects were so prolific that the insect ball at the trap was larger than the trap. This meant toads could get a feed of insects without going into the traps.

The jump in toad numbers in April was probably due to the onset of the dry season and much of the ephemeral surface water vanishing causing the toads to move in on the more permanent water sources. The significant jump in numbers in July-August coincides with smaller waterholes in creeks and shallow wetlands drying up. This would support the idea that toads will congregate on permanent water points but further research is needed to verify the extent of this. Observations indicate it is a very strong effect.

The capture of toads appears to be decreasing the toad population significantly at site 1 (dark bars) compared to the control site. The capture rate is increasing as the dry season progresses. Counts indicate approximately 74 % reduction to date.

Preliminary data analysis

The following statistical analysis looks at the results. Is there a significant change in numbers over time for Supertrap captures, trapped site observations, and control site observations?

Captures (monthly average)

There appears to be a significant 2nd order polynomial trend from January 2005 to March 2006 ($P < 0.0444$) suggesting an increase in toad captures during the dry season and a decline in toad captures during the wet season (Figure 4).

Figure 4: Captures (monthly average)

Regression Summary
capture.2 vs. month

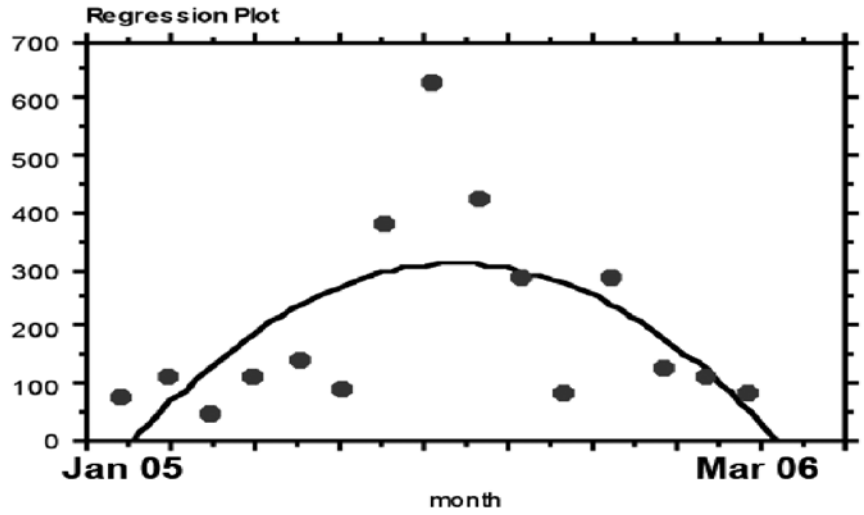
Count	15
Num. Missing	55
R	.636
R Squared	.405
Adjusted R Squared	.306
RMS Residual	138.822

ANOVA Table
capture.2 vs. month

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	2	157304.160	78652.080	4.081	.0444
Residual	12	231257.573	19271.464		
Total	14	388561.733			

Regression Coefficients
capture.2 vs. month

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	-8826529.259	3147049.544	-8826529.259	-2.805	.0159
month	.006	.002	392.957	2.804	.0159
month^2	-8.583E-13	3.062E-13	-392.834	-2.803	.0160



There appears to be a significant 2nd order polynomial trend from January 2005 to March 2006 ($P < 0.0198$) suggesting an increase in toad sightings during the dry season and a decline in toad sightings during the wet season at the trapped site.

Figure 5: Sightings trapped site (monthly average)

Regression Summary
c1avg vs. month

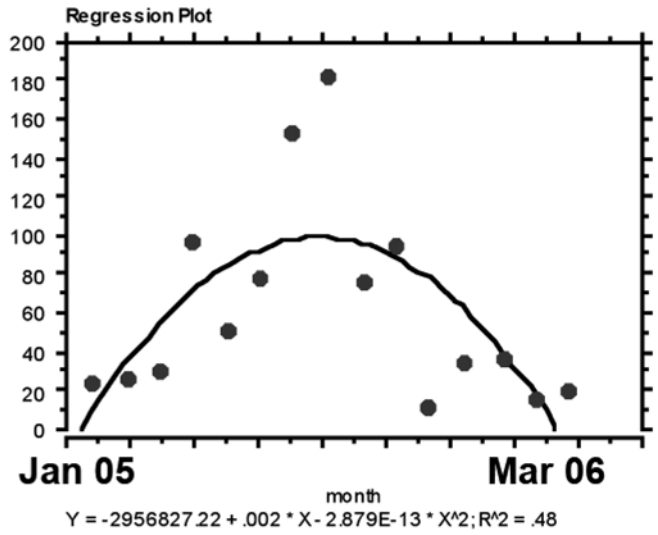
Count	15
Num. Missing	55
R	.693
R Squared	.480
Adjusted R Squared	.393
RMS Residual	40.185

ANOVA Table
c1avg vs. month

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	2	17885.132	8942.566	5.538	.0198
Residual	12	19378.081	1614.840		
Total	14	37263.213			

Regression Coefficients
c1avg vs. month

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	-2956827.220	910984.487	-2956827.220	-3.246	.0070
month	.002	.001	425.363	3.247	.0070
month^2	-2.879E-13	8.863E-14	-425.515	-3.248	.0070



There appears to be a significant 2nd order polynomial trend from January 2005 to March 2006 ($P < 0.0437$) suggesting an increase in toad sightings during the dry season and a decline in toad sightings during the wet season at the control site (Figures 5 and 6).

Figure 6: Sightings control site (monthly average)

Regression Summary
c2avg vs. month

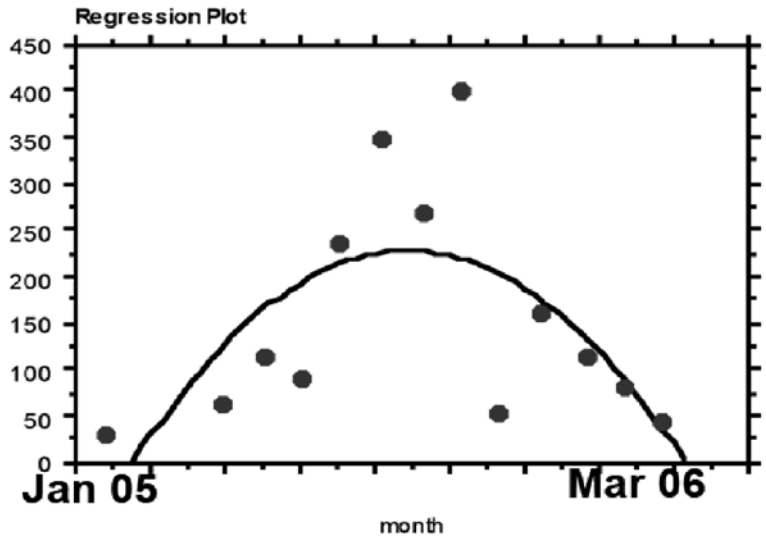
Count	13
Num. Missing	57
R	.682
R Squared	.465
Adjusted R Squared	.358
RMS Residual	96.912

ANOVA Table
c2avg vs. month

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	2	81740.604	40870.302	4.352	.0437
Residual	10	93919.907	9391.991		
Total	12	175660.511			

Regression Coefficients
c2avg vs. month

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	-6905040.676	2346523.964	-6905040.676	-2.943	.0147
month	.004	.001	396.480	2.942	.0147
month^2	-6.713E-13	2.282E-13	-396.430	-2.942	.0147



Is there a significant difference in densities between the trapped site and the control site?

A paired t-test (comparison of means) shows a strongly significant difference when considering all (raw) data, and a significant difference when considering monthly averages (Figure 7). However, this simply shows that there is a significant difference in the densities sighted at each location over time.

Figure 7: Individual datapoints: ***Paired t-test (P < 0.0001)***

Paired t-test
Hypothesized Difference = 0

Mean Diff	DF	t-Value	P-Value

Monthly average: Paired t-test (P < 0.0054)

Paired t-test
Hypothesized Difference = 0

Mean Diff	DF	t-Value	P-Value

Is there a correlation between captures and counts at the trapped site and the control site?

Captures V counts trapped site (monthly average)

There is a strong relationship (P < 0.0003) between Supertrap captures and the number of toad counts at the trapped site (Figure 8).

Figure 8: Captures vs. counts trapped site (monthly average)

Regression Summary
c1avg vs. capture.2

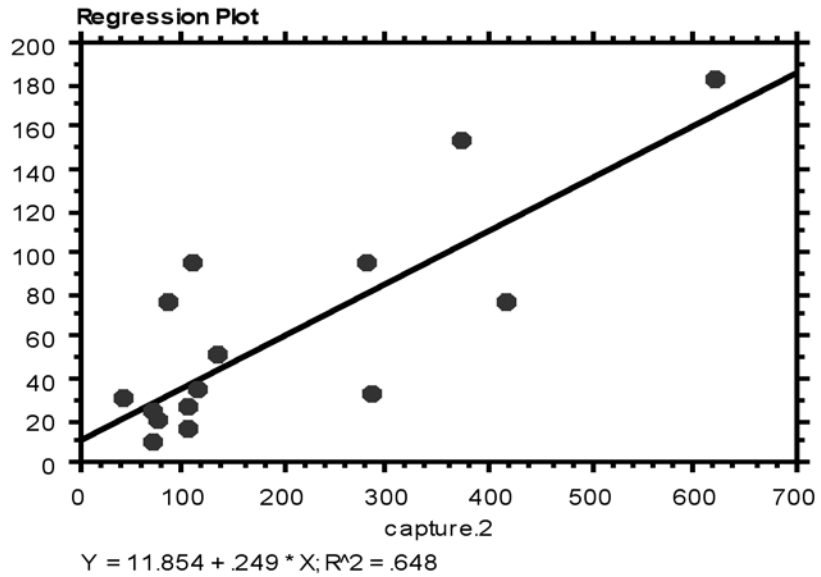
Count	15
Num. Missing	55
R	.805
R Squared	.648
Adjusted R Squared	.621
RMS Residual	31.782

ANOVA Table
c1avg vs. capture.2

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	24132.050	24132.050	23.891	.0003
Residual	13	13131.163	1010.089		
Total	14	37263.213			

Regression Coefficients
c1avg vs. capture.2

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	11.854	12.910	11.854	.918	.3752
capture.2	.249	.051	.805	4.888	.0003



Captures V counts control site (monthly average)

There is a strong relationship ($P < 0.0003$) between Supertrap captures and the number of toads spotted at the control site (Figure 9).

Figure 9: Captures vs. counts control site (monthly average)

Regression Summary
c2avg vs. capture.2

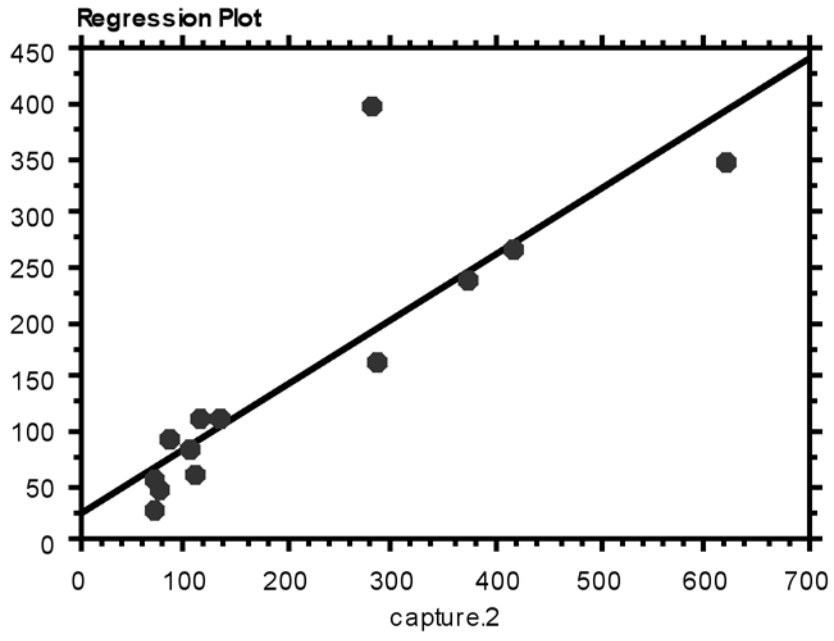
Count	13
Num. Missing	57
R	.844
RSquared	.712
Adjusted RSquared	.685
RMS Residual	67.851

ANOVA Table
c2avg vs. capture.2

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	125018.574	125018.574	27.155	.0003
Residual	11	50641.937	4603.812		
Total	12	175660.511			

Regression Coefficients
c2avg vs. capture.2

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	24.276	30.774	24.276	.789	.4469
capture.2	.594	.114	.844	5.211	.0003



Multiple regression – trap effect

The two figures above show that Supertrap captures show a strong relationship between toad counts at both trapped and control sites. However, a multiple regression (Figure 10) tests which of these two relationships is the strongest. Here, the relationship between captures and trapped site counts (count1) is much stronger than the relationship between captures and control site counts (count2). This suggests that the number of captures has a strong relationship with the number of toads seen at the trapped site, but much less so at the control site. This supports the hypothesis that toad trends at the trapped site are different to the control site, and suggests that trapping is affecting toad trends.

Figure 10: Multiple regression trap effect

Regression Summary
capture vs. 2 Independents

Count	42
Num. Missing	28
R	.469
R Squared	.220
Adjusted R Squared	.180
RMS Residual	52.376

ANOVA Table
capture vs. 2 Independents

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	2	30145.470	15072.735	5.495	.0079
Residual	39	106985.101	2743.208		
Total	41	137130.571			

Regression Coefficients
capture vs. 2 Independents

	Coefficient	Std. Error	Std. Coeff.	t-Value	P-Value
Intercept	22.930	13.014	22.930	1.762	.0859
count1	.414	.179	.452	2.309	.0263
count2	.008	.066	.023	.119	.9060

Observations

We have a number of observations and findings which we are hoping to follow through on with additional research and trials at other sites.

During the late dry season in the wet dry tropics cane toads are very susceptible to trapping with light based traps.

Male captures are much more prevalent than female captures close to water (less than 10m from edge) during the wet season.

There were differences in the counts at the trapping site early in the trial indicating that the trap was reducing the number of toads on that side of the dam. Consistently the count was lower on the trap side. In the week when the light failed and no toads were caught the numbers were more even.

A second trap was added as it was apparent the toads on the eastern side were not moving to the trap during the wet season when insects were plentiful. It was unclear what distance the traps will attract toads from and we had a trap on each side making a trap every 500 metres of shoreline approximately.

This trap was set 30 metres from the edge of the water to see if more females would be caught. Preliminary indications are that females toads are keeping back from the edge of the water but appear to be moving in to the edge more as the dry season sets in.

A third trap was added in late May to cover a specific location at a major refuge site near the dam.

Questions

1. Would other controls such as increasing the number of traps and manual control supplements increase the rate of toad removal?
2. What combination of lights needs to be used to maximise capture at different times of the year?
3. What other attractants can be used to supplement the effectiveness of the lights as lures (we have used dead toads with some positive outcomes)?
4. Are multiple small traps more effective than fewer large traps or vice-versa?

Conclusions

It appears that FrogWatch traps can be used to significantly reduce cane toad numbers around water bodies in tropical savannah and this should now be tested on a broader scale in significant wetlands in national parks.

The traps are humane and cane toad specific. Managing and monitoring of the traps is easy and traps only need checking once every few weeks. As long as water is maintained in the traps the toads appear to flourish in the traps coming out to feed on insects each night.

We are hoping to verify these results with additional research during 2006–07.

The field results of nine months of volunteer toad busting by the Kimberley Toad Busters 300 km east of the Northern Territory/Western Australian border

Sandra Boulter, Dean Goodgame and Lee Scott-Virtue, Kimberley Toadbusters.
www.kimberleyspecialists.com.au; www.canetoads.com.au

Abstract

In examining present and future factors that might impact on the natural and cultural Kimberley biodiversity, we realised that cane toads could be the most destructive force to hit our fragile ecosystems. To confirm our understanding of this threat, the Federal Government on listing cane toads as a 'key threatening process' to the nation's biodiversity, noted that the toads are one of the, 'world's worst invaders'. Lee Scott-Virtue looked over the border in 2003 at the cane toads amassing and heading towards us, and decided she would do something about it.

Burning practices in the Kimberley since European settlement, particularly since the introduction of aerial burning over a decade and a half ago, have substantially altered the plant and animal biodiversity of the Kimberley. These fire regimes, combined with poor natural and cultural resource management decisions, have degraded our upper catchments and led to increased threats of extinction of a number of animal species, especially those dependent on our aquatic systems. One aspect of the degradation is the silting up of many of our river and creek systems, therein changing systems from perennial, to annual with uncertain flows. There is a growing awareness that the Kimberley aquatic biodiversity, as well as the terrestrial biodiversity, is currently teetering on the 'balancing act of survival' as never witnessed before.

> "It is important to recognise that the pristine terrestrial and aquatic habitat systems of the Kimberley are already under threat. Current land-care and resource management policies undertaken by land and resource managers have had a detrimental impact on Kimberley biodiversity. Most of our plant and animal biodiversity is in a fragile state. The impact of the cane toad, if allowed to happen, will literally destroy one of the last unique biodiversity wilderness frontiers in Australia": Lee Scott-Virtue, Archaeologist/ Kimberley outback eco-tour operator/award winning environmental campaigner/founder and president of Kimberley Toad Busters and Kimberley Specialists in Research Inc.

The concern of Kimberley community groups, such as Kimberley Specialists in Research Inc. has led to an unprecedented action by local community members, not seen in the 70 years since the introduction of the cane toad to the Queensland sugar cane industry by CSIRO. This campaign commenced, as it clearly needed to, well before the cane toad was in a position to cross the Western Australian border.

Although the toads were still some 300 kilometres from the Western Australian border, Kimberley Specialists in Research (a non profit organisation campaigning on environmental issues in the Kimberley since 1988) set about alerting its local government (the Shire of Wyndham East Kimberley (SWEK)) of the impending impact. The State government conservation department, the Department of Conservation and Land Management (CALM) was the first to really heed to the community call. Under the leadership of Gae Mackae (CEO CALM Kununurra) and Noel Wilson (CEO Agriculture WA, Kununurra) a local Kununurra cane toad group, representing community, government and business, was established. This group was a coalition of the community, local business and government agencies based in the East Kimberley purpose built for communication.

Kimberley Specialists in Research Inc. organised a cane toad Forum, which was held in March 2005, with one of the outcomes resulting in the setting up of a volunteer Community Force, Kimberley Toad Busters (KTB). Since September 2005, the KTB have been travelling some 300 kilometres from the WA border, to bust cane toads, record cane toad behavioural patterns, record relevant field data that might assist in helping to delay/mitigate the impact on the Kimberley, and generally try and 'buy' time for the Kimberley. Furthermore, the Kimberley community needed recognition by Federal and State governments that the cane toad was now a national cross jurisdictional threat, not just a local issue to be tackled by each State or Territory, as the cane toad invaded their lands.

The KTBs hope that in presenting its findings to this forum, we assist in some way to the understanding of cane toads, so as to foster an early rather than later effective and safe control of cane toads so that we can keep them out of the Kimberley.

Introduction

This paper focuses on the extraordinary 'outcomes' of a community based volunteer force established in the town of Kununurra, East Kimberley, Western Australia, ten months 'down the track' with regular volunteer toad busting some 300 kilometres into the Northern Territory.

The 'wake-up' call to Kimberley residents was the much publicised devastating impact of the cane toad on the biodiversity on our national icon, Kakadu National Park. Research into the Frogwatch website and later consultation with Graeme Sawyer and Ian Morris, executives of Frogwatch, made it obvious that the cane toad, if allowed to cross the border into the Kimberley would expand its devastating impact from the Northern Territory to the Western Australian Kimberley ecosystems, therein decimating our terrestrial and aquatic plant species, already under threat from fire regimes (especially the aerial burning practices of the last 15 years) (see the proceedings published in 2002 from the remarkably successful 3 day fire forum Fire in the Kimberley organised and run by Kimberley Specialists in Research) and poor resource management decisions over many years.

It was apparent to Kimberley Specialists in Research Inc, the group that initiated the KTB, that we really know very little (despite the 70 years since the introduction of the cane toad by CSIRO) of the full extent, short-term and long-term, of the past and likely future impact of cane toads on our native vertebrate and/or invertebrate biodiversity. What had become apparent was that the cane toad did 'kill' a large number of native wildlife, and placed an indeterminate number of species under threat of extinction.

In conjunction with the Kununurra based Kimberley Wildlife Rescue and other community members, the volunteer campaign to hand catch and trap all stages of the cane toad life cycle, at the cane toad front 300kms away in the Northern Territory, through the Kimberley Toad Busters was established.

Who are the Kimberley Toad Busters?

The KTB was initiated and organised by the Kununurra based Kimberley Specialists in Research Inc. (KSIR), a non-profit environment research organisation set up by Lee Scott-Virtue in 1998. Discussion and consultation by KSIR with various local government organisations in the East Kimberley in late 2004 culminated in the establishment of the Kununurra cane toad Working Group (KCTWG), which promoted communication and coordination between government and the community for the coming campaign.

KSIR then fundraised, initiated and organised the Kununurra cane toad Forum held 19-20 March 2005. KSIR invited cane toad specialists from around Australia to inform the Kununurra community about what it truly faced if cane toads occupied the Kimberley. The papers from this forum have been published on the KSIR sponsored cane toad website www.canetoads.com.au and will be formally published once funding and workload permit. Regular fortnightly campaign newsletters are published by KSIR on this website. Accordingly, all field work by the KTBs and their field results have been shared through the website and through the Cane Toad Working Group, for anyone to easily access and acquaint themselves with our work.

KSIR organised the early cane toad busting and training, and funded (and still partially funds) the recurrent costs of cane toad busting, established the KTB in August 2005 and is gradually devolving the campaign responsibility from KSIR to the KTB who will shortly be incorporated. The KTB:

- Are all volunteers.
- Are the only Western Australian Toad Busting volunteer organisation working in the field at the cane toad front in the NT.
- Are focussed on stopping the cane toad from crossing into Western Australia.
- Have a volunteer multi-cultural organisation based in Kununurra.
- Have a membership base comprised of Aboriginal and non-Aboriginal individuals and groups from the East Kimberley.
- Are largely self-funded with donations and some sponsorship from CALM, SWEK, individuals and community business.
- Have an agreement with Biodiversity Protection WA to be its primary fundraiser.
- Are currently in the process of incorporating as a Community Group (we have a draft constitution, and an inaugural Board, which is meeting informally until incorporation is granted).

KTB as umbrella coordinator

Kimberley Toad Busters is a generic umbrella term for the multiple East Kimberley groups that have an interest in stopping the cane toad from crossing into Western Australia. Each weekend, KSIR and KTB coordinate and organise placement of all volunteer teams as concluded necessary by the previous week's field results, debriefing with CALM and our ever increasing data base. The field work exercises are coordinated by Lee Scott-Virtue with assistance as called for. The KTB groups include:

- KTB Research Advisory. Leader: Ade Meredith (scientist, collating data from all volunteer trapping groups, liaising with universities/research group).
- KTB Trapping Strategy. Leader: Chris Spurr (TAFE KTB trap making, TAFE KTB cane toad ranger course).
- KTB Safety Field Operational. Leader: Del Collins (Lions, CALM volunteer group)
- KTB Operational Field Strategy. Leader: Lee Scott-Virtue (Coordinate all groups' trapping location and activities on basis of previous weeks trapping results, weekly debrief of all groups results with CALM, constant updating of field strategies, KSIR and KSIT liaison).
- KTB cane toad 'Educational' Training. Leaders: Sarah Brett, Chris Spur & Ronnie Atkins (Kimberley Wildlife Centre, Kimberley Veterinary Centre, Education of outlying aboriginal communities about cane toads when undertaking veterinary work; CDEP ranger program with 20 aboriginal students).
- KTB Field Equipment Advisory. Leader: Dean Goodgame & Chris Spur (IT, website, store and distribute equipment and transport for all groups at front).

- KTB Euthanasia Control. Leader: Del Collins & Dr Sarah Brett (Liaise with Kimberley Vet Centre, volunteer safety).
- KTB Fund Raising, Communication and Public Relations. Leaders: Sandra Boulter & Lee Scott-Virtue.
- Kimberley Speleologists' Group. Leader: Dave Woods & John Cugley (Field trapping, data reports, GPS maps).
- SWEK Liaison. CEO: Peter Stubbs (Shire support, regular briefings to SWEK from KTB). Building Surveyor: Sharon Maclachlan (PUTart).
- Warrangari Team. Leader: Marianne Winton, Mirriuwong Aboriginal elder (working on natural Aboriginal fish poisons) Ronnie Atkins, Coordinator for Warrangari.

Kimberley Toad Busters goals

It was evident that KSIR needed to set in place a number of goals or objectives before embarking on setting up the 'volunteer' cane toad 'busting' campaign, which would clearly require training of our leaders.

Our goals are to:

- Keep the cane toad out of the Kimberley.
- Mitigate the impact on native biodiversity by allowing time for State government to undertake 'base-line' data research to ensure we know what is there before the cane toad 'hits'.
- Preserve our Kimberley biodiversity (by identifying 'safe islands of biodiversity') from the threats posed by cane toads.
- Ensure everyone in the Kimberley knows what a cane toad looks like and how to distinguish it from native frogs, and what to do if you find one.
- Continue to work with the Shire of Wyndham East Kimberley to ensure our Kimberley community can vigilantly and informatively combat any cane toads that cross the border.
- Establish a sustainable and ecologically aware community volunteer hand catching and trapping program that complements the CALM program and supports the KTB toad busting field activities, to stop the cane toad from crossing into Western Australia.
- Ensure all possible safety equipment available for each volunteer in the field, and insurance cover is obtained for volunteers.
- Run 'cane toad training and educational exercises' in the field to establish a sustainable volunteer leadership program.
- Put in place a methodology for volunteers to collect ecological field data as well as record general observations on cane toad behaviour in the Kimberley.
- Determine accurately where the cane toad front line incursions have been established, and to identify main colonisation and breeding areas.
- Determine a 'line of defence' and/or 'sacrificial zone' between the Western Australian border and the Northern Territory cane toad front in preparation for a 'final stand' by KTB against the cane toad .
- Build on and facilitate the work with Aboriginal communities in the Kimberley and the NT.
- Encourage participation of the Kununurra aboriginal community through involvement of community elders, aboriginal coordinators, schools, TAFE and CDEP
- Obtain secure funding for our very expensive recurrent costs for each week's toad busts (petrol, food, safety and communication, and toad catching and killing equipment).

It became evident, from the very beginnings of the establishment of the KTB, that it had triggered a response within the community not witnessed in Kununurra for a very long time, if ever. It was also evident that cultural boundaries had been 'traversed' in a way not witnessed before by the participants. The campaign has became a true 'community campaign', not just an individual issue with enormous and unanticipated positive social outcomes as well as the positive economic and environmental outcomes, thus being quite properly described as promoting sustainability in our community.

Kimberley Toad Buster outcomes to date

Unique program established, continuing and growing – learning as we go:

- 6 Toad Busting Leadership Training exercises undertaken at the cane toad front since September 2005.
- 34 weekend Toad Busts at the Victoria River cane toad Front Line.
- 492 individuals (children and adults) having participated in the volunteer toad busting activities.
- 738 community members registered as KTB volunteers.
- 14,9319 juvenile and adult cane toads caught (primarily by hand) and humanely disposed.
- Tens of thousands of eggs, tadpoles and metamorphs removed from the system.
- A working relationship established with scientists from several Universities in QLD, NSW (Sydney), and the NT.
- Trapping and safety strategies in place (but as ongoing work in progress as we campaign and learn).
- Barrier fencing materials being tested.
- Cultural barriers broken down and a 'unity' of community spirit and dedication to a cause never seen before in Kununurra.
- 19 East Kimberley Aboriginal Communities with cane toad Busting registered as an official CDEP activity.
- Cane Toad Busting now registered as a Lions and Leo activity providing insurance for all KTB including children 12 and over.
- Kimberley TAFE has registered cane toad trap making as a certified activity with an educational component in place.
- TAFE trained KTB (primarily aboriginal youth) now hitting the cane toad front every few weeks.
- 78 trained KTB leaders registered under the CALM volunteer program.
- Good and cooperative working relationship between CALM and KTB.
- Access to Bradshaw Military Base approved by Defence Department.
- Good working relationship with NT Government & Landholders (pastoralists and Aboriginal Communities).
- Permission to work in NT National Parks.

Wet season field work

After 10 months of field work we have now been able to collate and assess the field data from the wet season toad busting and provide the general observations listed below. It must be noted that this work has been done by volunteers with limited equipment and time constraints.

It is anticipated that the field data will provide us with some insight into how we plan our volunteer 'field attack' on the areas identified as 'potential cane toad front line incursion' threat areas.

We have identified:

- Major cane toad breeding colonisation fronts west of the Victoria River.
- Cane toad 'front-line incursions' west of the cane toad breeding colonising fronts.
- Wet season cane toad movement patterns in a diverse range of habitat systems.
- Valuable wet season adult/tadpole and metamorph cane toad habitat information.
- Best humane methods of disposing of large numbers of cane toads when captured.

We have experimented with and identified:

- Ways of capturing juvenile cane toads.
- Best methods of 'capturing' cane toad tadpoles and metamorphs.
- Best methods of identifying and 'toad busting' cane toad habitat systems during the day.

We have also:

- Observed and recorded new and valuable scientific information on cane toad tadpole behaviour/sexing juveniles/metamorph behaviour/adult cane toad behaviour.
- Observed and identified and recorded cane toad impacts on native fauna such as whistling ducks/ kite hawks/ olive python and other reptile species/ burrowing frog/ freshwater crocodile.
- Observed and recorded significant drops in numbers of first generation adult toads in areas being regularly 'busted' as well as a reduced number of tadpoles in areas being regularly busted.
- Experimented and recorded best field methods for 'busting' adult toads, eggs, tadpoles, metamorphs and juveniles both at night and during the day.
- Identified best wet season trapping methods for overnight catching and trapping.
- Identified best methods for 'disposal' of cane toads once euthanised to ensure least impact on native wildlife.

Wet season analysis

As a result of the ten months spent in the field by volunteers coordinated by the KTB, the collation of the field data has indicated a number of specific wet season and early dry season field results. KTB have roughly identified where the main cane toad 'front line(s)' is. In discussion and debriefing 'field' information sessions with CALM, at present the KTBs and CALM generally agree as follows:

- That there is no consistent 'front line' and that what we are seeing is a series of fronts. Will these successive front line invasions continue while food resources continue to be a problem? How many toads could one expect until diminishing food resources begin to play a role? This is difficult to determine without further more consistent monitoring. Despite the fact that the Victoria River Road House is now moving into its third year of cane toad occupation there still appears to be plenty of food resources to maintain breeding populations and successive 'waves'.

- That 'major front line' populations represent all generations but also feature a dominance of juveniles becoming adults. Their main identifying feature is the dominance of large mature toads generally second and third generation, and the generally even mix of females and males of this size.

- We are identifying 'colonising' advance frontline movement from the main population fronts by the dominance of large male toads (13-15cm in size) found in a single area (with few or no females) and that they are all calling (an amazing experience when you encounter it for the first time). These generally comprise very large numbers and make up about 98% of the colonising population. Examples of this phenomenon were seen at Esplanade Walk (busted at Christmas and for two consecutive weekends) and Brownies Creek over several weekends (only recently has the population mix changed and does this mean they have moved on or is it that we have wiped out the bulk of the adult male population?). Interestingly, when we first started work on Coolibah Station, the cane toad gender mix in the population (although all large and over a year old) was about even. While it is feasible to suggest that the male colonising front may have moved on, our volunteer toad busters working at Brownies Creek and the general area felt fairly confident they had 'busted' the main colonising population. We were also able to pinpoint dominant males calling along the Victoria River in two other locations between Coolibah Station and Brownies Creek, although we were unable to get to them at the time.

- Front line incursions have been identified as random or and to some degree a result of 'accidental' movement (such as those found at Timber Creek) and probably due to both large male and gravid females being swept along the Victoria River and other flooded tributaries coming off the Victoria River. There appears to be a deliberate movement by adult toads along the upper reaches of the Victoria River. Not being able to get into this area during the wet season means that we may have missed deliberate male colonising fronts moving against the flood. Until more recently we were still seeing the Victoria River as the main water highway but we feel the flooded periods have now changed the dynamics of this movement. Hence the incursion fronts being found in Jasper, Kidman Springs, Mooloolloo Station, etc. This has also been facilitated by the movement along the Victoria Highway and other road systems such as the Buchanan while they were wet.

- The KTB has concluded that maintaining trapping and toad busting (during the dry) in the main 'front' areas (and further east as well) are critical in containing as well as reducing successive 'waves' of adult toads into the problem incursion areas. It is also agreed that it is critical that we also bust all the problem incursion fronts at the same time to reduce successful breeding and to take as many of the adult breeding toads from the system as we possibly can; and

- KTB is working on a trapping strategy that gets traps into as many of the 'hotspots' and 'incursion areas' as possible, and to also maintain trapping in the 'front line' areas. We will continue to complement this with physical hand catching toad busting in areas that we have identified as major 'front line' and with areas that are well east behind the cane toad invasion waves. The more volunteers that we have the more we can do. The more recurrent funding we have, the more volunteers we can support in the field.

General field observations from the front line

The KTB observations in the field include:

- Colonising male toad fronts are distinctive and recognisable, and suggest that primarily only male toads move forward and once safely established begin 'calling' the mature females to join them. (Female adult toads, if present at all, were represented by less than 3% of the total population 'busted' in these colonising fronts).

- Female toads at the end of the dry were observed to dominate creek systems and other 'wetter' areas, and were 'busted' 'hibernating' in areas that were frequented by cattle and buffalo.
- Day time habitat systems of adult cane toads include buffalo hoof prints (Figure 1), grassy verges on dams and other still water bodies.
- Metamorphs and juveniles were found in hoof prints, grassy shaded areas etc.
- During the wet, males were seen to be moving in huge numbers ahead of the cane toad 'front' colonising areas, and then 'sitting' and 'calling'. (Higher humidity and temperature readings on these nights indicate this might have some bearing on this movement and the insistence of the male call?)
- Adult toads were seen to be swimming across large expanses of water as well as being able to 'submerge' for several minutes to avoid capture.
- Metamorphs were observed to move several hundred metres from water and to climb up into high, well watered escarpment areas;
- Cane toad egg transition to 'tadpole' appeared to be as little as 13 hours during the 'hotter' part of the wet. (This suggests that egg-to tadpole-metamorph-may depend on environment more than we have preciously estimated?)
- Native frogs, in the initial period of the cane toad arriving seemed able to work out a compromise. Several areas 'busted' by volunteers indicated that water holes were divided into separate areas, with cane toads dominating one and native frog species dominating another. It was observed by KTB that this 'situation' only sustained for the first period of 'toad' colonisation (for 3-5 weeks).
- Quantities of 'bait' fish were observed 'dead' on the surface of some creek systems containing cane toad eggs and tadpoles; and
- Within a few hours of cane toad eggs being deposited in a dam or other small isolated water systems (such as gravel pits), native tadpoles were often found dead on the surface of the water (Figure 2).

Conclusions

Figure 1: Day-time habitat of adult toads (hoofprint)

Figure 2: Dead native tadpoles

It has been a little over one year since the Kununurra community banded together, and participated in the Kununurra cane toad forum.

In this time, huge achievements have been made in educating the local community about the cane toad threat, its habitats and behaviour; developing strategies to fight its advance in the coming dry season; and implementing infrastructure and resources to put the fight to the next level.

The end of last year's dry season gave the toad busters their first experience with the cane toad battle. During this period many of us were able to explore first hand the area and topography where this unique desert battle will be fought in the coming dry season. This experience provided us our own first hand information as to the behaviour of the toads; develop ideas about weapons we could use in our arsenal, such as traps and nets; equipment needed such as vehicles, radios, generators and camping gear; the ideal safety equipment for our volunteers; and the myriad number of other sundry items needed to wage a successful campaign.

The last wet season has proved invaluable as a lead up to the coming fight. At the front line, tens of thousands of breeding toads were disposed of, and hundreds of thousands, perhaps millions of eggs were prevented from being laid ahead of the cane toad front. Likely invasion routes have been plotted, potential specific cane toad water course and water hole habitats have been identified, our strategies have been further refined, equipment procured and funding secured.

Through the work in the last dry season and the recent wet season many valuable working and education relationships have been established and solidified with the KTBs. These include with land holders such as local Aboriginal groups (Figure 3); Federal departments such as the DEH and the Defence Forces; State and Territory governments through WA CALM and the NT Parks and Wildlife; local government through SWEK; NT pastoralists; NT aboriginal communities; NT and WA and NT tourist operators.

The KTB is now in a position to begin what we believe to be the largest, best equipped and coordinated sustainable field on the ground campaign to stop or at worst significantly slow the advance of the cane toad in Australia's history.

The success of our campaign to date is the result of the hard work and dedication of the Kimberley community, especially Kununurra. This whole effort started in Kununurra and has largely been facilitated by local residents. It has brought the community together in a way that no other single project has before. Government departments, local business, shire, indigenous groups, community members of all persuasions are all working together in the field for a common purpose. This paper is a celebration of their efforts and a hope for their future and continued success.

Postscript: word of caution

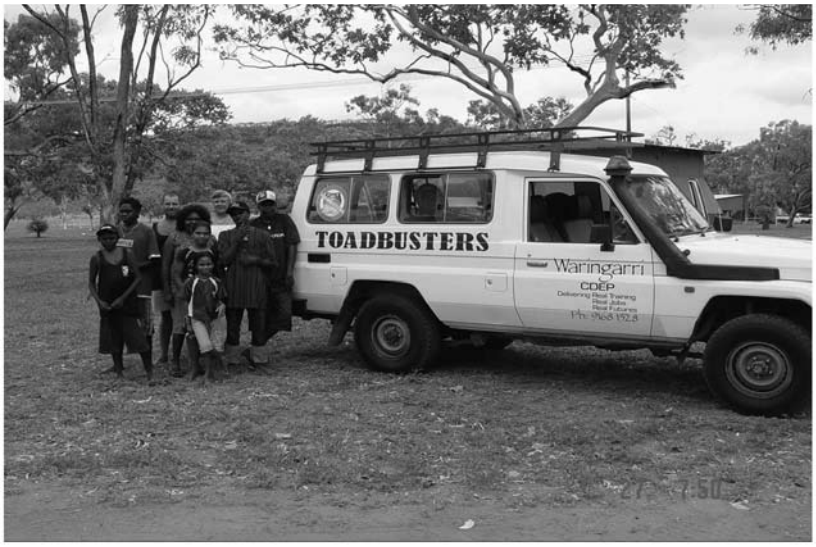
The real strength of this volunteer regional campaign is that it is being planned, directed and coordinated from Kununurra where we are all part of the effort; every individual has ownership and makes their contribution in close liaison with the KTB Board and KTB field coordinator, in liaison with CALM. Any attempt that succeeds in removing any part of the campaign decision making or the field strategy planning away from the Kununurra community groups and the KTB will result in a loss of ownership felt amongst the campaigners. This is likely to lead to a significant loss of the volunteer energy required for our success. If that happens, the Kimberley ecosystems and the fauna and flora they support are more likely to be diminished and perhaps even lost to this noxious menace.

Acknowledgements

- Our volunteers, aboriginal and non-aboriginal, children and adults, families and singles, teachers and students, government and non-government who are all in together.
- Kimberley Specialists in Research Inc. (over $50,000 in cash and well over that in kind), Kimberley Specialists in Tourism and Kimberley Wildlife Rescue Inc. for financial facilitation and organisation of the KTB and the in-kind financial input into organising the KTB volunteer group.

- Triple J for the donation of a 21 seater bus, fuel and trailer to get volunteers to the cane toad front and other financial donations of approximately $38,000.
- Conservation and Land Management (CALM) for $10,000 to Kimberley Specialists in Research for KTB on-ground activities and $5,000 towards the maintenance of the Community Website, www.canetoads.com.au.
- Biodiversity Protection WA for $77,000 towards traps and safety equipment for the volunteers (this has been a life saver – literally).
- Federal Government for $79,000 towards the purchase of a second hand 4W/D and quad bikes.
- Gae Mackae and her CALM team in Kununurra for their willingness to work with us.
- Peter Stubbs, CEO SWEK for his continued support for our program and for the provision of a Shire 4W/D vehicle for our voluntary weekend cane toad exercises.
- Coles for the provision of a 4W/D on weekends.
- Kimberley Echo – our local newspaper and editor Bruce Russell.
- Victoria River Roadhouse.
- All KTB Leaders and Community Groups who are increasingly taking on the organisation of individual teams to travel to the front line every weekend.
- Georgina and Ju Ju Wilson, and Annie Fitzgerald, our Aboriginal Co-ordinators for organising the Aboriginal membership component of KTB.
- Local Community individuals and groups that have assisted towards the in-kind and financial expense of getting volunteers to the cane toad front.

Figure 3: Aboriginal membership component of KTB



Maximising coordination of on-ground cane toad control effort

Dennis Beros, Campaign Manager, Stop the Toad Foundation Inc.

The purpose of this presentation is to make participants aware of the existence of, and reasons for the existence of, the Stop The Toad Foundation (STTF) Inc. – a WA based and WA incorporated non-government, not-for-profit organisation.

The title of the talk is "Maximising coordination of on-ground cane toad control effort". As just one of the players in the efforts to keep WA free of cane toads, the Foundation of course cannot insist upon the cooperation of other groups. However it can encourage cooperation and one of the ways it can do this is by having clear statements of its own intent and by inviting the input of others to its strategies and operational plans.

At the time of this presentation the Foundation is close to finalising its Strategic Plan, and within that plan nest a number of operational plans – most notably our 'Dry Season Strategy' for toad control.

I seek from you, the participants of this workshop, your approval to circulate these documents to you once finalised. (Nods of agreement & group thanked). You are invited to review the documents and comment on them, and I encourage you take the time to do so. The Strategic Plan is a substantial document but I implore you to at least use your 'FIND' function to search out keywords and scan the document for your areas of interest. I expect that at least some of the proposed activities of the Foundation will be of direct interest to you. The Foundation may be able to offer support to your projects (in principle, or perhaps even directly in some cases). There may also be situations where our activities in the field could be of assistance/interest/benefit to researchers.

The Strategic Plan will always remain a working document but will go through a preliminary comment period as a 'draft'.

The STTF Strategic Plan may be found at: http://www.stopthetoad.com/publications/sttf_strategicplan.php

Foundation Board

Chair : Robert Edel (Gadens Lawyers).

Vice-chair : Luc Longley.

Secretary : Chris Tallentire (Conservation Council of WA).

Treasurer : Kenneth Bradley (Volunteering WA).

WA Govt. rep. : Rita Saffioti.

Member : Dr Andrew Storey (University of WA)

Member : Russell Gueho (Northern Habitat – Broome).

Patron : Tim Winton.

The objectives of the STTF

1. To prevent the migration of cane toads into the Western Australia.
2. To fund, develop, install and operate toad trapping devices and other toad control mechanisms with the aim of preventing cane toads entering Western Australia.
3. To protect Western Australia's native fauna and flora from the infestation of cane toads.
4. To educate the public on the risks and danger posed by cane toads and the ways to prevent the migration of cane toads into Western Australia.
5. To conduct and finance research into the development of effective methods of controlling, reducing or eliminating cane toad populations.
6. To implement cane toad control measures in Western Australia in the event that populations of cane toads are established in the State.
7. To carry out activities that promote or to facilitate the above objectives, including fund raising activities.
8. To establish and maintain a public fund to be called the "Stop the Toad Fund" for the specific purpose of supporting the environmental objects/purposes of the Foundation. The Public Fund is established to receive all gifts of money or property for this purpose and any money received because of such gifts must be credited to the Public Fund Bank Account. The Public Fund must not receive any other money or property into the Public Fund Bank Account and it must also comply with subdivision 30-E of the Income Tax Assessment Act 1997.

What the STTF has done so far

1. Established the issue in the public mind.
2. Successfully sought a $500,000 WA Govt. contribution.
3. Lobbied the Aust. Govt. for support.
4. Generated donations from the public.
5. Established a trap-building program in Perth schools.
6. Engaged the corporate sector.
7. Engaged experts to contribute to our strategic planning.
8. Encouraged a coordinated whole-of-community effort .

Purposes of the draft Strategic Plan

1. A process for agreeing organisational direction and strategy between board members, staff, volunteers and key stakeholders.
2. A focus for consultation and debate with groups and individuals having an interest in cane toad control.

Purposes of the Strategic Plan

1. An overview of the Foundation's planned activities to inform and gain support in the outside world.
2. An overt statement of intent which can be reviewed and revised as circumstances change.
3. A checklist for Risk Management.

The Strategic Plan in essence

VISION

A cane toad free WA
with toad numbers in decline across Australia.

MISSION

To encourage all parties to make 'all efforts' to prevent the cane toad invasion of Western Australia and to work together to that aim through mutually agreed strategies, operations and procedures.

Because cane toads are closing on the WA border at such an alarming rate, the Foundation has resolved to throw everything it can at cane toad control efforts this year.

We must know this year whether or not we can have an impact on the toad's westward spread. The Foundation has a target of $1m in cash and in-kind support for its on-ground activities this year.

By way of example the following are the Key Objectives in the 'Operations and Training' section of the (draft) Strategic Plan.

Key Objective 1.1: Conduct surveillance on the cane toad front and the terrain it is moving through of sufficient detail and accuracy to enable decision making on when and where to best target on-ground control activities.

Sub-objective 1.1.1: Map and track the cane toad front.

Sub-objective 1.1.2: Evaluate terrain ahead of front.

Sub-objective 1.1.3: Monitor for incursions ahead of the front (hitchhikers).

Key Objective 1.2: Conduct on-ground cane toad control activities to deliver maximum impact on the advance of the front.

Sub-objective 1.2.1: Trapping and hand capture;

Sub-objective 1.2.2: Barriers;

Sub-objective 1.2.3: Other methodologies including life-stage approaches.

Key Objective 1.3: Design and implement a rapid response capability to effectively deal with toad incursions ahead of the front (hitchhikers), in concert with responsible government agencies.

Key Objective 1.4: Develop a culture of safety and implement and maintain robust safety procedures at all times for all Foundation staff and volunteers.

Key Objective 1.5: Develop a training program which ensures that all Foundation staff and volunteers are adequately prepared for all activities and all foreseeable contingencies.

Key Objective 1.6: Develop and promote sound holding, euthanasia and disposal procedures for toads captured.

Key Objective 1.7: Protect the environment in all phases of operations to the maximum extent possible.

More information on the STTF and its activities can be found at www.stopthetoad.com

SESSION 4: BIOCONTROL – CURRENT AND PROSPECTIVE

CSIRO biocontrol project: concept and progress

Tony Robinson, Nicole Siddon, Suze Tarmo, Damien Halliday, Thayalini Shanmuganathan and Daryl Venables. CSIRO Entomology.

Abstract

The formal search for natural pathogens that might act as biocontrol agents for cane toads (*Bufo marinus*) began in 1986 and after 10 years, despite considerable effort, no suitable biocontrol agent was identified. In 2000, CSIRO was successful in bidding for a government contract to renew the search for a biocontrol agent. Given the lack of success in finding naturally occurring viruses, the idea of using a virus that could be modified to contain a gene that would interfere with some essential process in the cane toad was proposed. In an experiment with bullfrogs (*Rana catesbiana*) (Maniatis *et al.* 1969) adult haemoglobin extracted from blood and inoculated into bullfrog tadpoles was able to change the outcome of metamorphosis. As a starting point for the experimental phase, attempts are being made in cane toads to reproduce the work done with bullfrogs as a demonstration of proof of principle. A cane toad breeding colony has been established and tadpoles have been inoculated with a recombinant adult β-globin. Initial experiments have shown that the levels of tadpole and adult haemoglobin can be altered in the subsequent metamorphosing adults. Other components of the work can be found in subsequent papers in this Proceedings (Pallister *et al*).

The search for natural pathogens

The formal search for natural pathogens that might act as biocontrol agents for cane toads (*Bufo marinus*) began in 1986 when COMCON, a council of Australian government ministers with responsibility for conservation and the environment, funded a project at James Cook University. Researchers were to compile a comprehensive review of all diseases recorded in the cane toad worldwide (http://www.jcu.edu.au/school/phtm/PHTM/frogs/bibliog.htm), survey the diseases and parasites in Australian anurans including detailed necropsies of cane toads from northern and eastern Australia and attempt the isolation and identification of infective agents as well as assessing their pathogenicity. Despite considerable effort no suitable biocontrol agent was identified.

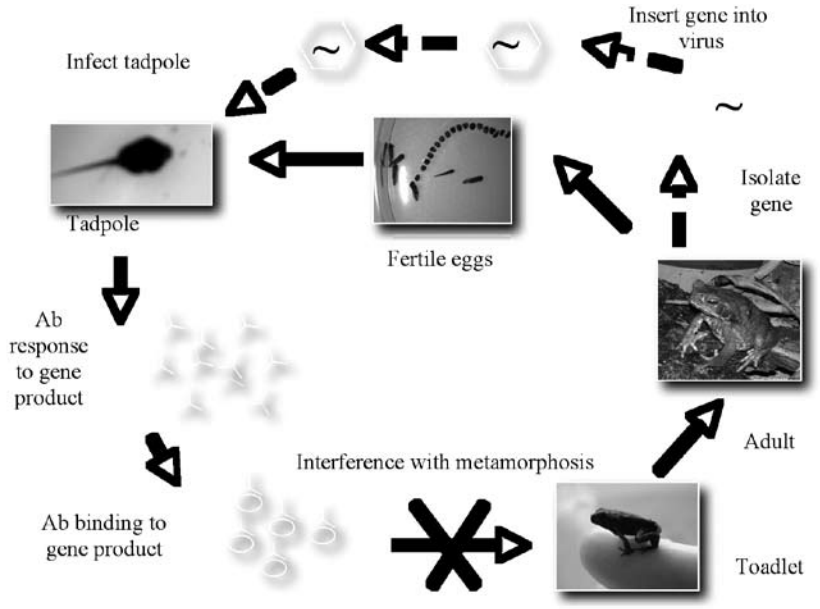
In March 1990, following a review of this work, it was decided to mount another search for potential biocontrol agents of cane toads, this time in their natural habitat in South America. CSIRO in collaboration with the Instituto Venezolano de Investigaciones Cientificas (IVIC) in Caracas isolated a number of bacteria and viruses from cane toads. The viruses were shipped back to the Australian Animal Health Laboratory for further characterisation. All the viruses were confirmed as viruses belonging to the genus Ranavirus in the Iridovirus family. These viruses are ubiquitous viruses of amphibians and have a broad host range and are unsuitable as biocontrol agents unless they could be modified in some way (Hyatt *et al.* 1998). The project was parked in 1996 when funding ceased.

A new approach

With the imminent arrival of cane toads into Kakadu National Park interest in cane toad control was renewed and in 2000 CSIRO was successful in bidding for a government contract to commence development of a biocontrol agent. Given the lack of success in finding naturally occurring viruses that would be suitable, the idea of using a virus that could be modified to contain a gene that would interfere with some essential process in the cane toad is to be explored (Figure 1).

We have begun by targeting the process of metamorphosis. In amphibians there are a number of genes that are switched on for the first time at metamorphosis (Flajnik *et al.* 1987) and it has been shown that the proteins that are produced by these genes are potentially immunogenic if introduced into tadpoles before this switch-on. In an experiment with bullfrogs (*Rana catesbiana*) adult haemoglobin extracted from blood and inoculated into bullfrog tadpoles was able to change the outcome of metamorphosis (Maniatis *et al.* 1969). The ensuing adults lacked proteins at the expected positions in polyacrylamide gels for adult globin or tadpole globin and instead appeared to be producing a third form of globin. The effect was postulated to be mediated by antibody to the adult globin.

Figure 1: Diagramatic representation of a strategy for interfering with metamorphosis in cane toads.



Establishment of a cane toad breeding colony

Essential to any study of this nature is a reliable source of experimental animals. We have established a cane toad breeding colony which can supply us with all the life stages of the cane toad that we need (Hamilton *et al. in press*). Adults are sourced from Queensland and on arrival are treated with Ivomectin to treat infestation with the lung roundworm, *Rhabdias sp*. Two other infections we have found in the toads are *Mucor amphibiorum* which causes a wasting disease in the toads and *Mycobacterium marinum* which causes skin ulcers. *M. marinum* infection can be controlled by ensuring there are no sharp objects in the toad enclosures that can cause skin punctures. For this reason we have discontinued the use of saw dust and wood shavings as bedding and replaced them with marine matting.

The food supply is mainly meal worms and crickets which can be obtained from commercial suppliers although this can be unreliable and we are now growing our own meal worms. We have been successful in raising metamorphs to young adults and to do this live moving food small enough for the toadlets to mouth is essential. We use "pin-head" crickets and early stage *Helicoverpa amigera* larvae.

For breeding, female adults are checked by palpation to ensure they are sufficiently gravid and they are then inoculated with synthetic gonadotrophin releasing hormone. An hour later the males are inoculated and the pair is placed in a plastic bin with the lid in place. The eggs are expressed and fertilised overnight. Many thousands of eggs are expressed and most are fertile. Tadpoles hatch in 48 hours and develop to metamorphs over 35 days.

Experiments with adult cane toad β-globin

As a starting point for the experimental phase, attempts are being made in cane toads to reproduce the work done with bullfrogs as a demonstration of proof of principle. We have isolated the gene encoding the ß haemoglobin chain and have produced milligram quantities *in vitro* with which to inoculate tadpoles. In initial experiments where we have inoculated tadpoles with this protein in Freund's complete adjuvant (FCA), we have shown that newly emerged metamorphs show a retention of tadpole globin RNA and a reduction in adult globin RNA. We are in the process of repeating these experiments at different time points and developing methods to detect haemoglobin protein in tissue extracts. If this result can be confirmed we have our proof of principle. As haemoglobin is a relatively conserved protein and is probably not a useful protein target in a biocontrol approach we have also screened for other proteins using microarray technology. Seven candidate proteins have been identified.

Why should there be an effect on RNA levels when the immune response is directed against protein or peptides in the context of major histo-compatibility (MHC) presenters? We have no easy explanation for this but it could be that the cells producing adult globin are being eliminated by the immune system during the lead up to metamorphosis. There is evidence that different haemopoetic cells are producing the different haemoglobins and the knock-out of one may trigger the retention of the other.

If we can find a suitable protein target which when compromised, can affect metamorphosis then a means to deliver this to toads needs to be found. Currently we are using a modified attenuated ranavirus as our delivery vehicle in laboratory experiments and the results of manipulations with this virus will be the subject of another paper in these Proceedings (Pallister *et al*).

Future work is aimed at continuing the search for and testing of target proteins, assessing their species-specificity, and also screening toads for other viruses that may be useful delivery vehicles in the field.

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Viral delivery of cane toad biological control

J. Pallister, R. Voysey, V. Olsen and A. Hyatt
CSIRO Livestock Industries Australian Animal Health Laboratory, PO Bag 24, Geelong, Victoria 3220, Australia

Abstract

In the absence of a cane toad specific pathogen, the Department of Environment and Heritage (DEH) sponsored project on biological control of cane toads aims to use a virus, *Bohle iridovirus* (BIV), to deliver a cane toad specific gene that will interfere with the metamorphosis of cane toad tadpoles. BIV is the only amphibian virus that has been isolated in Australia and grows well at the temperatures found in northern Australia; however the pathogenicity of the virus means it must be attenuated before it can be used. Pathogenicity testing in cane toad tadpoles showed that BIV was successfully attenuated by passaging 99 times in Vero cells, and also by construction of two recombinant BIV (rBIV) with the neomycin resistance gene inserted into either the BIV eukaryotic initiation factor 2α gene or the ribonucleotide reductase large subunit gene. Construction of these rBIV demonstrated the capacity of the virus to carry and express foreign DNA. A rBIV expressing the cane toad adult globin gene is the most attenuated virus we have produced so far. Cane toad tadpoles have been infected with this virus and we are currently monitoring the effects of the virus infection on the levels of mRNA for adult globin and tadpole globin in cane toad metamorphs.

Introduction

The aim of the DEH sponsored cane toad biological control project is to use a virus to deliver a cane toad specific gene, or genes, to cane toad tadpoles that will interfere with their metamorphosis. The project is carried out at CSIRO Australian Animal Health Laboratory (AAHL), Geelong and CSIRO Division of Entomology in Canberra. The project is split between the two groups, the Entomology group is searching for the cane toad specific genes and the AAHL group is developing a virus that will act as the delivery vehicle for the selected cane toad specific genes.

The virus: advantages and disadvantages

Viral delivery was chosen as the means of delivering cane toad biological control because a self replicating means of delivery was considered necessary to penetrate the vast and inaccessible areas of the Australian continent that the cane toad inhabits. The virus chosen for delivery of cane toad genes was BIV, a member of the family *Iridoviridae*. Ideally the virus chosen to eradicate cane toads would be a highly pathogenic one that only infected cane toads and no other species, but attempts to identify such a virus have been unsuccessful. Seven viruses isolated from Venezuelan cane toads were all ranaviruses (genus *Ranaviridae*, family *Iridoviridae*) and all were pathogenic in tadpoles of Australian native frogs (Hyatt *et. al.*, 2002).

In the absence of a naturally occurring cane toad specific virus, the approach taken at AAHL was to engineer a virus that was non pathogenic for any species it infected apart from the cane toad. The effect of the virus on the cane toad would be determined by the *Bufo marinus* gene carried by the virus. BIV was selected for development as a vector for the following reasons: (1) it is endemic and BIV is the only virus of amphibians that has been isolated in Australia (Speare and Smith, 1992) (2) BIV was isolated in Bohle in northern Queensland indicating that its range was likely to overlap that of the cane toad (3) it is a large double stranded DNA virus which means its genome is typically easier to manipulate than the genome of an RNA virus.

The large genome (the ranavirus genomes sequenced so far tend to be around 105 kb) (He *et. al.*, 2002, Tan *et. al.*, 2004) makes it more likely that the BIV genome will be able to carry relatively large amounts of foreign DNA and (4) BIV can survive desiccation for at least 6 weeks and grows well in the temperature range at which cane toad tadpoles thrive (25 - 30°C).

While BIV is well suited to the Australian environment, it has been shown experimentally to infect barramundi fingerlings (Moody *et. al.*, 1994), tortoise hatchlings (Ariel, 1997) and other native amphibians (Cullen and Owens, 2002) and so attenuation of the virus is very important. Finally, we know very little about the virus itself, and very little about its behaviour in the environment.

At AAHL we have specifically been working to:

1. Attenuate BIV.
2. Show that BIV can carry and express foreign DNA.
3. Show that BIV can deliver foreign DNA to cane toad tadpoles and generate a response to products of the foreign DNA.

Attenuation of virus

There are two methods used to attenuate viruses, one is the classical method used to produce many of our current vaccines, where a virus is passaged under non ideal conditions such as in a cell line from a different species, or at a non ideal temperature, to produce a variant of the original virus with altered growth characteristics. This method of attenuation is random, and the nature of the alteration that produces the attenuation is unknown. We passaged BIV 99 times in Vero cells (African green monkey kidney cells) and showed that the pathogenicity of the resulting virus (BV099) was reduced: 10^5 $TCID_{50}$ of wild type virus killed 12/12 frogs, while the same dose of BV099 killed 1/12 frogs. The remaining 11 frogs were challenged with the wild type virus and survived, indicating very importantly, that the attenuated virus had not lost its ability to infect frogs.

An alternative method of attenuating a virus is to delete known genes that are associated with virulence and that are non essential for virus replication. Using the sequence of non essential genes in other large double stranded DNA viruses we were able to identify four of these genes in BIV – the ribonucleotide reductase small subunit (rrss), the ribonucleotide reductase large subunit (rrls), thymidine kinase (TK) and eukaryotic initiation factor 2α (eIF-2α). We cloned each of these viral genes into a plasmid, inserted the neomycin resistance gene into the viral gene (growth of wild type virus is inhibited by neomycin), and transfected the resulting plasmid into BIV infected cells. Homologous recombination may then occur resulting in a virus that carries the neomycin resistance gene and an interrupted, non functional viral gene. If a recombinant virus expressing the neomycin resistance gene does result from the transfection, then we know that the interrupted virus gene was non essential for virus growth. If no recombinant virus results then it is possible that either the interrupted gene is essential, or we have inadvertently interrupted another gene that is essential. The recombinant virus can then be tested in animals to see if interruption of the viral gene results in attenuation of the virus. Recombinant viruses generated in this way can therefore identify non essential genes which are required as insertion sites for foreign DNA, and can also identify genes involved in virus pathogenicity.

Recombinant viruses

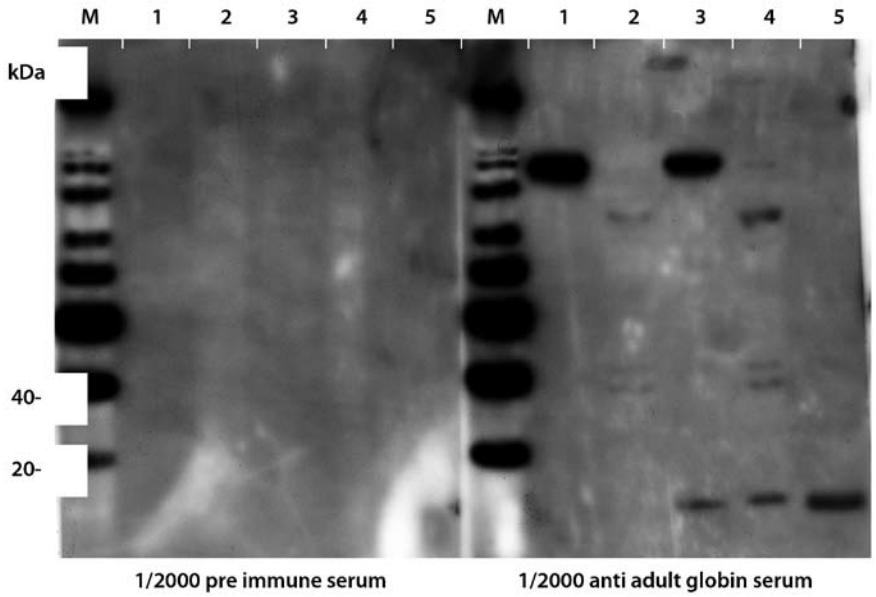
Using transfection, then plaque purification in the presence of neomycin to purify recombinant virus we successfully made viruses with the neomycin resistance gene inserted into the eIF-2α gene of BIV and the rrls gene of BIV. We were unable to purify viruses with the neomycin resistance gene inserted into the rrss or TK genes despite several attempts. Analysis by real time PCR showed that the neomycin gene had been inserted into both the rrss and the TK gene, but we couldn't purify the recombinant virus away from the wild type virus, indicating that the recombinant virus was lacking a vital function provided by the wild type virus. We proceeded to make a recombinant BIV (rBIV) with the cane toad adult globin (adglo) gene and the neomycin resistance gene inserted into the eIF-2α gene of BV099 (rBIV/adglo). A western blot was performed to confirm that adult globin was expressed by the virus (Figure 1). The pathogenicity of all three rBIV was then tested in cane toad tadpoles at doses of 10^2,10^3, 10^4 and 10^5 $TCID_{50}$/ml and compared to the wild type virus (Table 1).

Table 1. Mortalities in cane toad tadpoles infected with wild type (WT) BIV, rBIV with either the rrls or the TK gene interrupted by insertion of the neomycin gene (rrls/neor, TK/neo^r), and a rBIV with both the adult globin and the neomycin resistance gene inserted into the eIF-2α gene (eIF/adglo/neo^r).

Virus	**Percentage deaths**			
$TCID_{50}$/ml	WT BIV	rrls/neo^r	eIF/neo^r	eIF/adglo/neo^r
10^2	0	ND	ND	ND
10^3	90	25	10	18
10^4	100	40	30	20
10^5	ND	80	90	60

All three rBIV showed a reduction in pathogenicity compared with the wild type virus, and this was the most marked for rBIV/adglo at the 105 dose. Virus pathogenicity might be expected to vary between different batches of tadpoles as these are outbred populations but so far the pathogenicity of rBIV/adglo has been tested twice with around 10% variation in the outcome.

Figure 1: Western blot using pre immune serum and antiserum raised in rabbits to toad β globin. M (in both figures) is Magic Mark western blot markers. Lanes 1 and 2 respectively show the pellet and supernatant from BIV p100 with no inserts, Lanes 3 and 4, the pellet and supernatant from rBIV-eif/globin/neo and lane 5, toad β globin.



A rBIV is currently under construction that does not carry the neomycin resistance gene (a virus that carried antibiotic resistance genes would not be released into the environment), with the cane toad adult globin gene inserted into the BIV eIF-2α gene, and most of the rrls gene deleted. Herpesviruses deleted in the rrls gene and genes with an equivalent function to eIF-2α did not revert to wild type when tested in primates (Yazaki *et al* 1995).

Animal experiments

The ultimate test for the virus is its ability to deliver a cane toad gene and show a detectable effect in the metamorph. The original experiment carried out in the 1960s showed that administration of adult cane toad globin to tadpoles altered the expression of globin in the metamorphs. This experiment has since been repeated by the cane toad group at CSIRO Entomology who showed that mRNA for tadpole globin persisted in metamorphs that had been injected with adult globin as tadpoles, while control metamorphs (no adult globin injected) had no mRNA for tadpole globin. In a very preliminary experiment we allowed a small group of tadpoles to go through metamorphosis, including one group of uninfected animals, two groups infected with rBIV/adglo and one group infected with wild type BIV. The aim was, in part, to establish metamorph husbandry and also to gain some indication of the outcome. At stage 43 of metamorphosis (front legs present, tail curved), tadpoles were transferred from water tanks to a dry/wet tank and kept for 2-3 days until their tail was resorbed when they were euthanased for mRNA analysis. Table 2 shows the mortality rate in each group, where a mortality is recorded if the animal dies before reaching the stage of tail resorbption. In this experiment 20% of the negative control animals that received no virus died before reaching this stage, in contrast the mortality rates in the three groups that received virus varied from 80% to 100%. We are currently analysing the levels of mRNA for adult and tadpole globin in these metamorphs.

Table 2. Mortality in cane toad metamorphs previously infected with no virus, virus expressing adult globin or wild type BIV.

Virus	Deaths
None	1/5
rBIV/eIF/adglo/neo^r, group 1	4/5
rBIV/eIF/adglo/neo^r, group 2	6/6
Wild type BIV	4/4

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Spin-offs: What have been the direct and indirect outcomes from research into biocontrol of cane toads?

Alex Hyatt, Jackie Pallister, Veronica Olsen and Rhonda Voysey, CSIRO, Livestock Industries, Australian Animal Health Laboratory (AAHL),
5 Portarlington Road, Geelong Vic 3220
Private Bag 24, Australia (61) 0352275419, fax (61) 0352275555

The first Australian *Ranavirus*

On the 16th June 1985, Drs Jeremy Langdon and John Humphrey from the Australian Fish Health Reference Laboratory (now the AAHL Fish Diseases Laboratory) submitted cells which had been inoculated with tissue homogenates from moribund redfin perch (*Perca fluviatilis*) collected from Lake Nillacootie in Victoria. The samples were submitted to the electron microscopy laboratory of the Australian National Animal Health Laboratory (now AAHL). Ultrastructural examination of the cells led to the discovery of Australia's first 'iridovirus'[1] (Langdon *et al.* 1986).

Building of expertise

Subsequent studies revealed the basic morphogenesis of the virus, the approximate number of proteins and led to the production of antibodies and the first enzyme linked immunosorbant assay (ELISA) (Eaton *et al.*, 1991; Hyatt *et al.*, 1992). Following this work another sample was submitted from Dr Rick Speare who had isolated a virus from the ornate burrowing frog *Limnodynastes ornatus*. This was also identified as a virus belonging to the family *Iridoviridae* (Speare and Smith, 1992, Hengstberger *et al.*, 1993).

During this time period Dr Speare had also been investigating diseases of the cane toad, *Bufo marinus*. In recognition of the work conducted at AAHL, and of the understanding that researchers (from an Australian Government funded project into biocontrol of cane toads, 1991-1994) may have isolated ranaviruses from Venezuelean cane toads, Dr Speare invited Dr Hyatt to attend a National meeting on which was held at the CSIRO Division of Sustainable Ecosystems under the Chair of Dr Hugh Tyndale-Biscoe. From this meeting a second successful submission was made to the Federal Government which resulted in the AAHL joining the investigative team.

Assays and collaborations

The team at AAHL comprising of Dr Alex Hyatt, Dr Jacques Zuponavic, Dr John Humphrey/Dr Helen Parkes and Ms Suzie Daglas began the work of propagating the Venezuelean viruses, undertaking their characterisation, performing transmission experiments and developing further diagnostic assays with collaborations with colleagues including Dr Barbara Coupar, Dr Allan Gould and Dr Jackie Kattenbelt. The assays included the development of a polymerase chain reaction (PCR) assay for ranaviruses (Gould *et al.*, 1995) from conventional tissues and formalin-fixed tissues (Kattenbelt *et al.*, 2000) and two antibody detection ELISAs (Zupanovich *et al.*, 1998). During this period, collaborations were also established between AAHL and Dr Andrew Cunningham (Institute of Zoology, Zoological Society of London, Regent's Park London) who worked at AAHL characterizing ranaviruses responsible for amphibian declines in the United Kingdom. Phylogenetic analyses resulting from these studies identified demarcation criteria that could be used to group viruses within the genus *Ranavirus* (Family *Iridoviridae*). (Hyatt *et al.*, 2000)

Diagnostics, taxonomy and other uses of recombinant ranaviruses

Diagnostics

The initial diagnostic assays have been used routinely for the detection of piscine ranaviruses, namely *epizootic haematopoietic necrosis virus* (EHNV). During the mid 1990s work was undertaken to compare all 'iridoviruses' held at AAHL. The work (Hyatt *et al.*, 2000) showed that the piscine, amphibian and reptilian ranaviruses all reacted with specific polyclonal antibodies in an antigen capture ELISA. Phylogenetic analysis of the major capsid protein indicated a geographical and species grouping. This information was used in 1998 when a person was apprehended at Cairns International Airport attempting to smuggle green pythons (*Chondrapython viridis*) into Australia from Indonesia (Hyatt *et al.*, 2002). Analyses of tissues from the seized pythons (ELISA, PCR and electron microscopy), that had later died, revealed the animals were infected with a previously undescribed *Ranavirus*. We can only speculate what the impact may have been if these snakes had been released into captive or free-ranging reptilian populations. The event also highlighted the inherent danger of illegal and unregulated trade in wild life.

Also developed over this period of time were two antibody capture ELISAs (Zupanovic *et al.*, 1998). These ELISAs, a competitive and indirect, are important as no discriminating anti ranavirus antibodies have been identified. These assays were used to map the distribution of antibodies against ranaviruses in cane toads within Australia and Venezuela. The competitive assay has also been used to screen for antibodies against ranaviruses within captive amphibian populations in the U.K. More work is required to validate these assays in accordance with O.I.E. stated criteria (http://www.oie.int/eng/en_index.htm).

More recently members of the cane toad team have developed an advanced molecular assay which will provide rapid, sensitive and specific assays for the detection and discrimination of ranaviruses. Studies with our collaborators at CSIRO Entomology, should also result in generic diagnostic assays for herpesviruses and adenoviruses within Australia's amphibian populations.

Taxonomy of ranaviruses

The information generated over the past decade has resulted in the accumulation of knowledge which has led ranaviruses being identified to the taxonomic level of 'species'. Recent work has resulted in demarcation criteria being suggested for the genus and species (Hyatt and Whittington, 2002). The former of these has now been accepted and adapted by the International Committee on Taxonomy of Viruses (Fauquet *et al.*, 2005) and distinct species namely *Ambystoma tigrinum virus, Bohle iridovirus, Epizootic haematopoietic necrosis virus, European catfish virus, Frog virus 3* and *Santee-Cooper ranavirus* have been recognised.

Other uses of recombinant ranaviruses

The current work involving the development of a recombinant virus for controlling cane toads in Australia has generated a virus with the potential to be used as a vaccine (mono, or multi-valent) against diseases of amphibians, reptiles and fish. The demonstrated ability to attenuate the virus and insert foreign genes means a recombinant virus could potentially be used to vaccinate susceptible species against epizootic haematopoietic necrosis (a potentially fatal disease arising from ranavirus infection), other diseases of fresh water fin fish such as viral encephalopathy and retinopathy (a potential fatal disease arising from *Betanodavirus* infection) and theoretically any other disease found in vertebrate poikliotherms which are susceptible to *Ranavirus* or indeed *Megalocytivirus* (another genus of the family *Iridoviridae*) infections.

Indirect spin-offs

Whilst the cane toad research was being conducted at AAHL another national and international conservation dilemma was coming to the fore; this was the enigmatic declines of amphibians. Amphibians were declining at a rate faster than any other taxa (Millenium assessment report … ref) and this caused considerable concern in the Australian and the international scientific community. In Australia Keith McDonald (frog ecologist and taxonomist from the Queensland Department of Environment and Heritage) and Prof Rick Speare of James Cook University decided to investigate the problem within the upland frogs in Queensland. The team then expanded to include Alex Hyatt (AAHL) and a jointly supervised PhD student (Lee Berger, a veterinarian with an interest in wildlife) whose thesis was focused on the identification of infectious agents which may be responsible for the decline of Australian frogs. The research effort was designed to use the technology developed from previous *Ranavirus* studies in an attempt to identify the speculated infectious agent.

Work began in 1995 at the CSIRO AAHL. After spending considerable time looking for ranaviruses Lee Berger concentrated on skin parasites which were a common observation from moribund and dead frogs. Images of the parasitic agent were sent to a colleague (Dr Peter Daszak, a parasitologist) of Dr Andrew Cunningham (who was still working in the laboratory). From this came the eventual identification of the chytrid *Batrachochytrium dendrobatidis* (Bd). With the identification came major international grants (Integrated Research Challenges in Environmental Biology (IRCEB) grant IBN-9977063 from the National Science Foundation, USA) which in turn led to further funding at AAHL where the new cutting edge diagnostic and sampling assays for Bd were developed, validated, implemented and transferred to laboratories throughout Australia and overseas. This success led to the ability for many of the research activities identified in the Federal Government Threat Abatement Plan for "infection of amphibians with chytrid fungus resulting in chytridiomycosis" to be completed.

Summary

The biocontrol work so far conducted by CSIRO has resulted in the identification of infectious agents that can be used as potential biocontrol agents for the cane toad, *Bufo marinus*. It has also initiated a momentum in research that has advanced our appreciation of the diversity of infectious agents infecting amphibians, the development of a broad range of diagnostic assays and the development of potentially new generation vaccines. The momentum has also created a critical mass of infrastructure within Australia whereby new initiatives have been possible e.g. the discovery of Bd and development of new generation diagnostic assays. It is intended that any future work addressing infectious diseases of the herpetological fauna, including that inherent within the paper in *Science* 'Confronting Amphibian Declines and Extinctions' (Mendelson *et al.*, 2006), will directly or indirectly benefit from the research so far conducted into the biocontrol of cane toads.

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[1]Iridoviruses are large icosahedral viruses that belong to the viral family *Iridoviridae*.

Investigation of a pathogen for *Bufo marinus* in northern Argentina: could it be a trypanosome?

David Peacock, Animal and Plant Control Group, Department of Water, Land and Biodiversity Conservation, GPO Box 2834, Adelaide 5001, South Australia
Phone: 08 8303 9504; 0417-355 498
Email: peacock.david@saugov.sa.gov.au

Abstract

The strategy of seeking a biocontrol agent for *Bufo marinus* where this species is not present, but bioclimatically suited, was applied to a published CLIMEX modeling study for the species. Northern Argentina is identified as a region fitting this criteria and worthy of investigation. It is proposed that a search for a biocontrol agent for *B. marinus* be undertaken in this region utilizing captive *B. marinus* sourced from a neighbouring South American country and exposing them to local pathogen sources such as aquatic leeches and mosquitoes. *Trypanosoma cruzi* is recognized as a protozoa that has experimentally caused *B. marinus* mortality and hence anuran trypanosomes are considered worthy of special consideration as a potential pathogen. Factors such as a reported vectoring by a number of invertebrates, significant host specificity and absence from Australian *B. marinus*, is suggestive of the potential value of anuran trypanosomes as biocontrol agents. Identification of a non-pathogenic *B. marinus* or *Bufo* specific trypanosome may provide an agent worthy of consideration for genetic modification into a *B. marinus* biocontrol agent. This genetic approach is proposed to have further application to other pest species reported to host a species- or genus-specific trypanosome.

Introduction

The June 2005 report from the National Cane Toad Taskforce to the Vertebrate Pests Committee lists Priority 2 for the long-term control of cane toads (*B. marinus*) by a pathogen to be identification of a suitable pathogen through a review of the literature (Taylor and Edwards 2005 p. 95). This report further states that in the search for a cane toad pathogen, "… given the myxomatosis experience, a potential strategy would be to screen other *Bufo* spp. particularly in areas where *Bufo marinus* is not present for micro-organisms with the potential for biocontrol of *Bufo marinus*. By analogy with the myxomatosis experience, such micro organisms would not necessarily produce large scale mortality in their natural hosts" (Taylor and Edwards 2005 p. 52). In accordance with this priority and suggested potential strategy, a review was made of *B. marinus* literature. With regard to identification of potential pathogens, focus was particularly given to the review paper of Speare (1990), and for the identification of a suitable search area, to the CLIMEX modeling paper of Sutherst *et al.* (1996).

Assessment of the method suggested by Taylor and Edwards (2005 p. 52), being "… screen[ing] other *Bufo* spp. particularly in areas where *Bufo marinus* is not present for micro-organisms with the potential for biocontrol of *Bufo marinus*", compared to utilising *B. marinus* in a bioprospecting manner, being exposure of the toads to as many likely pathogen sources as possible, was made. Speare (1990) outlined the research of Niño (1925), reporting a trypanosome to have caused *B. marinus* mortality. This lead to my hypothesis that this mortality could provide an indication of an inherent susceptibility of *B. marinus* to trypanosome pathogens. A review was therefore further made of anuran trypanosome research and the potential benefits and vectors of a trypanosome pathogenic to *B. marinus*. In addition, the potential benefit of a non-pathogenic trypanosome specific to *B. marinus*, or the *Bufo* genus, was reviewed with regard to current genetic approaches to cane toad control.

Results

The paper of Sutherst *et al.* (1996) provides a map of the natural range of *B. marinus* in South and Central America as well as a predictive range based on CLIMEX modeling. Comparison of these maps indicates that the Corrientes region in northern Argentina (Figure 1) is bioclimatically highly favourable for permanent colonisation by cane toads, however it lacks any habitation by the species. This region approximately equates with the current southern range of *B. marinus* in Australia and although Lever (2001; citing Easteal *et al.* 1981) states Argentina to be a part of the species natural range, Argentinean scientists specialised in amphibians inform me the species does not occur at all in Argentina (Marcos Vaira and Monika Hamann *pers. comm*. 2005). It is unknown how long *B. marinus* has been in Paraguay, however based on its colonisation of Australia (Lever 2001, Phillips *et al.* 2006), it is considered highly likely to have had sufficient time to allow for colonisation of the bioclimatically suited neighbouring northern Argentina, if this was possible. If not due to inter-specific competition from the resident *B. paracnemis* or *B. arenarum*, absence of *B. marinus* from the Corrientes region may be due to a pathogen of these resident *Bufo*, or perhaps an associated environmental pathogen.

Figure 1: Natural range of Bufo marinus (from Lever 2001 p. 2) with proposed biocontrol search area

If absence of *B. marinus* is due to a pathogen, such a pathogen may be having minimal impact on the resident toad species, as proposed by the National Cane Toad Taskforce (Taylor and Edwards 2005), but may be fatal to any *B. marinus* attempting to colonise the region from adjoining Paraguay, or from elsewhere. Assessment of all the known parasites and viruses associated with *B. paracnemis* or *B. arenarum* that could be inhibiting any colonisation of the Corrientes area by *B. marinus* was not conducted. Alternatively, it was considered that the best strategy for testing the hypothesis that a *B. marinus* pathogen exists in the Corrientes region was to use this toad in a bioprospecting manner. Being open to all possible pathogens and their sources using a bioprospecting approach was supported by the following examples:

1. The serendipitous discovery of (+)-calanolide, found to stop replication of the AIDS virus, was achieved using a bioprospecting approach (Wilson 2002 p. 123-124).

2. The serendipitous discovery of myxomatosis as a pathogen for European rabbits (*Oryctolagus cuniculus*) was achieved through their inadvertent exposure to the myxoma virus likely from *Sylvilagus brasiliensis* (Taylor and Edwards 2005 p. 52); and
3. that "more than half of the total contribution to the biological control of Cactaceae has been with agents that do not include the target weed species in their native home range" (Moran and Zimmermann 1984 p. 297).

Drawing on the myxomatosis experience, as described in the National Cane Toad Taskforce 2005 report, where susceptible European rabbits (*O. cuniculus*) were introduced into the newly established Institute of Experimental Hygiene in Uruguay and inadvertently exposed to myxoma virus likely from *Sylvilagus brasiliensis* (Fenner and Fantini 1999), it is proposed that *B. marinus* be deliberately exposed to potential pathogens in northern Argentina. It is therefore proposed that South American cane toads be brought into a facility in Corrientes and held in toad-proof ponds, with water and pathogen sources, such as aquatic leeches, mosquitoes and arthropods, sourced from the Chaco and Corrientes provinces. Precedents exist for *B. marinus* being brought into Argentina for scientific study (R. Garraffo, National Institutes of Health, USA, *pers. comm*. 2006). Laboratory experiments, such as dosing cane toads with the blood, and hence blood protozoa, of the resident toads, and ultimately any suspect pathogens, would for example further test the hypothesis. [It would need to be decided whether some of the South American cane toads should first be treated with drugs to destroy their native blood protozoans, thereby somewhat replicating the lack of native blood protozoans Delvinquier and Freeland (1988) describe for Australian toads, and likely also the situation for the toads released elsewhere in the Pacific.]

In reviewing Speare (1990) it was noted that Niño (1925) showed *Trypanosoma cruzi* to have caused *B. marinus* mortality. In reviewing a translation of Niño (1925) it is questionable whether the toads were in fact *B. marinus*. The toads were sourced locally from the National Hipodromo in Palermo, Buenos Aires and this is contrary to advice that the species does not occur at all in Argentina (Marcos Vaira and Monika Hamann *pers. comm*. 2005). Notwithstanding this inconsistency, of the six *Bufo* dosed with a *Trypanosoma cruzi* suspension, one toad went missing and the remaining five all died, most in 3-12 days. Although this trypanosome has a broad mammalian host range also causing human mortality (Hoare 1972), it is hypothesized that this *Bufo* mortality may provide an indication of an inherent susceptibility to trypanosome pathogens. It is further hypothesized that a trypanosome pathogenic to *B. marinus*, and specific to *B. marinus* or the *Bufo* genus, may provide a valuable pathogen for the control of *B. marinus* in Australia and perhaps elsewhere in its naturalized range. Consequently an evaluation was made of trypanosomes as a potential biocontrol for *B. marinus*.

Trypanosomes are protozoa associated with some very serious insect transmitted human diseases, *Trypanosoma cruzi* causing Chagas disease, a disease of South and Central America, and two sub-species of *Trypanosoma brucei* causing sleeping sickness in Africa (eg. Hoare 1972). They have a rapid rate of multiplication when the vertebrate is first infected, rapid growth rate in some species (*T. rotatorium* reaching maturity in ~7 days; P. Hamilton *pers. comm*. 2005) and a capacity to also infect the tadpole life stage (Mackerras and Mackerras 1961). Pathogenicity in trypanosomes is reported primarily from mammal trypanosomes such as *T. cruzi* and *T. brucei*, with both species extensively researched for many years. They are also seemingly ubiquitous, widespread protozoa, which have host-parasite relationships with vertebrates around the world, including Australia (eg. Hoare 1972). In Australian vertebrates trypanosomes have been detected in species including the iconic platypus, common wombat and eastern grey kangaroo (Noyes *et al.* 1999), as well as birds (Mackerras and Mackerras 1959) such as the currawong (*Strepera sp*. – Hamilton *et al.* 2005a), a gecko and skinks (Mackerras 1961), tortoises (Mackerras and Mackerras 1961, Jakes *et al.* 2001), fish (Mackerras and Mackerras 1961) and frogs (Mackerras and Mackerras 1961, Hamilton *et al.* 2005a). The rabbit trypanosome, *Trypanosoma (Herpetosoma) nabiasi* has also recently been detected in European rabbits (*O. cuniculus*) within Australia (Hamilton *et al.* 2005b).

Discussion

In the search for a biocontrol agent for *B. marinus* there are many locations and numerous possible pathogens which could be investigated. After a review of the CLIMEX modeling of Sutherst *et al.* (1996) it was however considered to suggest that the Corrientes region of northern Argentina offers a tantalizing possibility for a biocontrol for *B. marinus*. A region identified as bioclimatically highly favourable for permanent colonisation by this toad species, yet not colonised despite the species relatively close proximity in Paraguay, suggests that a biocontrol for *B. marinus* may be preventing this colonisation. Similarly, there are many possible pathogens which could be effecting this exclusion of *B. marinus* and which could therefore be researched. However, there are few agents identified as being fatal to this toad and the experiments of Niño (1925) are also considered to offer another attractive possibility. Perhaps there is an agent pathogenic to *B. marinus* active in the Corrientes region, and perhaps it is a trypanosome? Based on these possibilities further investigation was made of trypanosomes as a potential biocontrol for *B. marinus*.

The four primary requirements of a potential biocontrol agent for *B. marinus* are considered to be:

1. Pathogenicity to *B. marinus*
2. Specificity to *B. marinus* or *Bufo*
3. Susceptibility of Australian populations of *B. marinus* to the pathogen
4. Availability of pathogen vectors.

A preliminary literature review indicates that a trypanosome pathogen would fulfil, or be likely to fulfil, these criteria and is therefore considered a suitable pathogen to investigate for the long-term biocontrol of the cane toad.

Pathogenicity to *Bufo marinus*

The experimental infection of an Argentinean *Bufo* species with *T. cruzi*, and subsequent toad mortality, is suggested to indicate that *Bufo* may have a degree of inherent susceptibility to trypanosomes. The research proposed seeks to identify another trypanosome species that is both pathogenic and also specific to *B. marinus* or *Bufo*. It should be noted that trypanosome pathogenicity can be increased in the laboratory with passage through its host (P. B. Hamilton, Bristol University, *pers. comm*. 2005).

Specificity to *Bufo marinus* or *Bufo*

Although Desser (2001) found no trypanosomes in three *B. marinus* from Costa Rica, Lehmann (1966) found unidentified trypanosomes in *B. marinus*, but apparently not in the cohabiting *B. spinulosus*, collected from 2 valleys in northern Peru. In addition, trypanosomes have been recorded in numerous other *Bufo* species - eg. *T. fallisi* from *B. americanus* (Martin and Desser 1991b) and *T. mega* from *B. viridis* (Ashour and Gaafar 1997).

Identifying trypanosomes by morphology is stated to be somewhat unreliable, with isozyme and DNA studies finding morphology to "not keep pace with molecular and biochemical changes", and naming of anuran trypanosomes is currently discouraged (Desser 2001 p. 158). Notwithstanding this caution, the following research indicates anuran *Trypanosoma* species to have restricted host specificity.

- "In 4 of the 7 species [of anurans] sampled …nine morphologically distinct trypanosomes were observed … Surprisingly, none of the trypanosome types was shared by different hosts, with the possible exception of the morphologically similar *Trypanosoma* sp. (a) … and *Trypanosoma* sp. (g) …" (Desser 2001 p. 157).
- "Another feature of anuran trypanosomes … is their host specificity, which appears quite strict from the limited information available. In their study of the host range for *T. fallisi* [*Trypanosoma fallisi*] of the American toad [*Bufo americanus*] in Ontario, Martin and Desser (1991b) were able to experimentally transmit only transient infections to another toad *Bufo valiceps*, and a tree frog *Hyla versicolor*. Rana species were not susceptible to infections with *T. fallisi*" (Desser 2001 p. 158).
- An experimental infection with *Trypanosoma rotatorium* from the frogs *Hyla crepitans* and *Leptodactylus insularum* failed to infect *B. marinus* (Delvinquier and Freeland 1988 - citing Ramos and Urdaneta-Morales 1977).

Susceptibility of the Australian populations of *B. marinus* to the pathogen

Delvinquier and Freeland (1988 p. 1) state that "*Bufo marinus* [in Australia] has not retained any of its native blood protozoans". This would suggest therefore that Australian *B. marinus* are a naïve population and would be susceptible to a trypanosome pathogen.

Availability of pathogen vectors

Hamilton *et. al*. (2005a) state trypanosomes of amphibians to be primarily transmitted by aquatic leeches. However, Australian terrestrial leeches (Haemadipsidae) "of the genera *Philaemon* and *Micobdella* have been observed on frogs, including this species [*Mixophyes fleayi*; trypanosome infected] (H. Hines, personal communication)" (Hamilton *et al.* 2005a p. 440). Haemadipsid leeches are widespread in Australia, from temperate forest in Victoria to tropical rainforest in northern Australia (Hamilton *et al.* 2005a p. 1). Martin and Desser (1991a) state that "even the brief aquatic sojourn of more terrestrial anurans, like the American toad [*Bufo americanus*], was shown to be sufficient for transmission of *Trypanosoma fallisi* by the leech vectors". Leeches are also a favourable vector as trypanosomes are recorded to have long-term survival of up to 52 days in haemadipsid leeches (Hamilton *et al.* 2005a – citing Richardson 1968), and even 12 months postulated for *T. fallisi* in its leech vector *Desserobdella picta* (Martin and Desser 1991a). Easteal and Floyd (1986 p. 32) state that "leeches can commonly be found" feeding on *B. marinus* in Australia, though similar observations have not been made in the Townsville and Heathlands regions of Queensland (R. Alford *pers. comm*. 2006).

A concern with Australian leech vectors is their susceptibility to B*. marinus* toxin. Crossland and Alford (1998) found that for the Australian aquatic leech *Goddardobdella elegans*, 60% (6 of 10) were killed through feeding on *B. marinus* tadpoles. Unfortunately this leech wasn't tested on *B. marinus* hatchlings or adults where, in adults at least, the toxin is considered to be restricted to parotoid glands and "small verucose skin glands that cover the entire back" (Meyer and Linde 1971 – cited in Lever 2001 p. 30). Although this toxin producing tissue is not considered to be preferred tissue from which leeches would feed, this needs to be confirmed. Alternatively, it would need to be determined whether *B. marinus* tadpoles, hatchlings and adults can successfully be infected with trypanosomes from a trypanosome-infected leech before any leech mortality occurs.

"In addition to leeches that are generally acknowledged to be the primary vectors [of anuran trypanosomes], there have been reports of phlebotomine [sandflies] serving as vectors of trypanosomes of [*Bufo*] toads. … Despite the fact that amphibian-feeding flies may provide a suitable habitat for replication, the parasites do not develop to the infective metacyclic trypomastigote stage seen in the leech vector" (Desser 2001). However, although the trypanosomes may not fully develop in these haematophagous insects, it seems plausible that

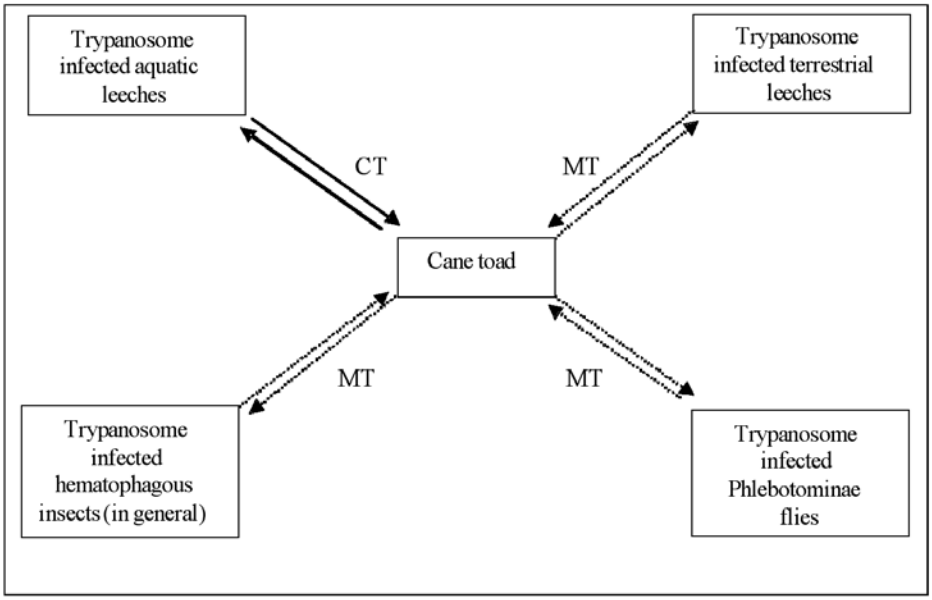
ingestion of epimastigotes by the normal anuran host (Ayala 1971) would allow transmission to occur. Similarly, it has been postulated that mosquitoes which have fed on trypanosome infected frogs may transmit the trypanosomes to other frogs by being ingested, the trypanosomes being found in the digestive tract rather than in the mouthparts or salivary glands of some of the mosquitoes tested (Ramos and Urdaneta-Morales 1977).

If phlebotomine sandflies are discovered to be a vector, or assist leeches in the vectoring of a pathogenic trypanosome, they (*Phlebotominae*) are known to cohabit with *B. marinus* in Australia, being widespread in the tropics (Lewis and Dyce 1988). In addition, if the postulation by Ramos and Urdaneta-Morales (1977) regarding batrachophilic mosquitoes transmitting trypanosomes to other frogs by being ingested by these anurans was supported, mosquitoes such as *Mimomyia elegans*, found to feed on *B. marinus* (van Beurden 1980), are common in the current and potential range of this toad.

Figure 2 describes possible transmission of pathogenic trypanosomes to *B. marinus* through documented and postulated vectors, being widespread and generally abundant in Australia.

Figure 2: Likely routes of cane toad infection with a trypanosome pathogen

CT = circular transmission; MT = mechanical transmission



Conclusion

History suggests the philosophy of being open to new ideas in the search for biocontrol agents and investing in educated 'long-shots' is a valuable strategy for success. This study therefore proposes that the focus for the search for a biocontrol for *B. marinus* be on the Corrientes region of northern Argentina. A research strategy which utilises *B. marinus* and their exposure to as many as possible of the region's pathogen sources is suggested to offer the best chance of identifying a *B. marinus* pathogen. However it is further suggested that special focus be given to trypanosomes as the potential pathogen, these protozoa being found to fulfil requirements for a biocontrol agent, especially in relation to the cane toad, *Bufo marinus*.

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Dr Patrick Hamilton (University of Bristol) and Dr Brian Cooke for their helpful comments on my original discussion paper before it was submitted to the IA CRC. Dr Thomas Spande and Dr Martin Garraffo (National Institutes of Health, Maryland, USA) for their helpful comments, contacts, references and ideas. Mr Peter Nielsen for recording an English translation of the Niño (1925) paper for my reference. Dr Ross Alford (James Cook University) for his observations on potential vectors. Dr Mónica Hamann (CECOAL-CONICET, Corrientes, Argentina) and Marcos Vaira (Museo de Ciencias Naturales, Argentina) for confirming cane toads are not present in Argentina and to Mónica and Dr Kehr (Corrientes, Argentina) for discussion of research hypotheses and project feasibility.

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SESSION 5: CURRENT AND PROSPECTIVE BIOCONTROL CONTINUED

Cane toads in decline due to disease?

Deborah Pergolotti, Frog Decline Reversal Project, Inc. www.fdrproject.org.au

Introduction

The Frog Decline Reversal Project, Inc. (FDR Project, Inc.) is a non-profit association formed in June 2000 and incorporated in February 2002. It is more widely known to the public by its rescue activity name Cairns Frog Hospital. The Cairns Frog Hospital (CFH) is a threat detection and care facility for diseased, injured, translocated, deformed and rescued frogs, tadpoles and cane toads and is run by volunteer carers experienced in amphibian husbandry. (Cane toads are not recovered but are collected for disease identification purposes.) The CFH also pursues pathology and researcher involvement in identifying the various conditions present on the animals. Symptoms for each species and condition are catalogued and full backgrounds collected for each animal prior to its arrival at the facility. Conditions and injuries which are treatable are corrected so that recovered animals can be returned to the wild to slow the speed of decline while threats are investigated.

Veterinary supervision and services are provided by several experienced wildlife vets and scientific assistance is provided by a range of relevant specialists including Associate Professor Rick Speare of James Cook University (JCU) and the JCU Amphibian Diseases Specialist Group. The FDR Project's activities are covered under several permits from Queensland Parks and Wildlife Service including Rehabilitation, Scientific Purposes and Educational Purposes. The organisation is a member of the Australian Wildlife Health Network, the Wildlife Disease Association, the Ecological Society of Australia, the Declining Amphibian Populations Task Force and several other environmental societies. The Founder of the FDR Project, Inc. currently holds a seat on the Conservation Sector Liaison Group of the Wet Tropics Management Authority and has received the Centenary Medal for her work in amphibian health.

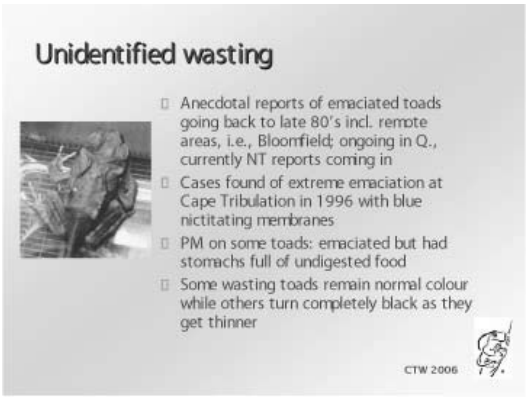
As a frog conservation organisation, the FDR Project, Inc. also provides community education and awareness, provides training to carers and veterinarians in frog rehabilitation, conducts field surveys, lobbies decision makers concerning the detection and investigation of wildlife diseases, and has an extensive website containing unique information unavailable anywhere else in the world. As the only setup of its kind in the country, the CFH is called upon by residents and carers from all around Australia and even overseas for help with distressed amphibians. Its setup has become an important model for consideration in effective amphibian disease surveillance and it is well placed to monitor the spread of and provide early warning for the five new amphibian disease threats it has uncovered, given proper support.

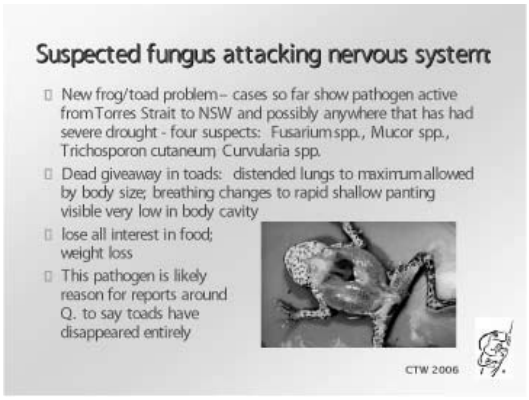
As of July 2006, the group has received and treated more than 1,660 juvenile and adult frogs, at least 30,000 rescued tadpoles and tadpoles randomly sampled for aquatic diseases (including cane toad tadpoles), and at least 100 sick and deformed cane toads. Although the CFH would like to target the collection of diseased and deformed toads in the same way as frogs are currently received, it does not have the financial support to handle the huge numbers of sick toads that would enter its receiving system.

Diseases being tracked by the FDR Project are detailed in the group's comprehensive website and some of these conditions target cane toads including:

1. *Mucor amphibiorum*
2. Chytrid fungus
3. Unidentified wasting
4. Unidentified nervous system disease (strong indications of a fungus)
5. Unidentified virus causing deformities and sudden death
6. Increased malformations, especially extra or malformed limbs.

Presentation given at the workshop:





"Redlynch" Virus:

- first known cases from Redlynch in Jan 03 but moving around fast
- found by sequencing at JCU but unidentified
  - causes mass die-offs at specific stages in tadpoles and sudden death of juveniles; bent/deformed tails common
  - Stunted growth in tads and frogs
  - the few survivors are deformed and malformed; reach 1/3rd normal size

CTW 2006

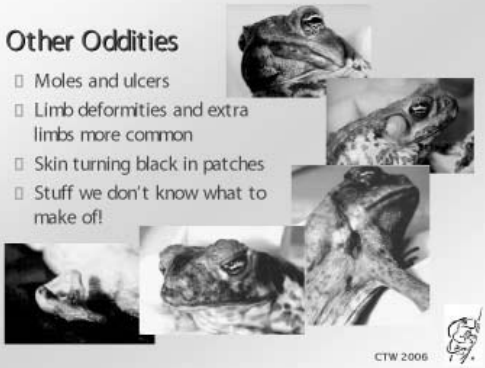
Deadly virulent to toads

- We can't show you photos of what "Redlynch virus" does to toads. It is so virulent, nearly 100% of them die early in rear leg growth stage or at metamorphosis!
- We have sampled at least 3,000 toad tads from RV affected water bodies and ALL but two died within a week (the two are eating well but extremely pale)
- The vast majority of toad tads we collect die before we even get them across town! They are DOA in the containers within an hour. Some survive for a week but most start to die within three days of collection; once die-off starts, all dead within 6 to 48 hrs.
- Finding toad tads to sample is getting difficult – they are absent entirely from available waterbodies or present in extraordinarily low numbers

CTW 2006

Other Oddities

- Moles and ulcers
- Limb deformities and extra limbs more common
- Skin turning black in patches
- Stuff we don't know what to make of!



CTW 2006

Personal parting thoughts:

- Millions of dollars are being spent on hi-tech experimental approaches that MIGHT possibly work they way they are envisioned, IF all goes well, around five to ten years from now.
- At the rate of decline we're seeing, what sort of threat will toads really pose in ten years time? Will their numbers justify the kind of money being spent while new problems are surfacing in so many native species?
- Therefore, shouldn't we be far more prudent in asking for taxpayer money using toad control as a justification - when there has been no attempt to identify diseases that are already killing off toads? (and frogs – don't forget them)
- Hi-tech expertise should be redirected towards inducing greater immunity/antibodies/vaccines to save frog/wildlife populations instead of exacerbating pathogen pollution!

CTW 2006

Follow up-notes to supplement the presentation (which was a summary only due to time constraints)

Efforts to identify the 'respiratory/nervous system' disease

"We sent several specimens showing specific clinical symptoms of the condition to two different accredited labs who agreed to accept the specimens (Department Primary Industries (DPI) Darwin and DPI TAS). Neither could find a cause of death because they were using standard histology practices which are not appropriate for isolating and identifying pathogens/mycotoxins which only attack the nervous system and not tissues. We then found a receptive mycologist at DPI in Townsville who was willing to try an alternate investigative method to determine what pathogens were present without using histology. We provided disinfected holding tanks which then held only the diseased animals and these were cultured for a range of bacteria and fungi. We also provided cane toads that were examined and cultures done on the skin lesions which are caused by the condition. A variety of bacteria and fungi were present on some individual cases but the majority of species detected were not consistent amongst all toads and containers. It is the four short listed fungi which were consistent in all toad skin lesions and all containers which held infected frogs.

We used a variety of related information to challenge or support the likelihood that one of the these four fungi might be the problematic one including contacts with amphibian disease researchers, soil microfungi specialists, clinical progression of the animals and response to a variety of treatments, variations in the progression of the symptoms based on geographic location and climate patterns, and information about the collection sites for each sick animal including soil type, local stressors and presence/status of other amphibians at the source location. Through a broad spectrum query process where each new piece of information was weighed against other collected information, we were able to build a temporary working profile on this disease problem which could be refined or challenged when further lab analysis could be obtained. More than 600 cases with this condition have been received making the observation of patterns and repetition obvious.

Since July 2002 when the disease first became apparent, we have chased as much lab work as we have been able to for a community group with limited support. It appears that there are only a few experts for the detection of mycotoxins in Australia and we were only able to locate one suitably qualified mycologist who would agree to provide molecular services for the work if compensated sufficiently. Considering that our previous attempts at securing government support for this work were unsuccessful, we were unable to proceed any further with isolation. However, recent negotiations with the Deparment of Environment and Heritage (DEH) have led to a Request for Tender and if this Tender proceeds to fruition, the 'respiratory/nervous system' disease will be investigated.

Efforts to identify a virus temporarily dubbed the "Redlynch" virus (after the suburb the first diseased tadpoles were found in)

Our group has experienced difficulty finding available virologists even when we are able to compensate them for the testing. We were able to secure the services of Leigh Owens at JCU School of Immunology and Virology to test several specimens affected by this unusual problem and he reported sequencing a virus that he was unable to identify, but he did not have time to pursue the work further. We have pursued other virus researchers to replicate the work but all contacted have declined because of workload.

However, while investigating the arrival in South Australia of diseased frogs presenting with the exact same symptoms we have described for the "Redlynch" virus, we learned that some of these animals were sent by an Adelaide vet clinic to the IDEXX veterinary lab where they

also found a virus in the liver that they could not identify. Their pathologist forwarded a sample onto the AAHL in Geelong and [a researcher there] informs me personally that he believed the virus to be a "herpes virus". Specimens are also still sitting in the queues at both AAHL and JCU but further work on this virus is likely to sit until the DEH tender for new amphibian diseases is approved and initiated.

On the evidence of decline in toad populations in Qld

A paper survey we did of Cairns residents specifically in December/January 2004 has not been compiled and analysed yet due to resource difficulties.

The fact that we have received thousands of reports from residents (and a member of the Cane Toad Task Force) across Qld over the past five years who have pointed out the very noticeable difference in toad numbers and dead toads on their own properties cannot be ignored. Our own survey work, especially when seeking toad tadpoles for disease surveillance, is drawing blanks because we are having difficulty finding them in water bodies. (It is hard to survey what isn't there anymore.) We have preserved diseased specimens for someone to look at and these would be included in the new DEH tender, if approved."

Daughterless cane toads

Professor Peter Koopman
Institute for Molecular Bioscience, The University of Queensland, Brisbane Queensland 4072.
Phone +61-(0)7-3346 2059, email p.koopman@imb.uq.edu.au

Abstract

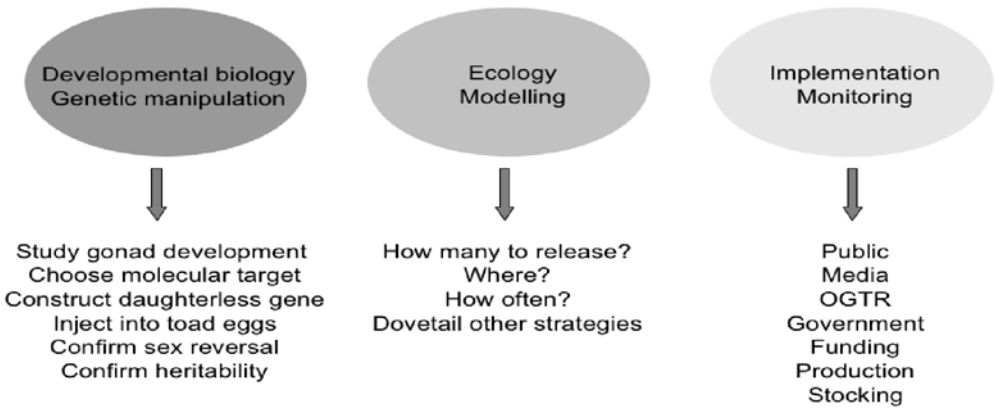
This paper outlines a genetic strategy – daughterless cane toads – designed to control the spread of the cane toad population, and perhaps eventually eradicate cane toads from Australia. The strategy is based on skewing of sex ratios in favour of males, thus limiting the number of females available for breeding. Major advantages of this strategy are that it is humane and non-lethal, and poses no threat to species other than the cane toad.

Introduction

Cane toads represent an increasing menace in Australian ecosystems, as elaborated in other papers in this volume. Current estimates are that the population of cane toads in Australia exceeds 200 million, with a firmly entrenched population base in Queensland, spreading rapidly across the north-west towards the Kimberley region of Western Australia, and southwards through New South Wales. The cane toads are well adapted to the Australian environment, compete effectively with native species for food supplies, and pose a threat to potential predators by virtue of their toxicity. These features, combined with their ability to migrate rapidly, make them a formidable pest species, and make their control or eradication a major challenge.

It is generally agreed that effective management of cane toads in Australia will call for a multidisciplinary approach, and that no single strategy should be regarded as a silver bullet. The present paper describes a genetic strategy that we refer to as "daughterless cane toads" (Figure 1). This is a highly sophisticated genetic strategy that has many advantages over other possible solutions to the cane toad problem, and has the potential to eradicate cane toads completely from the Australian ecosystems in a safe and humane manner. If successful, the strategy will be a major coup for genetic technologies in Australia and worldwide.

Figure 1: Overview of the daughterless toad strategy



Principles of the daughterless cane toad strategy

Daughterless cane toads is a sex skewing strategy based on limiting the number of females in the wild population. In this strategy, a genetically modified strain of cane toads will be produced that generates only male offspring. Male tadpole progeny of daughterless females will remain male and develop into fertile male toads. Female progeny tadpoles, on the other hand, will reverse their sex and also develop into fertile adult male toads. Thus as a result, the population will become overwhelmingly biased towards males and will limit its own numbers by virtue of the inability of males to find a breeding partner. It is relevant to note that the spread of the cane toad population appears to depend most critically on females, which lay between 7,000 and 30,000 eggs in a single clutch.

A significant feature of this strategy is that all the offspring of daughterless toads will be capable of spreading the daughterless gene to the following generation. Thus, the cane toads themselves will spread the agent of their own demise. However, because the spread of the daughterless gene will be diluted in further generations, restocking of the wild population will be required in order to ensure penetration of the daughterless gene throughout the population.

The daughterless strategy is based on the hypothesis that sex in cane toads is regulated, as it is in all other vertebrate species studied to date, by a network of key genes that cause the gonads to develop as testes or ovaries. The differentiation of testes or ovaries normally holds the key to whether an organism develops other secondary characteristics of maleness or femaleness. My research group has a wealth of experience studying the genetic and cellular mechanisms that underlie sex determination and gonadal development in mammals. This knowledge holds the key to the design and implementation of the daughterless strategy in cane toads.

To generate the daughterless strain of cane toads, we will use one of two molecular genetic approaches. The first of these would be to add the function of a male sex-determining gene to the cane toad genome. This would allow the male sex determination pathway to function normally in genetically male tadpoles, but would override the intrinsic female sex-determining program in genetically female tadpoles. The alternative approach would involve disrupting the function of female sex determining genes. This would have no effect in genetically male tadpoles, but would block the intrinsic female sex-determining program in genetically female tadpoles, with the likely effect that they would adopt the male sex differentiation pathway instead.

Precedents for both approaches exist. Blocking the female pathway of development by genetic interference with the function of the female specific steroidogenic enzyme aromatase forms the basis of the daughterless carp strategy currently being implemented by Dr Ron Thresher and colleagues at CSIRO, Hobart. This approach has recently yielded encouraging results in laboratory trials using *Medaka*. Conversely, we and others have shown that male sex determination can be induced in genetically female mice by adding the function of a male sex-determining gene such as the Y-chromosome sex determinant *Sry*, or the key vertebrate male sex determining gene *Sox9*. This approach has also been successful in reversing the sex of fish species such as *Medaka* and *Tilapia*. Adding the function of a male sex determining gene is currently our preferred approach in the daughterless cane toad project, based on the reasoning that sex reversal can be achieved this way even if the transgene is not 100% effective, whereas the same cannot be said for an approach based on blocking female development.

Logistics of making daughterless toads

Because we have only recently embarked on this project, the emphasis of this report is on the planned strategy for making daughterless toads. The first phase of this project involves combining our accumulated knowledge relating to the developmental biology of vertebrate sexual development with our experience in genetic manipulation to make daughterless toads in the laboratory. We have chosen a sex reversal approach, as described above, in preference

to alternative approaches such as female lethal, in the belief that sex reversal will allow more rapid dissemination of the daughterless gene through the wild population because of greater numbers of genetically manipulated offspring surviving and reproducing to pass the gene on. This phase of the work will require four distinct steps as described in the following sections.

Step 1: Finding the genetic target

In this project, we need to balance a desire to achieve rapid results with a desire to work in a methodical and scientific fashion. To balance these competing needs, we will follow two tracks in parallel. In what might be termed a fast-track approach, we will use our knowledge of mammalian sex determining genes and our experience in creating sex reversed transgenic mice, to design a daughterless gene construct with a high likelihood of being effective in cane toads. We have extensive knowledge of gene regulatory elements that are useful in driving transgene expression to the correct cells of the developing gonad at the appropriate time of gonadal development, and are reasonably confident that these regulatory elements may be useful in directing daughterless gene expression in the cane toad. We also know of a number of mammalian male sex-determining genes which, when introduced as transgenes into fertilised eggs, are capable of effecting testicular development even in chromosomally female mice. A number of these genes are known to be very highly conserved in evolution, and hence are likely to play a role in cane toad sexual development. Therefore, we are able to make an informed decision regarding the design of a DNA construct that can be deployed rapidly in initial attempts to make daughterless cane toads.

In parallel with these efforts, we will undertake extensive studies of the genetics and biology of sexual development in the cane toad, adopting more methodical and stepwise approaches. First, this will involve confirmation that sex is under genetic control in the cane toad. Sex appears to be chromosomally determined in a number of amphibian species, but, surprisingly, genetic control of sex determination has not been demonstrated conclusively in the cane toad. We will use cytogenetic techniques to look for chromosomal differences between male and female toads.

Alternatively, or in addition to genetic control, sex may be determined in cane toads by the temperature at which the larvae are reared. Temperature dependent sex determination is a feature of many reptilian species, and has not been formally excluded as a mechanism of sex determination in cane toads. We will therefore undertake experiments in the laboratory to determine whether sex ratios are affected by the rearing temperature of larvae, and study the effects of incubation temperature on the expression of known or suspected sex-determining genes.

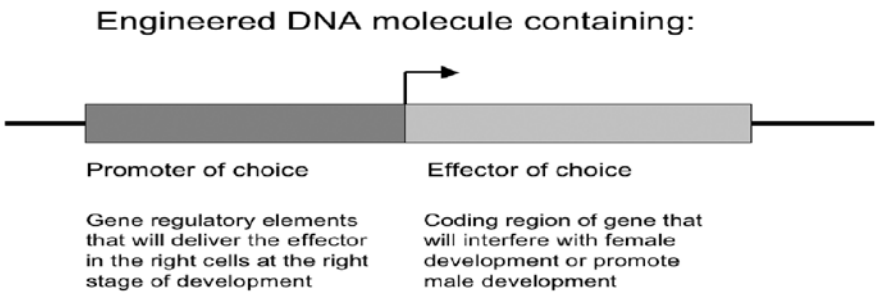
Thirdly, we will study how the application of exogenous sex hormones can affect sex determination in cane toad larvae. Use of hormones such as aromatase, testosterone, anti-Müllerian hormone, and estradiol, as well as the use of inhibitors such as aromatase inhibitor, are known to be effective in causing sex reversal, for example in fish, several amphibian species and wallabies. Determining which hormones are effective in bringing about sex reversal in cane toads may provide new information relating to intervention points that can be exploited in the daughterless cane toad strategy.

Finally, we will undertake detailed embryological studies to determine the stage at which gonads differentiate in the cane toad. We will also study in detail the expression of genes known to be important for sex determination and gonadal development in other species. That is, we will determine which genes are expressed during gonadal development in the cane toad, when they are expressed, and in which cells and tissues. Information from these studies will be critical for the intelligent design of the transgene that will form the basis of the daughterless cane toad strategy. This will become particularly important if the fast-track approach described above proves ineffective in generating sex reversed cane toads.

Step 2: Making the daughterless gene construct

The second step in this phase of work will be the construction of the transgene that will bring about sex reversal in the cane toad. This transgene, popularly referred to as the daughterless gene, will be an engineered DNA molecule with two parts (Figure 2). The effector part of the molecule will be the coding sequence of a gene, identified in step 1 above, that will promote male development in all offspring. The other part of the DNA molecule will be a promoter or enhancer containing gene regulatory elements that will cause the effector to be expressed in the right cells at the right stage of development to bring about female-to-male sex reversal. This DNA construct will be made using standard molecular laboratory techniques.

Figure 2: Construction of the "daughterless" gene.



Step 3: Generating transgenic toads

We will use the DNA construct described above to make transgenic cane toad larvae. This will involve microinjection of the DNA into fertilized cane toad eggs, following standard protocols developed in laboratory species such as *Xenopus laevis*. We expect that adapting this technology to *Bufo marinus* (cane toads) will pose minimal technical difficulties. If transgenic cane toads can be derived by this technique, we will in the longer term need to explore methods for making transgenic toads in bulk, to satisfy the requirements of large-scale restocking of daughterless toads in the wild.

Step 4: Determining fertility and heritability

After microinjection, tadpoles will be reared to sexual maturity and tested for sex reversal. This will involve comparing the transgene status (i.e., transgenic vs nontransgenic), genotype (i.e., genetically male vs genetically female), and sexual phenotype (i.e., male vs female), to look for evidence that incorporation and expression of the daughterless gene does in fact lead to sex reversal of cane toads. In addition, transgenic toads will be bred to ensure that the daughterless gene is heritable, a basic requirement for the success of the daughterless strategy overall.

Pros and cons of daughterless technology

The daughterless approach offers a number of extremely important advantages over other existing or possible strategies for cane toad management. These are briefly summarised as follows:

Daughterless technology is non-toxic.

Unlike viral or toxin-based approaches, the daughterless transgene will not lead to pain or morbidity in toads. Further, the daughterless strategy does not involve the release of any potentially harmful virus or toxic substance into the environment. Concern has been expressed that deleterious viruses might spread to South America and pose a threat to cane toads there; this would be an undesirable outcome since cane toads are native to South America. However, in the unlikely event that one or more daughterless toads should make their way to South America, these toads would pose no threat to the overall indigenous population since the success of the daughterless strategy relies on vigorous population restocking. Although not normally associated with genetic modification, the words clean and green can aptly be applied to the daughterless cane toad strategy.

The daughterless gene will be 100% species-specific.

It is spread by the toads themselves by mating, and who it is spread to depends entirely on who the cane toads made with. Obviously, cane toads mate only with other cane toads, ensuring the safety of other amphibian species, and indeed, all other species in the environment.

The effective agent in this strategy, the daughterless gene, is spread by the toads themselves.

The more the toads breed, the further the gene spreads. In this strategy, the toads do the work.

This is an ingenious solution that relies on cutting-edge genetic technologies.

If successful will place Australia at the forefront of genetic pest management strategies worldwide.

Public support for this strategy is high.

Not only are cane toads viewed as public enemy No. 1 in many parts of Australia, but also the public is captivated by the ingenuity of the daughterless solution. If successful, this strategy will go a large way towards public acceptance of genetic modification technologies for delivering positive outcomes for the Australian public.

This is not to say that the daughterless strategy has no drawbacks. Certainly, this technology is untested as a means of pest control. We will need to pay particular attention to the fitness and fertility of the daughterless strain in comparison to their wild type brethren, in order to ensure that the daughterless strain and hence the daughterless gene, is able to penetrate the population effectively. Further, as generations go by, the transgene will be diluted, and heavy restocking will be required to ensure penetration of the daughterless gene through the population. A clear drawback in this regard is that implementation of the daughterless program will require release of additional toads into the wild, and therefore may be seen to be adding to the problem in the short-term.

Will daughterless cane toads work, and when?

Developing daughterless cane toads in the laboratory is only one of several major strands that will need to proceed in parallel if this strategy is to be successful. Clearly, there is a pressing need for detailed ecological modeling of the impacts of daughterless cane toads. In particular, it will be necessary to thoroughly plan where the daughterless toads should best be released, how many will need to be released, when, and how often. The accompanying paper by Dr. Thresher addresses some of these issues.

In addition, further work will be required to ensure complete public acceptance of this strategy, approval from gene technology regulators, political backing, and further funding. To date, media and public support has been overwhelming. The issue of funding is particularly relevant because it is likely to impact on the numbers of daughterless toads that can be produced in the long-term, and more importantly the numbers of hands available to deploy the toads.

As mentioned at the outset, it is unlikely that any single strategy on its own will be likely to succeed in eradicating the cane toad. To maximize the likelihood of success, this approach will need to be combined with other strategies, which may include trapping and/or sex specific culling. The optimal combination of strategies is likely to become clearer in coming years as different possibilities are developed.

Acknowledgements

I thank Ron Thresher for the inspiration behind this project, and for continued encouragement, collaboration and constructive criticism. Thanks also to John Abramyan and Zara Borg for insightful discussions and comments on the manuscript. Financial support from the Invasive Animals CRC and the Queensland State Government is acknowledged.

Comparative analysis of genetic options for controlling invasive populations of the cane toad, *Bufo marinus*

Ronald E. Thresher and Nicholas Bax
Invasive Animals Cooperative Research Centre and CSIRO Division of Marine and Atmospheric Research
GPO Box 1538, Hobart, Tasmania, 7001 Australia

Abstract

We use a genetic/population model to investigate the strengths, weaknesses and potential for physical removal and five genetic techniques (sterile male release, "daughterless", female-specific lethality, female-specific sterility and a "Trojan gene") to eradicate cane toads. Results suggest that daughterless and female-specific lethality and sterility can all lead to pest extinction within 100 years of stocking, at seemingly reasonable levels of effort, but also that all control methods are affected by uncertainties in the degree of density dependence in cane toad populations.

Introduction

Methods to manage invasive species range from ignoring them and hoping they will go away through to a variety of options for physical removal, biocides and biological control (Thresher & Kuris, 2004). For well established and widely distributed pests, such as the cane toad, the only realistic options in the past have been augmentative and classical biological control, and sterile male release programs, both of which have significant constraints on their application (Whitten and Foster 1975). As a result, most invasive pests remain uncontrolled.

In the 1960s, entomologists speculated that genetic techniques could also be a powerful means of controlling pest populations (Hamilton 1967) based on the observation that meiotic drive had apparently driven some insect populations to extinction. Practical development of such techniques lied fallow, however, until recent developments in recombinant genetics stimulated renewed interest in the field (e.g., Thomas *et al.* 2000). Currently, at least three recombinant methods for pest control (repressible male sterility, virally vectored immunocontraception, and female-biased sex ratio distortion) are being tested in the laboratory. Another widely publicised study has speculated that the escape of even one carrier of a "Trojan gene" (a construct that pleiotropically enhances mating advantage while otherwise reducing fitness) could cause species extinction (Muir and Howard 2002), a considerable risk if a carrier accidentally escapes, but also a potentially powerful means of pest control. Although such methods are still in the early stages of development, focus group analysis indicates that such genetic techniques, if they affect only the pest population, are more acceptable to the public and managers than, for example, classical biological control (Thresher& Kuris, 2004).

In theory, any of these genetic methods could be used to control cane toads. However, whether any would be effective in the "real world" is not clear. As the development of such technologies is expensive, prior to investment it is useful to model the potential of, and potential constraints on, such genetic techniques, as well as on conventional approaches to managing pests. In this paper, we use a realistically parameterised genetic/population dynamics model to assess whether any of the genetic methods discussed in the literature will drive cane toads to extinction under real world conditions, and then use sensitivity analysis to quantify these conclusions in the face of current uncertainties in the ecology and behaviour of the toads.

Methods

Specifically, we looked at four options:

1. A gene construct that biases offspring sex ratios towards males ("daughterless").
2. One that sterilises only females ('female sterile").
3. One that causes pre-maturational female-specific mortality ("female lethal").
4. A trojan gene.

We focus on constructs that affect females on the basis that female, rather than male fecundity is the factor that usually constrains population growth. For the Trojan gene method, we set the mating success of male carriers 4 times higher than that of wild type males, but lowered the first-year survival of their offspring by 0.5%, a combination that Muir and Howard (2002) indicate as most likely to lead to population extinction.

We also included in the comparison stocking of a neutral gene as a control, and both physical removal of the toads and the release of sterile males as alternative or complementary management options. We did not include externally vectored recombinant effects, such as virally vectored immunocontraception or toxins produced using recombinant approaches, on the basis that their effectiveness would depend only in part on the population dynamics of the toad, and more on the dynamics of the vectoring organism or baiting strategy.

Information on the population dynamics of the toad is based on Lampo & De Leo (1998) and R. Shine (pers. comm.). Model details are described elsewhere (Bax and Thresher, ms).

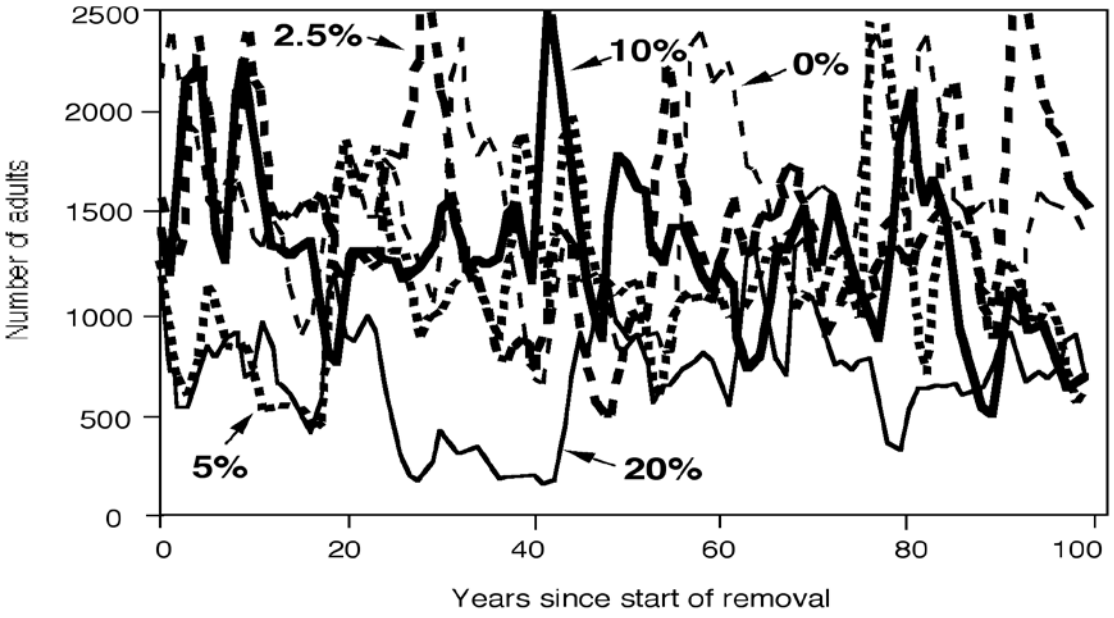
In brief, we use an age-structured deterministic model to simulate recruitment, mortality, sex ratios and gene frequencies in a freely interbreeding population, i.e., we assume no emigration and immigration. Initial conditions specify an annual mean recruitment of 1000 individuals at carrying capacity, a sex ratio of 1:1, a maximum age (95% mortality) of 5 years, a constant post-metamorphic mortality rate, and an age at maturity (95% mature) of 2 years. Recruitment (number of individuals surviving metamorphosis) was related to adult population size by means of a discrete logistic (Ricker) model. Lampo & De Leo (1998) suggest strong density dependence in the egg and larval stage, but only slight density dependence among juveniles and adults. We captured this in the model by using as our baseline a Ricker parameter of 1.25, which specifies low recruitment to the juvenile stage when populations are at less than half carrying capacity (due to the small number of breeding females) but which stabilises close to asymptotically at larger population sizes due to competition among pre-metamorphs.

We also assume recruitment is strongly affected by rainfall (proxied as the Southern Oscillation Index), on the basis that high water levels increase the number of available breeding ponds and resources for developing tadpoles. Extinction was arbitrarily defined as when the number of viable females falls to 1% of initial population size, and we limited the model runs to a maximum of 100 years, as any method that took longer than this to control cane toads would probably not be attractive to managers or the public.

Results

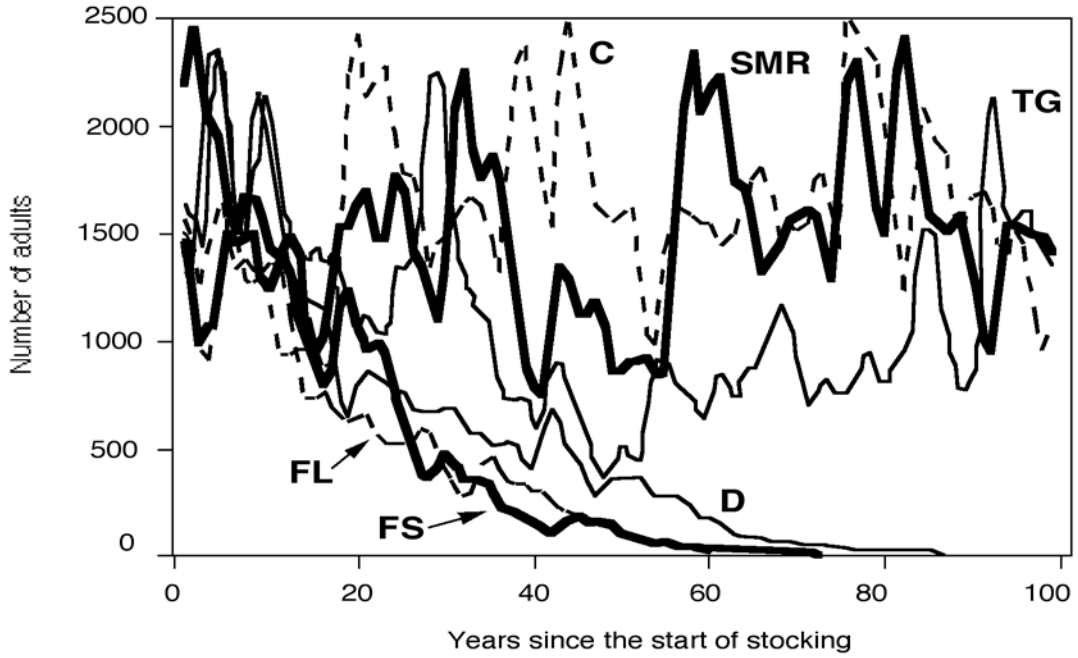
The effects of physical removal on the modelled toad population are shown in Figure 1, for removal levels of up to 20% of the breeding population each year. Physically removing toads reduces immediate population density, obviously, but this is largely over-ridden by inter-annual differences in breeding success and, due to density dependence, does not result in a significant long-term population decline. For the modelled population, physical removal does not lead to extinction until > 40% of adult toads is removed each year.

Figure 1: Effects of physical removal (poisoning, baiting, trapping) on the number of adult cane toads, at annual removal levels ranging from 0 to 20% of mean number of adult toads.



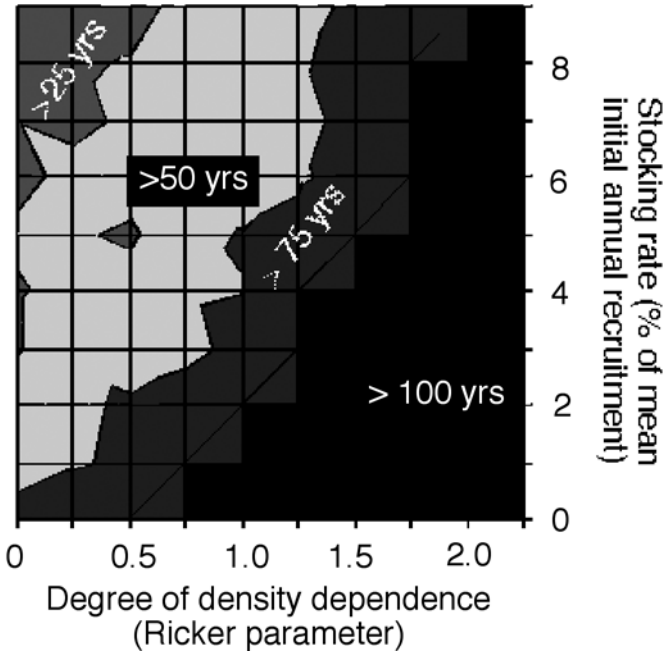
A baseline comparison of the effectiveness of the genetic methods, including sterile male release, is shown in Figure 2, which is based on an annual stocking rate of carriers (or sterile males) equal to 5% of initial mean annual recruitment and a genetic construct that causes the specified phenotype for about three generations. Releasing sterile males at this level has no effect on toad numbers relative to the control. In contrast, stocking carriers of female-lethal, female-sterile or daughterless constructs causes population extinction in less than 80 years. The minimum annual mean stocking rates required to achieve extinction are similar for female-lethal and female-sterile constructs (at about 2.5% of natural recruitment levels) and is slightly higher for a daughterless construct (3%). To achieve extinction using a sterile male release program requires annual releases of males >50% of the number of wild type males and takes about 90 years. The Trojan gene approach results in a rapid replacement of wild type toads (see Bax and Thresher, ms.), but does not lead to population extinction even at stocking rates as high as 70% of mean recruitment.

Figure 2: The effect of six genetic control methods on numbers of adult cane toads, all at an annual stocking rate equal to 5% of mean annual recruitment, no leakage and no fitness effects of the construct, and a copy number of 8. C = control (stocking of neutral gene); SMR = Sterile male release; TG = Trojan gene; FL = Female-specific lethal; FS = female-specific sterility; D = daughterless.



Sensitivity analysis shows that all control methods (including physical removal) are strongly affected by the degree of density dependence in the population (the higher the level of density dependence, the higher the stocking/removal rate required to achieve extinction)(Figure 3) and by fitness effects of the construct on carriers (the lower the fitness of carriers relative to the wild types, the higher the required stocking rate), but largely unaffected by the magnitude of environmental effects on annual recruitment or, within a reasonable range, mortality rates/ maximum age of post-metamorphs.

Figure 3: Number of years to population extinction as a function of stocking rate (0-9% of initial annual recruitment) and degree of density dependence (Ricker parameter ranging from 0 [no density dependence] to 2.25 [strong density dependence]) for the modelled cane toad population.



Models are not the real world, but can have heuristic value in indicating the strengths and weaknesses of, and constraints on, different approaches that, in this case, can potentially be used to control the toads. Our analyses suggest that physical removal of the post-metamorphs can result in lower toad densities, but requires extreme levels of effort if it is to be used to eradicate the toads. A similar conclusion can be drawn with regard to a sterile male release program. Both of these conclusions are consistent with long-term experience with other animal pests (Whitten and Foster 1975). By comparison, three of the genetic approaches we've modelled – daughterless, female-specific sterility and female-specific lethality – can all lead to population extinction within a relatively short time and at seemingly modest levels of stocking effort. The genetic mechanisms that could result in these options being available in cane toads have not yet been investigated, but may be similar to those presently being explored in fish. The models also suggest strongly that more detailed information on the degree and nature of density dependence in cane toad populations is important in refining predictions of the efficacy of any attempt to control the pests. Finally, the model thus far does not include effects of population fragmentation. All control methods will be ineffective when applied to small areas subject to immigration above a nominal level. Spatially explicit models will be needed not only to assess the efficacy of any control method on a well-established population like the cane toad, but also to design optimal release strategies (by taking advantage of source-sink dynamics) for genetic technologies.

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Modelling potential control strategies for cane toads

Hamish McCallum, School of Integrative Biology, The University of Queensland, Brisbane 4072.
h.mccallum@uq.edu.au

Abstract

This paper is an overview of what current ecological theory and current modelling suggests about potential control strategies for cane toads. The key points are as follows:

1. Preventing the establishment of new populations or preventing invasion require different strategies than reduction of the density of an existing population. In general, preventing invasion is likely to be the more feasible option. This paper is largely concerned with preventing invasion.
2. There are no known cases of the elimination of an established population of a vertebrate pest from a large area. Elimination of the cane toad on a broad scale is not a viable objective.
3. Density dependence in anurans, including cane toads, acts most strongly in the tadpole stage. This means that control strategies that reduce the tadpole population density may have limited effects on adult toad population density.
4. Baits, lures and traps may be able to prevent toads invading discrete areas. However, these control methods would need to remove about 25% of the toads present, each and every month, until the population was eliminated.
5. Development of a transmissible pathogen that decreases reproductive output is a feasible control strategy. If such an agent were engineered from an existing pathogen such that the impact on toad survival was decreased, but the impact on fecundity increased, it might be able to spread successfully through toad populations.
6. Sterile male release has been successfully used in insects, but in cases where only females cause ecological or economic damage. It has been investigated to control lampreys in the North American Great Lakes. After 30 years, there is no clear indication of wide-scale success. For cane toads, a simple model suggests that continued annual releases over five to 10 years of around 100 times the size of an existing toad population would be required to eliminate an invasion.
7. Similarly, models predict that daughterless male release could be effective at preventing a toad population from becoming established, but would require a release of about 100 times the size of the existing population. The models do not address the question of reducing the size of an established population.

Introduction: Prevention of establishment versus control of existing populations

There are two potential objectives of a control strategy. One may be to reduce population density in an area in which cane toads already exist. Alternatively, the objective may be to prevent cane toads from establishing in a particular area, or to slow or prevent the rate of invasion into new areas. The first objective is likely difficult than the second. Each objective requires quite different control strategies and quite different modelling methods. Whilst there have been some cases of vertebrate pest populations being reduced on a broad scale by the use of biological agents (the control of rabbit populations by myxomatosis is perhaps the classic example (Fenner 1994)) or by direct poisoning or shooting, there are no cases in which any vertebrate pest has been eliminated from a large area by any form of control once it has become established.

The feral cat population on the small sub-Antarctic Marion Island was exterminated by a control program that included the infectious disease feline panleucopaenia, but shooting was needed to eliminate the last few individuals (Van Rensburg *et al.* 1987).

Preventing populations becoming established in discrete habitats such as islands or perhaps even reducing the rate of spread is a more plausible objective than eliminating large, established populations. Invasion biology theory and also empirical data suggests that there is frequently a lag phase following the first arrival of invasive species, during which its rate of increase is relatively small (Sakai *et al.* 2001). Thus, a smaller absolute number of individuals needs to be removed in order to prevent establishment of a population than needs to be removed to reduce an established population. Furthermore, the proportion of individuals present that needs to be removed to reduce the rate of increase below the replacement level is likely to be rather smaller in a recently established population than in a well established population.

In the particular case of biological control, different approaches are more likely to be applicable when attempting to prevent establishment and when attempting to reduce existing populations. "Innundative" approaches require the swamping of the population to be controlled by a large number of control organisms. Usually, the organisms are predators or parasitoids, but sterile male release is an example of this type of strategy. Because the number of control organisms needs to be sufficiently large relative to the pest population, these approaches are likely to be successful only in the initial stages of establishment, or in local, contained populations. Conversely, release of an infectious control agent is likely to be most successful in well-established populations. In general, host specific pathogens (and any acceptable biological control agent is likely to need to be host specific) will have a threshold host population below which it cannot spread within the population. Thus, it is unlikely that such a biological control agent will be successful in a recently established low-density population and also it is unlikely that such an agent will be capable of driving its host population to extinction (McCallum and Dobson 1995). There are some important exceptions to this generalisation which I will discuss below.

Density dependence and compensatory mortality

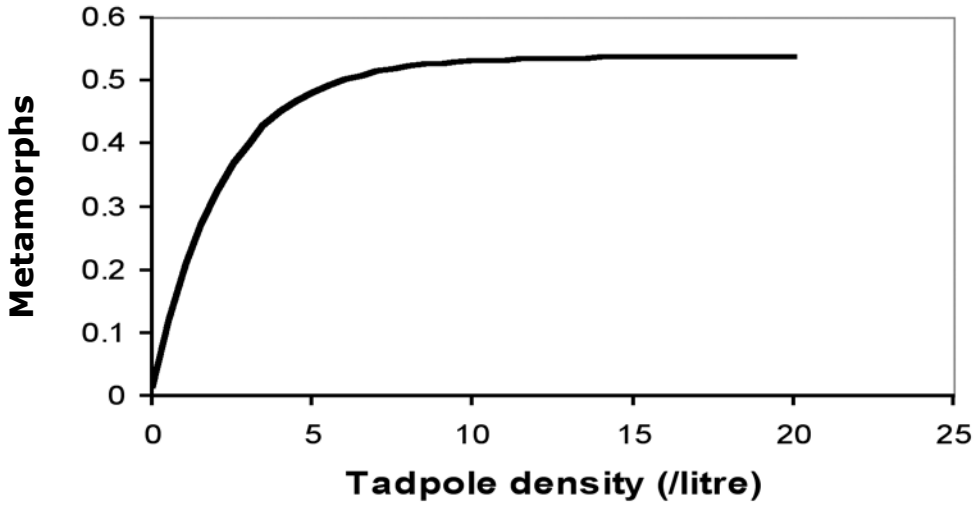
All populations, except in phases of initial growth following establishment, are subject to some form of density dependent regulation, which determines the long-term average population density. There has been extensive debate in ecological literature over density dependence. In essence, the debate is over whether density dependence operates at most population densities and at most times, or whether it occurs only when environmental variation means that resource limitation is particularly important, or only when population densities become extremely high. The axiomatic fact that density dependence must operate in all real populations at least some of the time is no longer under serious debate (Turchin 1995).

Density dependence has important implications for strategies designed to reduce the population density of an established population. In most populations, it operates most strongly during a particular life history stage. In the case of anurans (Govindarajulu *et al.* 2005), including cane toads (Lampo and De Leo 1998), the clearest evidence for density dependence is in the tadpole stage, in which intraspecific competition and predation can lead to decreased survival and lower growth and maturation rates. As this density dependence is largely a function of crowding in the small water bodies within which the larvae live, it is likely to operate even at low overall mean population densities.

If the proposed control strategy is applied to a life history stage before the one at which most density dependence operates, then it is likely to have a relatively small effect on the adult population size, even if it causes very substantial mortality indeed. Figure 1 shows how a substantial reduction in tadpole density in a discrete water body (perhaps via a sterility-inducing agent) may have only a minor impact on the number of toads reaching metamorphosis.

Conversely, if the control strategy is applied at a life history stage after the one at which density dependence is at its strongest, then it is likely to decrease the overall population density. In the case of cane toads, it is the adults that have the greatest impact on the biodiversity and on humans. Density-dependence acting primarily on the tadpole stage means that control strategies which reduce fertility of adults or target tadpole maturation may achieve large decreases in fertility or tadpole survival without a correspondingly large decrease in adult population density and therefore without a decrease in toad impact.

Figure 1: Cane toad metamorph production as a function of initial tadpole density. Recalculated from Lampo & De Leo (1998), in turn based on field data from Hearnden (1991). A reduction of tadpole density from 20 to 5 individuals per litre would lead to very little change in the number of metamorphs produced



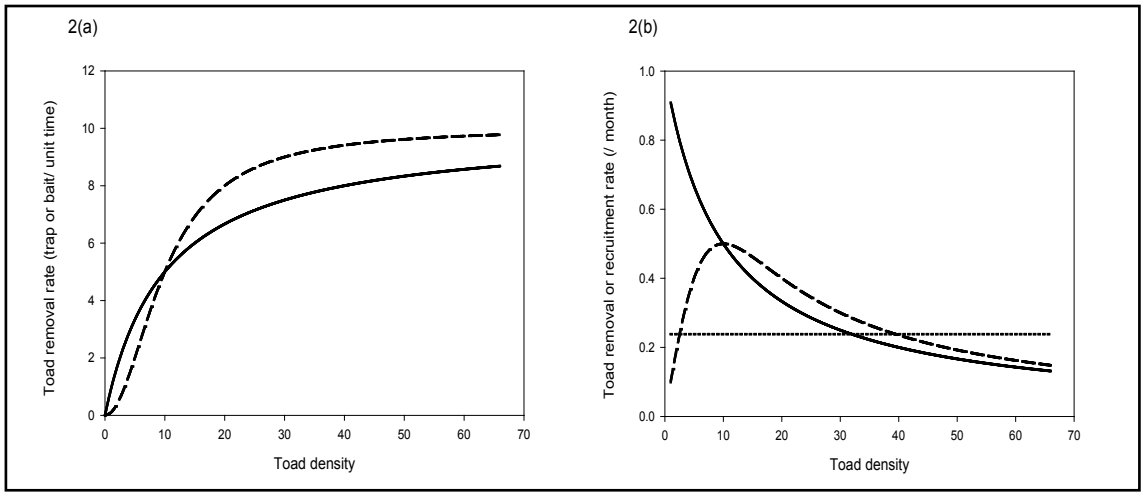
Baits, lures and traps

The population dynamics of the influence of baits, lures and traps on a host population can be understood within the framework of predator-prey modelling (Sinclair *et al.* 1998, McCallum 2000), with the human operating the device functioning as the predator. In a predator-prey interaction, the prey density has two influences on the predator. First, there is the numerical response, which is the way that the predator population size depends on the prey density. In the context of cane toad control, at very low toad densities, there is probably little incentive to employ control strategies. However, once toad density reaches a certain level, then financial and logistical constraints are likely to mean that the control effort reaches a plateau. We would therefore expect a numerical response that initially increases linearly (or perhaps even faster than linearly) with cane toad density, but which then asymptotes. The functional response is the way in which the number of prey items consumed per predator per unit time varies with prey density. For example, the number of toads caught per trap, per night is the functional response of the trap. For most baits, lures or traps it is likely that the functional response will be approximately linear at low toad densities: the more toads that are present, the more likely a bait is to be consumed or a toad is to encounter a trap. Again, one would expect the relationship to asymptote at high population densities. At some point, traps will become full or baits will all be consumed. In the context of predator prey theory, this means that the total response, which is the number of prey removed per unit of time as a function of prey density, will therefore follow either a simple asymptotic curve or probably an S-shaped relationship (see Figure 2(a)).

This can be converted into the increment in the prey death rate produced by the control strategy simply by dividing by prey density (see Figure 2(b)). Whilst the quantitative details of whether a

given control strategy can be effective will need to be determined, the overall qualitative pattern is that, because the death rate is inversely density dependent in high population densities, it is highly unlikely that these strategies will be effective in well-established populations. However, in low-density populations, it may be possible to eliminate toads or perhaps to maintain them at a very low level. The intrinsic rate of increase for cane toads in Australia has been roughly estimated at about 2.86 y^{-1} (Lampo and De Leo 1998). This means that for any baiting or trapping program to prevent population increase, it would need to remove toads at least at this rate: each toad present in the area must have a probability of being caught each month of about 25%, or about 5.5% per week.

Figure 2: Total responses to baits or traps. 2(a) toad removal rates per trap or bait as a function of toad density. Solid line: type II response, dashed line, type III response. 2(b) death rates per toad from traps or baits as a function of toad density. The dotted line represents the intrinsic rate of increase of the toad population. If removal rates are above the intrinsic rate of increase for the particular toad density, the population will decline. For both types II and III, the removal program would be effective only at densities < 40-50 toads/unit area. In the case of the Type III response, the toads could be maintained at a low density of about 2 /unit area, whereas if the Type II response existed, they could be eliminated.



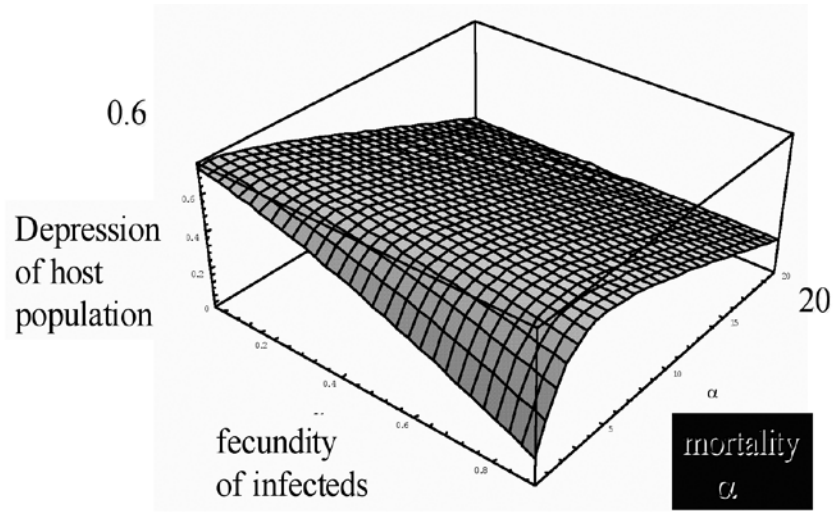
Transmissible biological control agents

Myxomatosis and rabbit haemorrhagic disease are probably the biological control agents that immediately spring to mind when considering the control of vertebrate pests. Both of these act to increase mortality in infected individuals. Whilst both have produced substantial reductions in rabbit population density, the rapid evolution of resistance in rabbits and the simultaneous reduction in the virulence of the myxomatosis virus (Ross 1982, Fenner and Fantini 1999) illustrate the limitations of mortality increasing agents as permanent control mechanisms. Further, if a pathogen is directly transmitted between hosts, conventional theory suggests that it should have density dependent transmission dynamics. This means that there is a threshold host population below which the disease cannot be maintained in the host population. Because highly pathogenic agents kill the host rapidly, they spread relatively poorly, with the result that the threshold population density is fairly high. Further, selection will operate on the pathogen to reduce virulence because pathogen strains that rapidly kill the host will have less time to transmit to other hosts than the pathogens that kill the host slowly. These conclusions do not apply if there is a reservoir species which can maintain a high force of infection onto the susceptible species, even as the population density of the susceptible species declines

towards extinction (McCallum and Dobson 1995). However, it is highly likely that any potential transmissible biological control agent will need to be highly host specific to reduce the impacts on other anurans.

There are some theoretical advantages in control agents that act to reduce fecundity rather than to increase mortality. Figure 3, from McCallum and Dobson (1995) shows that a pathogen which reduces fecundity alone can have a greater impact on the equilibrium host population density than one that impacts only on survival. This figure assumes density dependent transmission dynamics. The reason for this pattern is simply that a sterilised infected host remains in the population and able to infect other hosts, whereas a host that is killed is removed from the population. This also has implications for the evolution of resistance. In a homogeneously mixing population, a pathogen strain that reduces fecundity is not at a selective disadvantage relative to one that does not affect fecundity, whereas a pathogen strain that decreases survivorship is at a selective disadvantage relative to the wild type. In a spatially structured population, this conclusion is not quite as clear cut, because a sterilising pathogen strain will locally be surrounded by a lower population density than a wild type pathogen (Hood *et al.* 2000), which places the sterilising strain at a selective disadvantage. Selection on the hosts should operate to produce reduced susceptibility to a sterilising control agent, just as it should towards a lethal control agent. Nevertheless, it would be expected that natural selection should cause a less rapid decrease in the efficiency of biological control for a sterilising biological control than for a lethal control.

Figure 3: Effect of a pathogen on host population density as a function of effect on host mortality and effect on fecundity. From McCallum & Dobson (1995).



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One general point that should perhaps be more widely recognised is that a strain of an existing pathogen that was engineered to reduce the effects on host mortality, but to increase the impact on host fecundity, could both be capable of replacing the existing pathogen and have a greater impact on the population size than the existing pathogen.

Sterile male release

Sterile male release is a technique that is well accepted for control of insect pests (Knipling 1979). It relies on releasing large numbers of sterile males, relative to the number of fertile males in the population, with the objective of producing a high frequency of unsuccessful matings. It has been used with most success on initial invasions. Without doing formal modelling, there are several features of cane toad biology which are likely to be problematical when considering sterile male release. First, for almost all the insects for which it has been successfully used, it is the females that cause the economic damage. This means that adding large numbers of males to the population does not have any negative consequences. Second, the insects are usually very short lived so that the large number of sterile males released in any given year does not increase the average population size in the long-term. Neither of these conditions applies to cane toads. Males are likely to cause just as much environmental damage as females and any sterile animals released would persist in the population for some time.

There appears to be one example in which the technique has been tried for a vertebrate pest species. The Great Lakes in North America have substantial problems with parasitic lampreys, which entered the Lakes following the opening of the St Lawrence Seaway. Sterile male release, using chemically sterilized lampreys that have been captured from the wild, has been explored for the last 30 years (Bergstedt *et al.* 2003, Twohey *et al.* 2003, Bergstedt and Twohey 2005), with some, but limited, success. A trial in Lake Superior ran from 1987-1997, but was discontinued when it failed to produce any impact on lamprey populations. A second trial in St Mary's River, which produces most of the lampreys in Lake Huron, has demonstrated an impact on lamprey recruitment, but as yet, no clear impact on adult lamprey populations or lamprey damage to fish stocks.

The lamprey project operates under a conceptual framework of a hierarchy of nine hypotheses or preconditions, all of which must be satisfied for successful lamprey control. They can be paraphrased for cane toads as follows:

H1 : Male cane toads are successfully sterilized.

H2 : Sterilized males can be successfully released to mix with normal males.

H3 : Sterilized males compete for and successfully mate with females in the field, equivalent to normal males.

H4 : Sterility reduces tadpole production in individual spawnings.

H5 : Tadpole production is reduced in individual water bodies or cane toad habitats.

H6 : Production of metamorphs is reduced in individual water bodies or cane toad habitats.

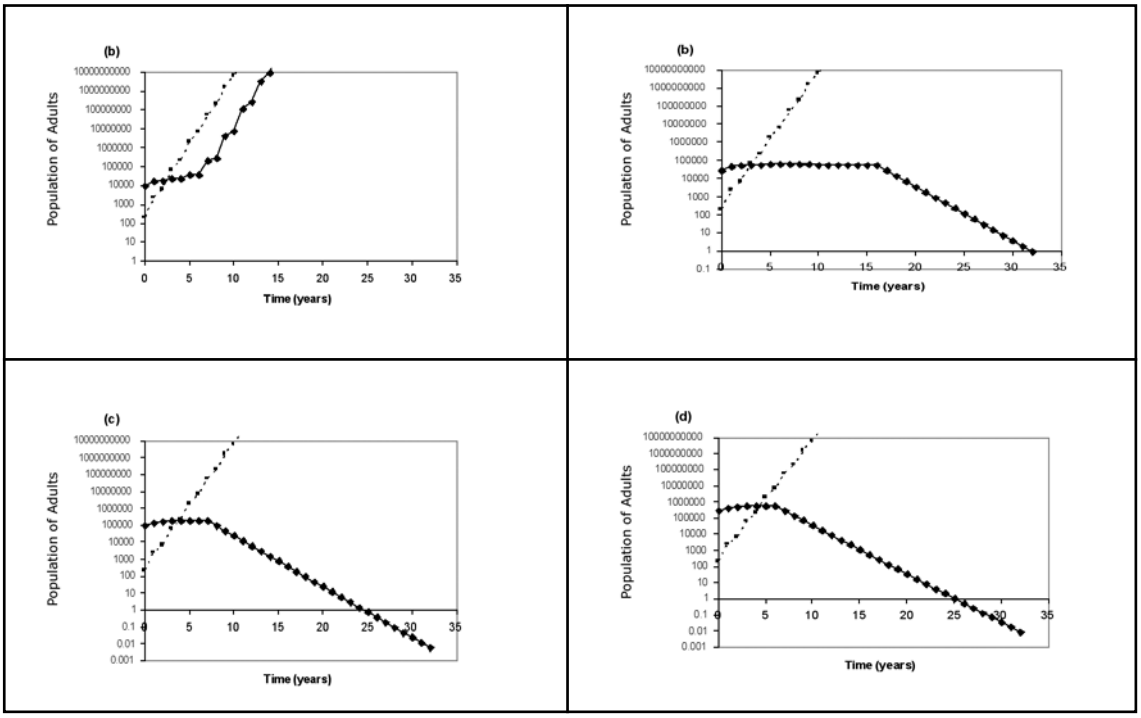
H7 : Adult cane toad recruitment is reduced in individual water bodies or cane toad habitats.

H8 : Cane toad populations are reduced on a regional basis.

H9 : Damage to biodiversity etc from cane toads is reduced.

Figure 4 shows the results of some preliminary modeling of sterile male release. The model is adapted from one of Lampo & De Leo (1998) and uses the parameter values they estimated for northern Australia. The model divides the toad population into juvenile and adult stages and assumes that metamorphosis occurs in one year from egg laying, but toads do not become reproductive until year 2. In this version of the model, I have omitted any density-dependence.

Figure 4: A simple stage-structured model of control of recently-established cane toad population by sterile male release, based on Lampo & De Leo (1998). Parameter values are: clutch size, 15,000; egg survival, 72%; tadpole survival to metamorphosis, 5%; juvenile survival 10%; annual adult survival 50%. Population size before control is 200 adults (a) 10,000 males released in year 0 and subsequent years, until female population <50; (b) 30,000 males released. (c) 100,000 males released; (d) 300,000 males released



The model shows that sterile male release can achieve control, but (with these parameters) only if about 300 times more sterile adult males are released each year as there are toads present at the start of the control program. The usual rule of thumb for sterile male release is that control requires a ratio of sterile:fertile males equal to the finite (per generation) rate of increase (Knipling 1979, Klassen *et al.* 2004). For the parameter values in this model, the finite rate of increase is about 30. However, the control can only begin to work once the recruits from the year in which control began reach maturity. This puts a one year delay into the system. Coupled with the exponential increase of toads and the fact that adults may survive several years, the delay means that the number of sterile toads that must be released is increased by a factor of about 10.

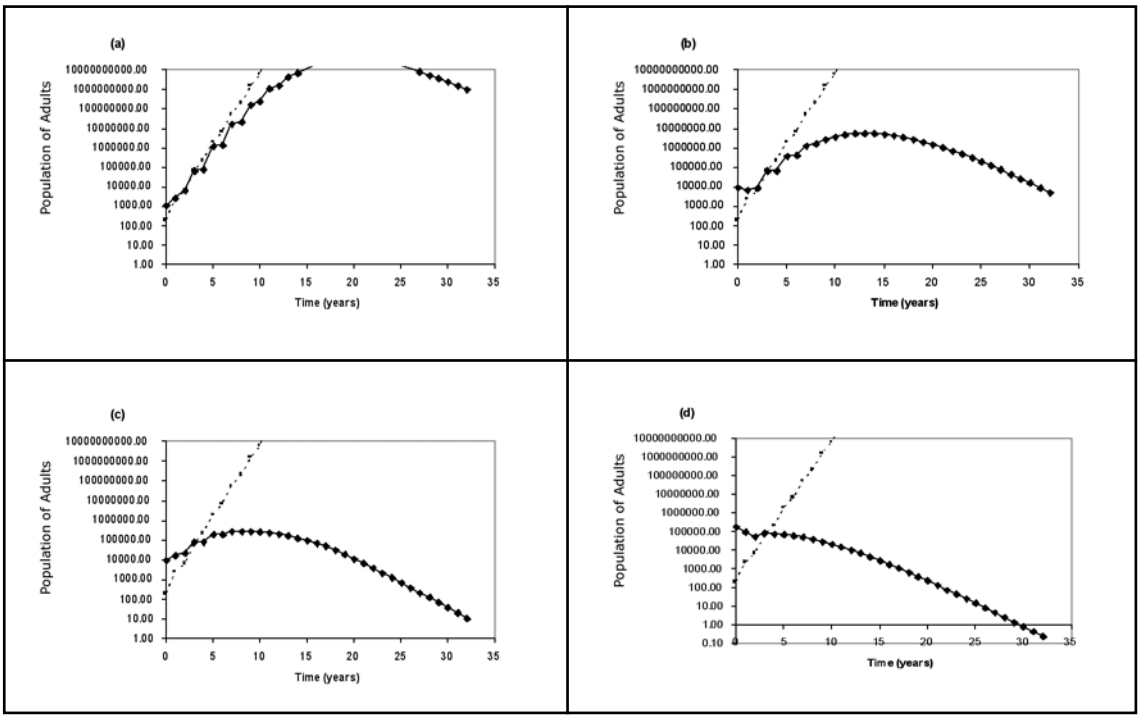
The initial stages of sterile male release will therefore increase the toad population by at least two orders of magnitude. Whether this would be acceptable is debatable. With these parameter values, the controlled population could be reduced to below the population size that would be reached without control after about 4 years, but would not be reduced to below the starting point until about 20 years after control commenced. Interestingly, too many sterile males released actually increases the time until control is established.

“Daughterless males”

Daughterless male technology has been suggested as a possible control mechanism for European carp populations in Australia (Davis *et al.* 1999, Brown and Walker 2004). In principle, the approach is quite similar to sterile male release: the objective is to have a high proportion of matings producing offspring with zero fitness. The main difference is that, with sterile male release, large numbers of sterile individuals need to be released, which subsequently produce no offspring in the F1 generation. In the case of daughterless males, they do produce offspring in the F1 and subsequent generations, but as these are all male, their long-term fitness will be zero. The benefit of this approach relative to sterile male release is that it may mean fewer individuals need to be released into the population, but an obvious cost is that control may take longer to achieve. Models of this strategy in insects suggest that it is much more efficient than sterile male release (Schliekelman *et al.* 2005). Nevertheless, in these models, control requires releasing more modified males than there are wild type males present and takes several generations to achieve. With a long-lived species such as the cane toad, this is a clear disadvantage. Further, as the daughterless gene has reduced fitness, it will inevitably be selected against. In the case of carp, an ingenious suggestion has been made that the daughterless gene should be inducible, so that it is selectively neutral until triggered by an agent of some type (Davis *et al.* 1999). For carp, this could be a substance added to the water in a given river or impoundment. Technically, chemical inducement would be harder to achieve satisfactorily in cane toads, which will inhabit a large number of disconnected water bodies in most environments. A further twist in the daughterless male approach is the notion that the inducible gene could be associated with under-dominance (Davis *et al.* 2001). This might allow the daughterless gene to become fixed or almost fixed in the population before it was induced, thus precipitating almost complete population collapse when finally triggered. A limitation of the current modelling has been undertaken in this area is that it is primarily concerned with modelling gene frequency. Driving an under-dominant allele to fixation is likely to require a large increase in the total population size in order to bring the frequency past the seperatrix.

Figure 5 shows a simple model of a daughterless male control program, which has the objective of controlling a recently-established population. For comparison with the sterile male release model, the parameter values and structure are almost identical. I have assumed that matings with daughterless males generate only further daughterless males so that the male tadpole production from a daughterless male mating is the same as total tadpole production from a normal mating. The model predicts that daughterless technology produces similar results to sterile male release, and again needs very large numbers of daughterless males released relative to the normal males present (Fig 5(a)). Comparing a continued daughterless release with a sterile release of the same size, the qualitative pattern is the same (Fig 5(b)). However, daughterless male release can work as a one-off control, although a very large release indeed is required (Figs 5(c) and (d).). This would initially increase the toad population by 3 orders of magnitude (x 1000!). Whether this would be acceptable is doubtful.

Figure 5: A simple deterministic model of daughterless male release to control a recently-established population. The model structure is as in figure 4, with the same parameter values. (a) One-off release of 1,000 daughterless males. (b) One-off release of 10,000 daughterless males. (c) Release of 10,000 daughterless males each year until adult females< 50; (d) One-off release of 200,000 daughterless males.



Discussion and future directions

Ecological theory and these simple models both emphasise that control of cane toads will not be easy. Both sterile male and daughterless male approaches can only control toad population invasions if releases that are very large relative to the current population size are employed. The early stages of a control program would therefore initially make the toad problem in the local area much worse. It is possible that sophisticated approaches with inducible genes might alleviate this problem to some extent (see Davis *et al.* 1999) but achieving adequate introgression of an inducible gene into a toad population by techniques such as under-dominance (Davis *et al.* 2001) will still require large, sustained releases of transgenic individuals.

The models in this paper of sterile male release and daughterless male release do not include density-dependence. As they are designed to consider control of initial invasions, they are intended to apply to situations before toad density has increased sufficiently for density-dependent mortality to be important. They do not address the question of reduction of population size in already-established populations. As discussed above, density dependence in tadpoles may be important even in populations at overall low density, because tadpole density in small water bodies may be locally quite high. To model control of established populations, it will be critical to consider density-dependent mortality. This will be a major future direction for this project. For the reasons discussed above, density dependent mortality is likely to make control more difficult to achieve. However, daughterless male technology may be much more effective in a closed population that is currently close to carrying capacity. A further limitation of the current work is that it does not consider spatial aspects at all. Clearly, understanding the rate of invasion across a landscape requires a spatially-explicit model.

Furthermore, there are important qualitative effects that spatial dynamics can have on the coevolution of hosts and pathogens (Carlsson-Graner and Thrall 2002, Thrall and Burdon 2003). Again, these are questions for the future.

The current models are deterministic (that is, they include no random components) and the results I have shown are based on a single set of toad demographic parameters. Future work will need to include extensive sensitivity analysis, in which the effect of varying the parameters across their plausible range is investigated. It will also be necessary to include the effects of environmental variability on the predictions of these models.

In theory, the ideal control agent would be a transmissible fertility-reducing agent. For such an agent to be able to propagate successfully through existing cane toad populations, it would either need to be a pathogen novel to cane toads in Australia or one that has a higher basic reproductive rate R0 than the wild type. An agent engineered to reduce impact on mortality but increase impact on fecundity might fulfil this condition and be able to substantially suppress toad populations. However, the technical, ethical and legal difficulties involved in releasing a genetically modified pathogen into a wild population should not be underestimated (McCallum and Hocking 2005).

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Van Rensburg, P. J. J., J. D. Skinner, and R. J. Van Aarde. 1987. Effects of feline panleucopaenia on the population characteristics of feral cats Figure 5

Control of cane toads by sterile male release and inherited sterility

Michael Mahony and John Clulow. School of Environmental and Life Sciences, The Newcastle University, University Drive, Callaghan 2308 NSW.
Phone 02 49216014
Michael.mahony@newcastle.edu.au

Abstract

We consider the possibility of biological control of the invasive cane toad by application of a sterile male release program. Our approach was initiated by two primary observations, 1) sterile male release programs (commonly called the sterile insect technique) have been successful in controlling a number of insect pests in area wide programs, and 2) sterility occurs naturally in frogs under certain circumstances indicating that there are methods to produce sterile cane toads.

Sterile male release as a form of biological control is a species specific and environmentally non-polluting method of population control that relies on the mass rearing, sterilization and release of a large number of individuals with fitness equivalent to wild type animals. Released sterile males compete for and mate with wild females, reducing their reproductive output and, ultimately, if enough sterile males are released for a sufficient length of time, eradication of the population is achieved.

Sterile adult frogs have been detected in nature and indicate that there are means by which sterility can be induced in the cane toad. Specific cases involve the occurrence of chromosomal variants in the number of haploid genome complements. The majority of animals are diploid (2n) having two of each chromosome per nuclei. In a small number of cases species have evolved by duplication of entire genomes (polyploidization) which persist because the level of duplication is even (usually 4n) and therefore meiosis is balanced. Where even numbered polyploid taxa (4n) interact with their diploid progenitors (2n) they form triploid hybrids (4n x 2n = 3n) which are sterile due to uneven chromosome numbers. In other situations rare sterile triploids occur within diploid species.

Triploids grow and develop similar to diploids and display normal fitness, however the occurrence of uneven numbered chromosome complements means that meiosis is disrupted and the animal sterile.

We have investigated the development of a genetically modified stock of cane toads that would be ideal for a male-only sterile release program. To that end we have developed a method for making sterile male via triploid and tetraploidy. We outline the methods that would be necessary to produce only sterile males for a control program.

Background

Population control by sterile male release fulfils the most basic requirements of any form of biological control – species specificity. This is because mating in most species is species specific to the males and females of that species. This means that if there is a mechanism by which the majority of males can be rendered sterile and the mating system is non-promiscuous (i.e., not polyandrous) then most matings will fail to produce offspring. An allied approach to the sterile male technique is the inherited sterile offspring technique, also referred to as delayed sterility, F_1 sterility, and partial sterility. This approach relies on a mechanism that produces fertile males whose offspring, rather than the parent, are infertile such that they would provide

a means of driving down the size of a population. These approaches operating over one and two generations rely on similar genetic constructs to the male sterility technique but are likely to have different demographic outcomes if applied in nature, i.e., delayed but also possibly amplified effects on population growth rates. The daughterless male technique is an example of this technique.

Specificity is the great strength of the sterile male technique. Unlike an introduced disease (either natural or genetically engineered) or poison that must be tested against a wide variety of native animals to ensure safety and specificity, the sterile male approach can be implemented with little risk. In the case of application to cane toads there is no requirement to test whether the technique affects native frogs or other native organisms. This in turn eliminates a considerable amount of time and cost in the deployment of the biological control agent. There is the added advantage of biosecurity, because with the sterile male approach there is no pathogen that can escape or be transported from the locality or country of release.

The release of sterile males to control pest populations in area-wide programs has been most effectively applied to insects (e.g. screwworm fly). The general approach is to swamp a population with sterile males so that the eggs of females are not fertilized. The approach relies on mass rearing and release of sterile males. The released males compete for mates with the wild males; any wild female mating with a released sterile male has no progeny and if a sufficient proportion of females produce no offspring the population declines. If sufficient sterile males can be released for a long enough period, the target population will be controlled or eradicated.

The sterile male method works most effectively in organisms that are not highly mobile, where reproduction is restricted to single pair copulation (non-promiscuous), where reproductive output is high, and the life cycle relatively short (Alphey and Andreasen 2005). 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. The cane toad has a high reproductive output (up to 40,000 eggs per mating), reproduction for the female, as far as is known, is restricted to one single partner per mating (sperm competition is not likely), and perhaps only one mating per season, and adults are relatively sedentary around established breeding sites. A male may achieve several matings in a season.

The sterile male and sterile offspring techniques, if effective, control highly fecund species, such as the cane toad, by reducing reproductive potential. In 1994 we suggested investigation of this mode of biological control for the cane toad (CSIRO Cane Toad Taskforce). Our objectives were to: 1) investigate genetic means to produce sterile male cane toads that have libidos equal to or greater than normal males, and; 2) produce male toads that confer sterility on their offspring.

Two primary observations led us to propose this approach. The first was the successful application of the sterile insect technique (SIT) in the control of insect pests. The second was the observation of sterile frogs in nature. In extremely rare events sterile frogs result from hybridisation between bisexual tetraploid and diploid species (Mahony and Robinson, 1980,Mahony 1986), and occasionally occur within diploid species (Green *et al.* 1984). Their occurrence indicated that sterility could be produced in male frogs and by extension that the technique of sterile male release was possible. We will briefly review the SIT technique and the observation of male sterility in frogs and then bring these themes together to develop the proposal that sterile male release provides a safe method for the biological control of cane toads.

Biological control and the sterile insect technique

Successful, field applied SIT programs have been conducted against the New World screwworm fly (*Cochliomyia hominivorax*)(Knipling 1985, Lindquist 1993, Wyss 2000), the Mediterranean fruit fly (*Ceratitis capitata*)(Hendrichs *et al.* 1995), and the tsetse fly (*Glossina spp*.)(Masangi *et al.* 2000). SIT has also been applied to ten species of disease vector mosquitoes in the period between 1960 and 1990 (see summary table in Benedict and Robinson 2003) and is being used, has been used, or is being developed for as many as 18 agricultural pests, mostly dipterans but also ticks and weevils (see papers in Graham *et al.* 1985). In most of these cases releases were aimed at answering specific research questions and did not anticipate immediate population suppression, and consequently many were of limited scale of release and area of application. Nonetheless several successful outcomes of suppression and eradication have been reported. Although the screwworm eradication program is the most visible and successful use of SIT, a number of other insect species have also been subjected to the release of sterile insects with varying success (Gould and Schliekelman 2004).

New possibilities for the SIT are apparent with the application of newly developed DNA recombinant technology (transgenics). Benedict and Robinson (2003) report that members of all major genera of disease vector mosquitoes can be routinely transformed genetically and they argue that SIT using transgenic material could provide an essentially safe and efficient form of widespread population control.

SIT and the New World screw-worm (*Cochliomyia hominivorax*)

The most widely reported and successful application of SIT is in the control and eradication of the New World screwworm, *Cochliomyia hominivorax*, from the United States, Mexico, Central America and also in Libya where an outbreak in 1988 was eradicated after an international effort sponsored by United Nations Food and Agriculture Organisation (FAD/IAEA 1990). The screwworm was successfully eradicated from United States in 1966. In 1972, Mexico and the US initiated a screwworm eradication program in order to establish a biological barrier farther south, covering the area from the US-Mexican border to the Isthmus of Tehuantepec with sterile screwworm flies. This area is now protected from reinvasion from South America by the release of a relatively small number of sterile flies across a narrow barrier zone (Benedict and Robinson 2003).

The screwworm is an obligate parasite of mammals, including humans. The female fly typically lays an egg mass containing 200-300 eggs on or near an open wound on a living animal and females are be capable of laying a mass of eggs once every three days for up to 10 or 11 times during their approximately month long adult life span.

In the screwworm fly the SIT takes advantage of two biological factors: 1) the male screwworm fly is sexually aggressive, and 2) the female mates only once in her lifetime. To obtain sterile males, pupae are exposed to a low dose of atomic radiation, which results in the production of aneuploid gametes from the ovaries in females and the testes in males without affecting other organs. This results in developmentally normal but sterile adult flies. Males and females can be identified and separated at the pupal stage and this means that only sterile males are released in control programs. In the field, the program disperses sterile flies where screwworm flies occur. The total number of individuals in the wild population will be reduced in the next generation if sufficient numbers of sterile males are released, so that most indigenous females are mated by them. Thus, continued releases of viable sterile males in overwhelming numbers over several consecutive generations will progressively reduce the wild population and eventually result in eradication.

Sexual sterility in insects can be induced in target pest species by various means of chemical and physical agents including alkylating agents, antimetabolites, X-rays, gamma rays and neutrons. In current practice, sexual sterility is induced with radiation emitted from radioisotopes such as caesium-137 and cobalt-60. The dosage of radiation applied must have no significant adverse effect on the males' longevity, searching behaviour and mating ability.

Genetic lethal sterility

It is pertinent to ask why SIT has not been widely applied to the control of insects, especially those with implications to human health such as the mosquito vectors of malaria, filariasis and dengue, given the outstanding success in controlling the screwworm. Unfortunately the induction of sterility, as achieved in the screwworm, by the application of a low dose of radiation during the pupal or adult stage, is not possible in all insects. For example, a dose of 5500 rad (55 Gy) is sufficient to guarantee 100% sterility of screwworm flies. However, even after doses exceeding 50,000 rad (500 Gy), some lepidopteran and coleopteran pests will still produce F1 progeny. Nevertheless, investigators have found that these F1 progeny do exhibit levels of sterility equal to or higher than those of their treated parents. It has been suggested this inherited (or delayed) sterility could be of use in control programs. Other means of causing sterility such as chemical mutagens have negative environmental complications at the release phase (Bracken and Dondale 1972). It is critical that mass produced sterile males must be able to compete for matings with wild females and that the mechanism of sterilization does not reduce male fitness. For SIT to be practical, it is also essential that a cost effective means of separating out sterile females is developed (Alphey and Andreasen 2003).

Several successful SIT applications to control insect vectors of human diseases have been reported but a widespread application has not been achieved (reviewed by Alphey and Andreasen 2003). Benedict and Robinson (2003) conclude that the failure of sterile mosquito releases was due to: 1) production of sterile males below required levels due to the absence of separate sexing; 2) loss of male fitness of sterile males; and 3) immigration into release areas. Alphey and Andreasen (2003) concur with this assessment and consider that SIT in mosquitoes could be highly effective against isolated and marginal populations, and with modest improvements could be a major weapon against insect disease vectors.

To overcome these hurdles a number of approaches have been proposed to produce large numbers of males that will be sterile or that pass on a lethal gene which renders the offspring unviable. One such approach is the development by genetic engineering of a conditional dominant lethal strain that would avoid the need for irradiation (Thomas *et al.* 2000, Alphey and Andreasen 2003). It is termed conditional because the strain can be raised in the laboratory under defined conditions such as the requirement for a specific substrate in the diet (e.g., tetracycline) which is absent in nature. Thus when males are released carrying a homozygous dominant mutant for the genetic construct they mate but all offspring die because they express the lethal gene. Thomas *et al.* (2000) termed this form of SIT "release of insects carrying a dominant lethal" (RIDL), because the insects are not sterile. They demonstrated the feasibility of RIDL in the laboratory and constructed the system in *Drosophila melanogaster*. In order to achieve the objective of releasing only males, two approaches have been developed; either a resistant factor or an induced lethal factor is constructed in females. Alphey and Andreasen (2000) developed a repressible gene expression system in which the insects are reared on a diet supplemented with a repressor chemical (e.g., tetracycline) until the final generation, at which time the repressor is withdrawn and the females die, leaving a male only population for release. They termed this approach a genetic sexing mechanism (GSM) and others have reported effective construction of such strains in *Drospohila* (Heinrich and Scott 2000). With these new developments there is renewed interest in the prospect of widespread control of insect vectors of disease.

Observations of sterility in adult amphibians found in nature

Investigations of the cytogenetics of Australian amphibians revealed that natural bisexual polyploidy has occurred among species in the largely desert distributed genus *Neobatrachus* (Mahony and Robinson 1980, Mahony and Roberts 1986). Bisexual polyploidy typically involves naturally occurring species with even ploidy levels, such as tetraploids (4n), hexaploids (6n) and decaploids (8n). Within *Neobatrachus* there are four tetraploid and five diploid taxa. Tetraploids reproduce through diploid eggs and sperm that are the result of 'normal meiosis' (Mahony 1986). Distribution of the various diploid and tetraploid taxa is complex with examples of broad sympatry, allopatry and parapatry among various species (Mahony 1986).

In several zones of parapatry, where diploid and tetraploid taxa interact, triploid individuals (3n) are detected in breeding congresses. Triploid frogs result from the hybridization of diploid and tetraploid frogs (Mahony 1986). Importantly the triploid individuals grow to maturity without apparent abnormalities and both sexes are found in breeding congresses. At one site on the Eyre Peninsula in South Australia triploid x diploid and triploid x tetraploid matings resulted in eggs masses that failed to develop. No observations of triploid x triploid crosses were made but there is no reason why they would not occur. This location has typical characteristics of a hybrid zone with the interspecific interactions restricted to a narrow ecotone and triploids are only detected in this zone. Unfortunately the zone is difficult to study because of its remote location and semiarid habitats, which mean that rainfall and thus breeding is unpredictable.

No analysis of the mating calls of the triploids was conducted, but evidence of cross matings suggests this was not a barrier. In frogs mating calls have a function of pre-mating isolation and selection, and form an important behavioural component at the time of mate selection.

Bisexual polyploidy is rare in animals; there are some 36 described cases, mostly within the amphibia (King 1990, Stock *et al.* 2002). Examples are limited to a small number of genera which have multiple polyploid species such as *Neobatrachus*. Other significant examples are within the African genus *Xenopus*, South American *Odontophyrynus*, North American *Hyla*, and Central Asian *Bufo* (Stock *et al.* 2002). Its occurrence in phylogenetically distant taxa indicates that it has evolved independently on several occasions within the amphibia.

In addition to the occurrence of even numbered polyploidy there are a small number of reports of naturally occurring triploids among diploid populations of *Rana pipiens* (Richards and Nace 1977), *Leioplema hoschtetteri* (Green *et al.* 1984), *Rana palustris* (Wiley and Bracewell 1986), and *Eupsophus vertebralis* (Formas, 1994). In these cases triploid individuals are rare examples within a normal diploid sexual species. Where diploid and tetraploid taxa occur sympatrically, as in *Neobatrachus*, triploids resulting from hybridization has been reported in *Phyllomedusa tetraploidea – P. distincta* (Hadda *et al.* 1994), *Hyla versicolor - H. chrysocelis* (Gerhardt *et al.* 1994), *Rana esculenta* complex (Gunther *et al.* 1979), and *Odontophyrnus americanus – O. cultripes* (Ruiz *et al.* 1980) and *Bufo viridis* (Stock *et al.* 2002).

Knowledge of the origin of naturally occurring bisexual polyploidy is significant in the current context of understanding sterile triploidy. Investigations indicate that in some cases the origin of polyploidy is related to hybridity and the two diploid parental taxa can be identified by karyology. These cases are classed as allopolyploids, and examples are the various polyploid species of *Xenopus* (Tymowska *et al.* 2000). In other cases the parental taxa cannot be identified by karyology and the mechanism of origin may involve duplication of the genome within a species.

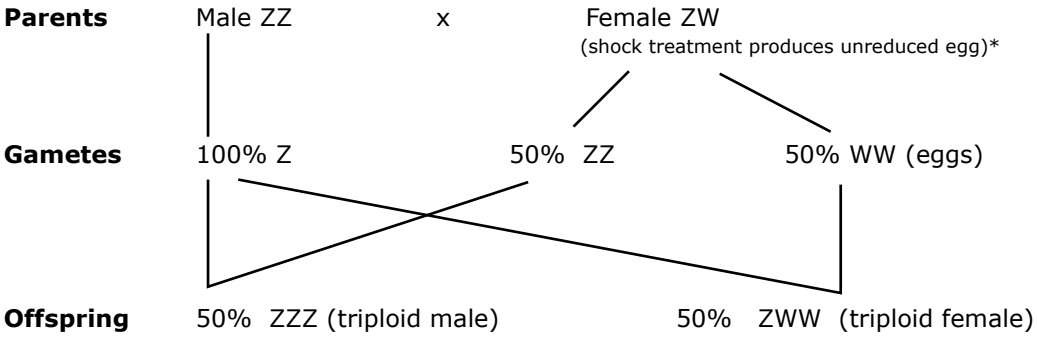
These are classed as autopolyploids. There is a significant cytological difference between these two classes which is pertinent to considerations here. At meiosis I in allopolyploids bivalent pairs are formed between the homologous chromosomes from the parental taxa, and reductional division at meiosis I proceeds as in diploid individuals. In autotpolyploids, multivalents and bivalents form, with the implication that the multiple copies of the chromosomes are homologues. Crossing over between homologous chromosomes occurs at pachytene of prophase and is terminalised during metaphase in the amphibia, and the formation of even numbered multivalents and bivalents results in equally balanced chromosome division at meiosis I and therefore also at meiosis II. If an uneven number of chromosomes are involved in synapsis at pachytene of meiosis I the process is either aborted or the products are genetically unbalanced, and this is what occurs in triploids despite the fact that the chromosomes are homologous.

The combination of genetic sterility with normal libido provides a critical component of the potential for biological control via triploidy. Cytological investigations of spermatogenesis in triploid adult males, collected from nature, resulting from hybridisation between *N. pictus* (2n) x *N. centralis* (4n) revealed that synapsis at pachytene of meiosis I was disrupted, uneven products resulted and although some cells proceeded to meiosis II spermatozoon were deformed (Mahony 1986). Histological examination of the testes of triploids confirmed the presence of functional Sertoli and Leydig cells and interstitial tissue. This indicates that as with all other somatic tissues of the animal, which divide by mitosis where there is no stage of synapsis between homologues, cell division and growth can proceed without disruption. Cells within the interstitial tissue of the testis are responsible for the production of the male hormone testosterone and are directly related to the libido of the animal. These cytological observations support those of the behaviour and morphology of the triploid animals. Triploid individuals are active in breeding congresses; they have secondary sexual morphological characters (nuptial pads) and behaviour (male calling).

Artificial methods to produce triploids

Production of sterile triploid frogs was first reported in studies investigating the outcome of hybridisation in crosses among diploid species of *Xenopus* (Muller 1977). These examples demonstrate a means by which allotriploids could be produced and the genetic mechanism involved. Subsequent studies found that by preventing the second division of meiosis by chemical means (use of Cytochaslin B to disrupt the meiotic spindle fibres)(Allen and Stanley 1979), or the use of various shock treatments (Kondo and Kashiwagi 2004) to prevent the extrusion of the second polar body at the time of fertilization of an oocyte, it was possible to retain a diploid maternal contribution that when combined with a haploid sperm resulted in triploid offspring (Kawamura *et al.* 1983, Purdom 1983). Genetically, the diploid oocyte is homozygous since the chromosomes are derived from the non-reduction of the haploid chromosome complement at meiosis II. Once fertilized by a haploid sperm the result is an autotriploid (see Figure 1). We predict that these individuals will be viable individuals similar to the triploids observed within *Neobatrachus* (see above).

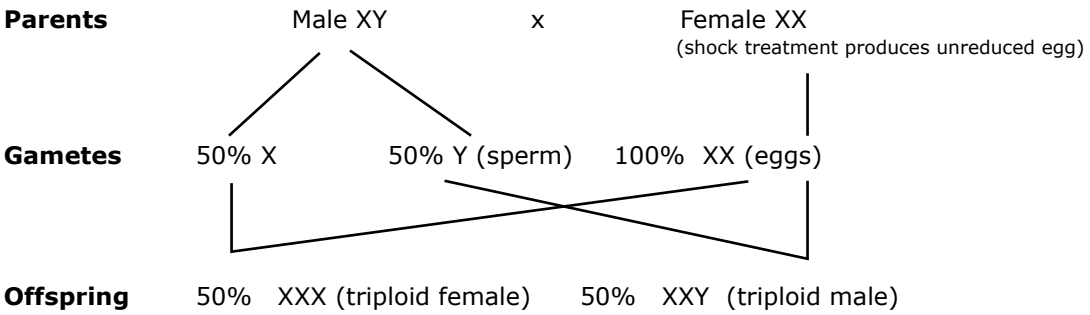
Figure 1a: Production of triploid males by using shock treatment to prevent diakinesis at meiosis II (assuming the female is the heterogametic sex, dominant – W mechanism)



50% of the offspring would be triploid males and 50% triploid females. All would be sterile

*(this assumes the shock treatment prevents diakinesis of meiosis II and the egg remains diploid)

Figure 1b: Production of triploid males by using shock treatment to prevent diakinesis at meiosis II (assuming the male is the heterogametic sex, dominant – Y mechanism)



Producing triploids by physical shock treatment of oocytes at fertilization has been reported in a number of amphibians. Shock treatment usually involves sudden temperature or pressure changes (see Nishioka & Ueda, 1983, and reference therein). This effectively produces a diploid egg; with the incorporation of the sperm nucleus the zygote will be triploid. We have successfully used cold temperature shock to produce triploid cane toads. This method as we employ it is not optimal and the use of pressure treatment, as used on a large scale in the aquaculture industry, may be more effective.

Mature triploids have been obtained in numerous urodeles and anurans (see Kashiwagi, 1993, for a review). For example in three species of *Hyla* and four species of *Rana*, triploids were obtained by exposing eggs to low temperatures of 0 – 2°C for two hours, 20 minutes after insemination (Nishioka1972, Nishioka & Ueda 1983, Kawamura 1951a,b, Kawamura *et al.* 1983, Kashiwagi 1993). Standard practice in the production of triploid salmon and trout is to use of hydrostatic pressure for a period of two hours, thirty minutes after artificial fertilisation (Purdom, 1983), but heat shock has also been successfully applied (Johnstone 1985, Purdom *et al.* 1985).

Using cold shock on artificially inseminated eggs of *Rana rugosa*, Kashiwagi (1993) produced 82% triploid offspring. The majority of these were raised to sexual maturity. No significant differences were observed between the triploids and control diploids in development and growth rate. All the triploids were male or hermaphrodites, which transformed into males, indicating that in this species the male is the heterogametic sex (dominant-Y system). IVF using sperm from eleven of these triploid males with eggs from normal diploid females resulted in 6% forming tadpoles, of which only one reached metamorphosis, i.e., they are effectively sterile. Chromosome counts revealed that the majority of the tadpoles were aneuploid.

Production of sterile triploids by crossing diploids to a tetraploid parental line

Another means of producing sterile triploids is to produce a tetraploid strain which can be backcrossed to diploids (see Figures 2 & 3), in a similar fashion to that observed in nature when tetraploids and diploids hybridise (see discussion above on hybrids in *Neobatrachus*). Kondo and Kashiwagi (2004) produced viable and fertile autotetraploid and allotetraploid frogs using diploid parental stock of *Rana nigromaculata* and *R. porosa brevipoda*.

Figure 2: Production of tetraploid individuals from normal diploids by prevention of first cleavage division in the zygote. A represents the haploid genome

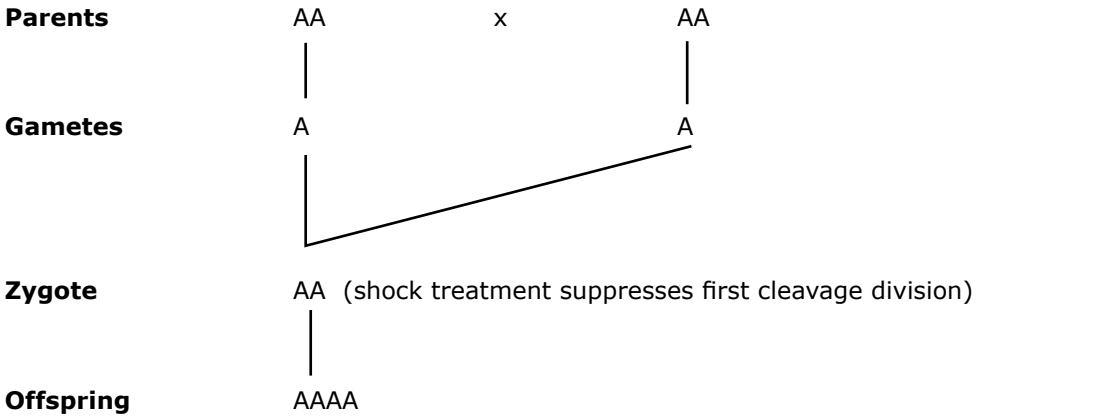
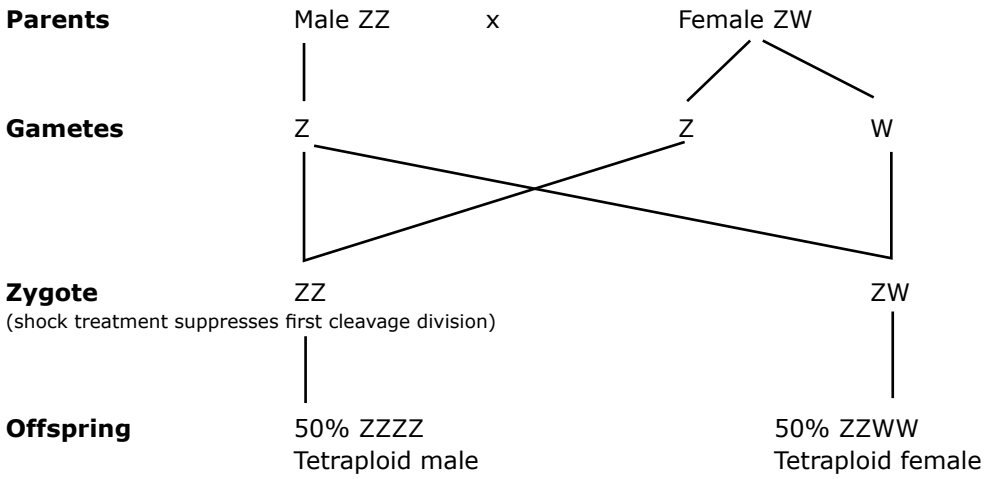


Figure 3: Sex determination in tetraploid individuals produced by prevention of first cleavage division in the zygote (assuming the female is the heterogametic sex, dominant – W mechanism)



Sex determination and polyploidy

Muller (1925) proposed that the rarity of polyploidy in animals was because polyploidization would interfere with sex determination. Whenever sex is determined by the ratio of X-chromosomes to autosomes (as in *Drosophila*), newly arisen polyploid species will produce offspring with unbalanced ratios, leading to disrupted sexual development and sterility. More commonly among animals sex is determined by the presence or absence of a dominant-Y chromosome in species with male heterogamy in XX/XY species (or dominant-W chromosome in species with female heterogamy in ZZ/ZW species)(Otto and Whitten 2000). With a dominant-Y (or W) system polyploidy is less likely to disrupt sexual development. It remains unclear why polyploidy is uncommon in the tetrapod vertebrates but it is possible that in species with heteromorphic sex chromosomes and a degenerate Y (or W) the ratio of autosomal to X gene products (dosage compensation) is disrupted (Orr 1990). This hypothesis is supported by the absence of heteromorphic sex chromosomes in those examples among amphibians where bisexual polyploid occurs (Mahony 1986).

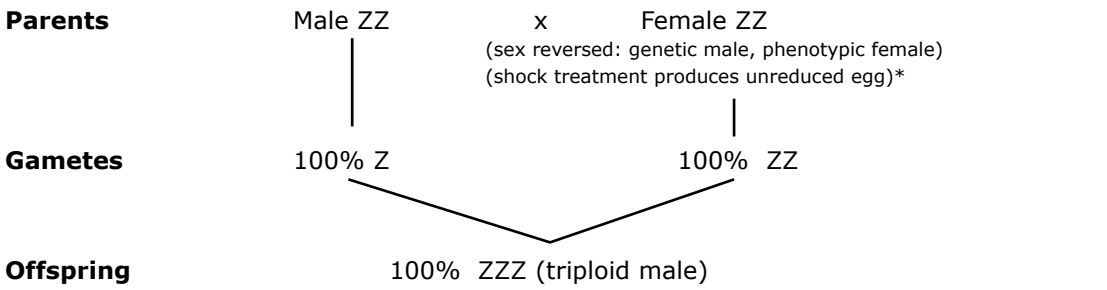
Sex determination in amphibia and implications for the production of triploid male cane toads

Knowledge of the sex determining system of the cane toad (*Bufo marinus*) would be critical to development of sterile triploids as a bio-control technique. All amphibians that have been examined possess a genetic mechanism of sex determination. Examples of male heterogamety (XX/XY) and female heterogamety (ZZ/ZW) have been reported throughout the amphibia (Schmid *et al.* 1991). Heteromorphic sex chromosomes have been identified in about 50 species, and breeding tests have established the heterogametic sex in a further 15 species where heteromorphic sex chromosomes are absent (Schmid *et al.* 1991, Mahony 1986). Hillis and Green (1990) have presented evidence based on phylogenetic analyses that the underlying ancestral pattern of genetic sex determination in amphibians involves ZW sex chromosomes. Schmid (1978) reported the karyotype of *Bufo marinus* and found that there is no heteromorphic sex chromosome pair. Thus it is not known whether *B. marinus* has male or female heterogametic sex determination. Sex reversal experiments involving the application of hormones during development have demonstrated that *Bufo variegata* has a ZZ/ZW system

(Gallien 1974). Studies using surgical techniques to reverse sex have also indicated a ZZ/ZW system in three species; *B. vulgaris* (Ponse 1941), *B. bufo* and *B. japonicus* (Ponse 1942,Muto 1952). Castrated adult males resulted in the Bidders organ (see below) being converted into a functional ovary and fertile females. In the case of castrated males (i.e., sex reversed to form a phenotypic female) all offspring in crosses to normal males produced male offspring, providing evidence of a WZ/ZZ system (dominant-W system).

Muto (1952) found that the majority of triploids raised from cold-treated or heat-treated eggs were females. Thus it is highly probably that in these species triploid females are ZZW or ZWW, and males ZZZ. Lacroix *et al.* (1990) demonstrated that triploids (ZZW) of the Urodele *Pleurodeles waltl* were female which indicates that the W chromosome has a feminizing influence and appears to be dominant. If this is also the case in *B. marinus* it will be necessary to produce a stock of sex-reversed females (genetically male ZZ, but phenotypically female) (see Figure 4). This can be achieved by surgical removal of the testes in the sexually mature male toad. The Bidders organ, which is located in the anterior part of the testes, is the incompletely involuted cortex of the embryonic gonad. It has been compared to the rudimentary ovary. Furthermore, the Mullerian duct has been conserved. When the testis is removed, the Bidder's organ develops into a functional ovary and the Mullerian duct enlarges (Schmid *et al.* 1991).

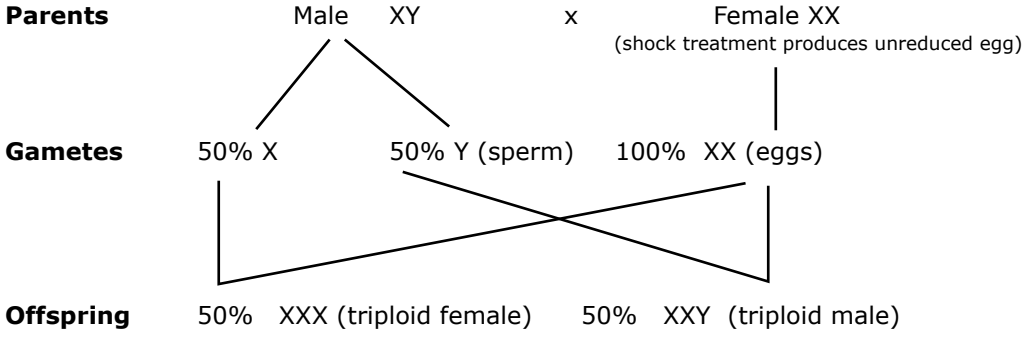
Figure 4: Production of triploid males using sex reversed females (assuming the female is the heterogametic sex, dominant – W mechanism)



All offspring would be triploid males

*(this assumes the shock treatment prevents diakinesis of meiosis II and the egg remains diploid)

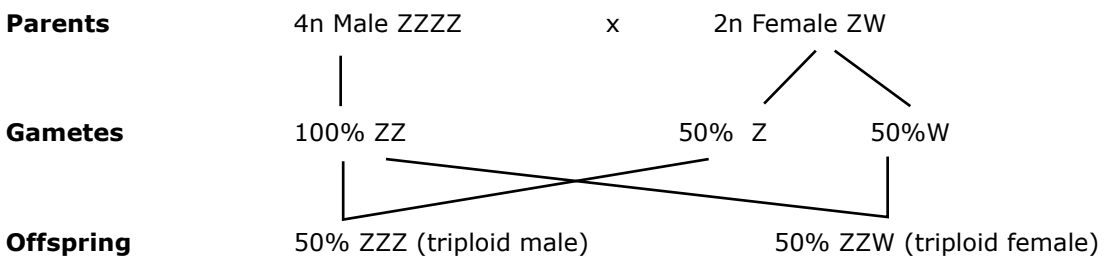
Figure 5: Production of triploid males (assuming the male is the heterogametic sex, dominant – Y mechanism)



Bufonids do not differentiate into male or female until after metamorphosis. All tadpoles develop ovaries which become compressed into a Bidders organ in front of the definitive gonad (ovary or testis) at metamorphosis (Wallace *et al.* 1999). It is the occurrence of the Bidders organ that has made possible the surgical sex reversal experiments conducted in bufonids (see Ponse 1941). Presence of the Bidders organ enables important manipulation of sex in bufonids and this could prove valuable in the development of sterile males. Thus if *B. marinus* has a ZZ/ZW sex determination system, crossing a sex reversed female with a normal male (ZZ sex reversed female x ZZ male) will produce all male offspring (see Figure 4), and if the sex determination involves a XX/XY system the outcome is 50% triploid males and 50% triploid females (see figure 5).

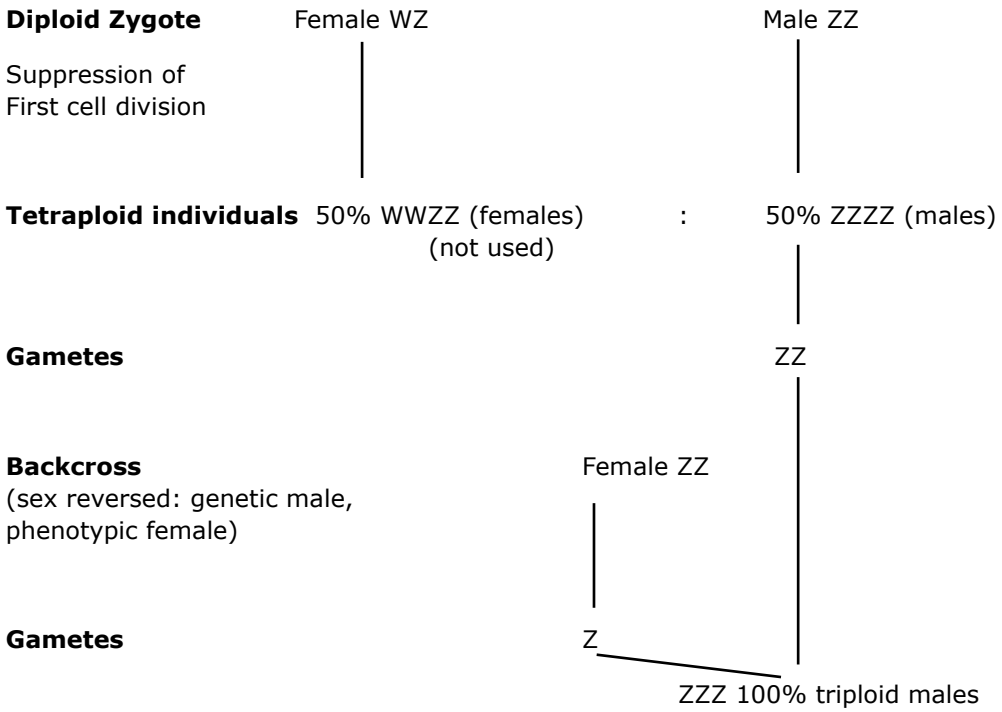
With female heterogamety (dominant-W mechanism in ZZ/ZW species) a triploid formed by suppressing meiosis II will result in 50% of the oocytes carrying ZZ and 50% WW chromosome complement. When fertilized by a sperm carrying Z the triploid offspring will be 50% ZZZ and all male and 50% WWZ which will presumably be female (see Figure 1a). If production of triploids is via a tetraploid stock of females with a chromosome complement of WZZZ, WWZZ or WZZZ the ratios of males to females in triploid offspring will vary depending on the number of W chromosomes in the adult female (see Figures 6 & 7). If the tetraploid is feminised (i.e., genetically male but phenotypically female, with ZZZZ chromosomes) the outcome will be all male triploids.

Figure 6. Outcome of releasing fertile tetraploid males into a diploid population (assuming the female is the heterogametic sex, dominant – W mechanism)



All offspring would be sterile

Figure 7: Production of triploid males by backcrossing tetraploid males with sex reversed diploid females (assuming the female is the heterogametic sex, dominant – W mechanism)



Production of sterile triploid male cane toads

In the early 1990s we investigated the possibility of producing triploid *B. marinus* and identified a number of methods where sterility with high libido might be achieved by altering the chromosomal make up of toads. Similar methods have been successfully applied in aquaculture (fish and shell fish)(Purdom 1983, Purdom *et al.* 1985, Johnstone 1985) where triploidy is used along with sex reversal to produce all female stock. A significant feature of the aquaculture success is that it shows the methods can be geared up to large-scale production that would be necessary in a control program. Our studies showed that it is possible to create triploid cane toad tadpoles whose growth and development through to metamorphosis was normal.

Our studies did not address whether the population dynamics of the toad are amenable to the sterile male approach. We adopted the position that it is first necessary to determine whether sterile males can be produced, before the question of population dynamics could be investigated with experimental trials. Furthermore, the success of SIT in insects indicated that under certain conditions sterile male release is effective in eradicating populations.

Advantages of the triploid sterile male approach for cane toads:

1. It does not involve the introduction or release of viral pathogens or a requirement for the testing of specificities of any pathogen (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).
2. It does not require a vector and the research effort necessary to identify an appropriate vector (i.e., there is no need to have a means to spread an introduced disease).
3. It does not involve the release or development of genetically engineered pathogens, or transgenic individuals.
4. There are no national or international biosecurity concerns associated with the deployment of sterile males or males that confer sterility on their offspring.
5. It does not involve the field use of toxins or poisons or the testing of specificities of any poison (i.e., it does not involve the need to test a large array of native animals to ascertain whether a poison is harmless to them).
6. The method of producing triploids does not require any harmful reagents.
7. It is humane and safe, and there are no identified impacts on native species, habitats or food chains.

Disadvantages of this approach:

1. The greatest problem for this approach is that its effectiveness is related to the density of sterile animals needed to reduce and eventually remove populations. Competition is intense in cane toad populations, particularly during the larval stage, such that a very small percentage of individuals survive to metamorphose and then reach adulthood. If the reproductive output of most of the females was neutralised by sterile males the few females to achieve normal matings might replace the reproductive potential of the others because their offspring would face reduced competition. However, this same limitation applies to all non-disseminating mechanisms of control whether it is a poison, traps, or non-disseminating viral genetically modified organisms.
2. Large numbers of sterile animals would be released into nature.

Introduction of sterile males into wild populations: two approaches

Release of sterile males

There are a range of possible scenarios in terms of approaches to testing and implementing cane road releases. It is self evident that a range of laboratory, enclosure and field trials would be necessary to test the effectiveness of the sterile male system. Nonetheless one of the strengths of the sterile male release approach is its safety, because it can be reversed simply by termination of the release program.

We consider the following basic steps necessary:

1. Large numbers of tadpoles that would grow into sterile males would be released into known breeding ponds. These would metamorphose as sterile males. The only animals released would be triploids that grow only into sterile adult males.
2. 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, thus it would be desirable to release 'sterile tadpoles' early in the breeding season.

3. The release could be at the advancing zone of the toad to act as a buffer or in areas where control was considered highly desirable, including islands and critical conservation areas.

Release of males that produce sterile offspring

Large numbers of tadpoles that grow into fertile tetraploid males that produce sterile offspring would be released into breeding ponds. The approach would be to produce tetraploid males that when mated with wild type females (diploid females) result in offspring that are triploid. Such offspring (both male and female) would be sterile and act in the manner described above.

What research is needed?

Proof of Concept:

We conducted research in 1992 with funding of $25k from the CSIRO/ANZEC Cane Toad Research Advisory Committee to determine whether sterile male cane toads with normal libido could be produced.

The research objective was to determine whether this could 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. It was recognized that to achieve the research outcome a number of questions needed to be answered.

1. *Can triploid cane toads be produced?*
   We demonstrated that using the simple method of cooling toad eggs immediately after fertilization could produce triploid toads. The technology that would be 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?*
   We have grown a small number of triploid toads 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. We have not grown young toads through to adulthood to confirm that later development is possible.
3. *Do triploid toads have normal libido?*
   If adult triploids toads can be produced (we believe they can) we would need to assess whether they have normal libido (hormone profiles and microscopic examination of testes).
4. *Are triploid adult males sterile?*
   Proof is required that any sperm produced are abnormal and incapable of fertilization and the production of viable embryos.

Another related matter that would be clarified in these studies and one that offers considerable potential for other means of biological control of toads is elucidating the means of sex determination in the cane toad. The sex determination mechanism in cane toads is not known and requires investigation. For the biocontrol method we propose it is critical that only sterile males are produced. The sex mechanism will determine the way in which sterile males are produced for release.

Is it possible to breed and produce large numbers of sterile male cane toads for release, as in the example of aquaculture? It is important to be aware that triploid salmon and trout are produced in the tens of thousands in aquaculture for release into natural waters. There are several commercial and environmental reasons that hatcheries produce these animals. The important take home message is that large numbers of sterile males can be produced on a routine basis, and sex reversed stock can be produced and maintained, because it is already done in commercial hatcheries.

Acknowledgements

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The planned eradication of cane toads off Viwa Island, Fiji

Craig Morley[1], Joape Kuruyawa[1] & Chris Denny[2]
[1]. Division of Biology, School of Biology, Chemistry and Environmental Science, University of the South Pacific
PO Box 1168, SUVA FIJI.
[2]. Pacific Invasives Initiative, SGES/TAMAKI Campus, University of Auckland, Private Bag 92019, AUCKLAND, NEW ZEALAND.

Abstract

Fiji has been identified as a hotspot in the Pacific region because of its high biological diversity and endemism. Invasive alien species (IAS) are known to have a negative ecological and economic impact, particularly on islands. To help restore the biodiversity on Viwa Island, Fiji, it is planned to eradicate several invasive species (cane toads, Pacific rats, feral cats and feral dogs). This project will also have numerous socio-economic benefits for the people of Viwa by providing employment, improving their water supply, improving health standards, and creating ecotourism opportunities. More importantly, this project will provide a model for an effective community-based conservation management programme for the Pacific.

Results from this project have the potential to demonstrate to Fiji (and other Pacific countries) that conservation in the South Pacific is beneficial and that the eradication of IAS is achievable. The mammalian eradications will be conducted first in 2006 and the planned cane toad eradication will start in 2007. The eradication of cane toads will be a world first from a tropical island and will require the development of many different effective eradication methods and technologies.

The accessibility of Viwa Island to Suva and the University of the South Pacific (USP), coupled with the islands easy terrain and small size, makes it an ideal site for achieving the planned eradications, awareness-raising, community education and research objectives. Viwa also has the potential to become an important ecotourism destination where people can view several rare endangered species, such as the Fijian ground frog, crested iguana and ground birds. The island could also be established as Fiji's first community-based wildlife sanctuary.

Introduction

IAS are non-native organisms that cause, or have the potential to cause, harm to the environment, economies and/or human health (Clavero & Garcia-Berthou 2005; Veitch & Clout 2002). Islands are particularly vulnerable to IAS, as more species have gone extinct on islands than in any other ecosystem type in recent times (Atkinson 1985; Richman *et al.* 1988: Simberloff 1995). Fortunately, the successful eradication of invasive species, like rats (*Rattus spp*.) and cats (*Felis catus*) from islands (Towns and Broome 2003), means significant biodiversity conservation goals can now be achieved (Courchamp *et al.* 2003).

The proposal to eradicate cane toads (*Bufo marinus*) and invasive mammals (rats, feral cats, and feral dogs, *Canis familiaris*) from Viwa Island, in Fiji, is in line with the main objectives set out in the Fiji Biodiversity Strategy and Action Plan (FBSAP) on protecting and conserving Fiji's biodiversity (Anon 1999). Managing the threat posed by IAS in the FBSAP is considered a high priority because of the significant effect these pest species have on Fiji's fragile insular ecosystems. Both cane toads and rats are listed as problem species in the FBSAP but it was only after discussions in 2002 with experts that the idea to eradicate the invasive mammals and cane toads was formulated.

The planned eradications on Viwa would help protect the endangered Fijian ground frog (*Platymantis vitianus*) which is found on only four mongoose-free islands in Fiji (and a small

population recently rediscovered on Vanua Levu by Alifereti Naikatini: Morrison *et al.* 2004). Fijian ground frogs are impacted by the Pacific rat (*Rattus exulans*), feral cats and cane toads (IUCN 2004). Cane toads are extremely abundant on Viwa (population estimates suggest 250,000+; N. Thomas *unpubl. data*) and they compete with Fijian ground frogs for food, as well as preying upon juveniles and adults (Phil Bishop, *pers. comm*.). Furthermore, there is ample evidence that on other islands Pacific rats have contributed to the decline and extinction of a range of herpetological species (Towns and Broome 2003).

The removal of these invasive species is also expected to benefit a range of other species from Viwa Island. These include the banded iguana (*Brachylophus fasciatus*), Pacific boa (*Candoia bibroni*), oceanic gecko (*Gehyra oceanica*), Pacific slender-toed gecko (*Nactus pelagicus*), several skink species (*Emoia cyanura, E. impar, Lipinia noctua* and *E. concolor*) and birds including the golden dove (*Chrysoenas luteovirens*), many-coloured fruit dove (*Ptilinopus perousii*) and banded rail (*Gallirallus philippensis*).

Background

Viwa Island is relatively small (60 ha) and is approximately 30kms northeast of Suva. The island is easily traversed with many tracks developed and maintained by the local residents. Viwa has one main settlement with 25 houses and 120 people living on the island. The main source of drinking water is rainwater collected in tanks from their roofs. There are nine permanent man-made ponds on the island, but only one is fed by groundwater. These ponds are used for bathing and washing clothes but only as a last resort when rainwater is limited during the dry season. Some people also have deep wells with brackish freshwater which is also used for washing dishes and clothes.

Although researchers have worked on Viwa Island in the past (e.g. Ryan 1984) little is known about the basic biology and ecology of the Fiji ground frog although some information has been published on their natural history and general distribution (Barbour 1923; Gorham 1967; Ryan 1985; Morrison 2003). To increase the understanding of the distribution of this endangered species, USP students surveyed four islands in Fiji for the frog in 2002-3 with funding from BP Conservation (Kuruyawa *et al.* 2004). This species was also recently found on Vanua Levu in the Wasali Reserve by Alifereti Naikatini (Morrison *et al.* 2004).

A feasibility study by Morley *et al.* (2004) concluded that the eradication of cane toads (and other mammals) would be valuable and potentially feasible on Viwa Island and they recommended preparing an eradication plan. This work was coordinated by the Pacific Island Initiative (PII) with funds provided by Conservation International.

The people of Viwa have given their permission to proceed with the eradication work as a strong relationship has been established by the USP researchers over the past 4 years. In the past 3 years a new health dispensary has been constructed with money from USP, pathways across the island (for their school children) and around the village have been laid (with money from the British High Commission and labour from Raleigh International), and a new community hall has been built for the many guests they receive on the island (with money raised by the community).

Project context

This project aims to enhance the livelihoods of the people of Viwa in a several ways:

1. By improving their water reticulation. Water is a limiting factor for both the people of Viwa and cane toads. Cane toads require water for breeding and it is, therefore, necessary to stop cane toads gaining access to water while increasing the storage capacity for the villagers.

2. By reducing illnesses through improved water quality and a reduction in animal-borne diseases.
3. By providing income towards community projects (e.g. a new school boat and education costs).
4. By offering skill-sharing and youth training opportunities.
5. By creating the potential for ecotourism by setting Viwa up as a "living" community-based wildlife sanctuary.

This project presents a unique opportunity to showcase how the people of Fiji can improve their standard of living by removing cane toads and rats and restoring their natural environment. The continued support of Viwa residents is critical to the success of the project and as long as everyone is fully consulted and involved in every step of the process then this community-based conservation project will provide an excellent demonstration project.

In addition, there are a number of international, regional and national strategies, policies and plans that this project will contribute too; for example; 1) Fiji is a contracting party to the Convention on Biological Diversity (Article 8h), 2) the Fijian ground frog is listed as endangered because its extent of occurrence is less than 5,000 km², its distribution is severely fragmented, and there is continuing decline in the number of mature individuals in Fiji (IUCN 2004), 3) the island forests of Fiji have been included in the World Wildlife Fund's Global 200 list of the most outstanding examples of the world's ecosystems and, 4) Conservation Internationals Critical Ecosystem Partnership Fund Ecosystem Profile for the Polynesia-Micronesia Hotspot identifies Fiji as an important 'Hotspot'.

Stakeholders

As there are a number of stakeholders involved in this project, two Stakeholder Committees have been formed. The Resident Stakeholder Committee (RSC) is comprised of residents from Viwa, plus the project coordinator and project manager. The second committee (the Viwa Stakeholder Committee) is comprised of all the members of RSC plus the representatives of non-government organisations and Fiji government. These committees oversee all activities on Viwa and provide a contact point for each agency. Either committee can raise their concerns and issues with the project manager, and help develop solutions to achieve resolution should any conflicts arise. Each person on the committee has been selected because they are able to effectively and authoritatively communicate with the people they represent.

Project design

The primary goal of this project is to restore and protect the native biodiversity of Viwa Island and enhance the sustainability of livelihoods of men, women and children on Viwa. Our first objective is to eradicate selected invasive alien species from Viwa Island (invasive mammals and cane toads) and our second objective is to enhance the quality and sustainability of the livelihoods of Viwa residents.

The cane toad work

Although there are some projects that aim to control the spread of cane toads (e.g. Frogwatch, Australia) there appears to be no successful cane toad eradication programmes on record. Therefore, it will be necessary to adopt an adaptive research approach to develop a range of tools to achieve a successful eradication. A range of mechanical, chemical, and biological methods will be trialled and tested before a suitable methodology is selected and deployed across Viwa Island.

It is likely that some tools will be used universally to achieve the initial knock down, whereas others will be used in the closing stages of the eradication project to remove the last individuals.

For the rat eradication, a grid network of 25 x 25m has been set-up with some additional stations in areas of dense vegetation and intense rat density. These stations are individually numbered so that accurate records of rat bait take can be kept. The GPS layout of the bait stations will be in a grid-like pattern along parallel lines to ensure that the coverage is even and methodical. A good track infrastructure is important as this reduces the risk of missing bait stations during checking and allows for the data to be related to the bait stations. We aim to utilise the same infrastructure for the cane toad eradication. But instead of rat bait stations we will employ a variety of traps (see Table 1).

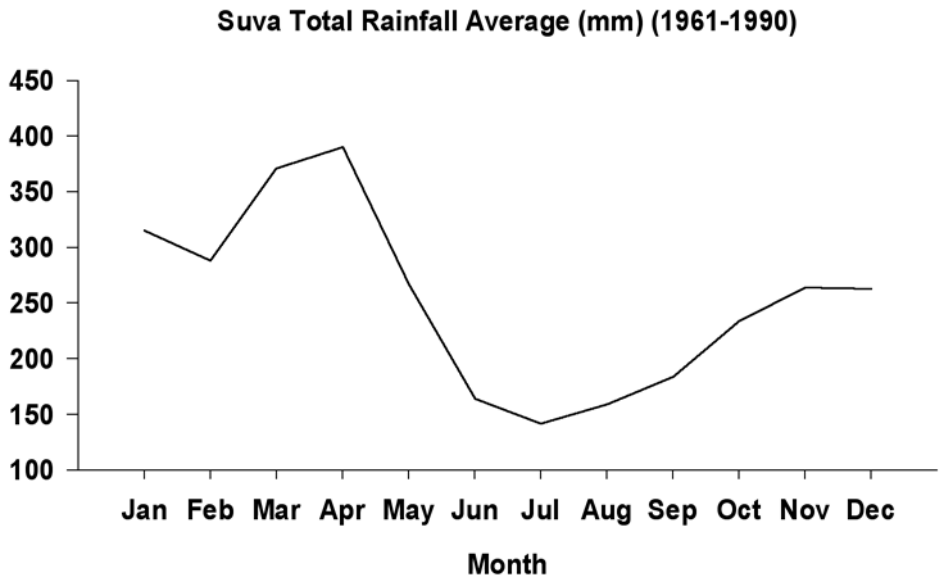
The eradication of cane toads will require multiple methods that focus on 1) the eggs, larvae and tadpoles, 2) the metamorphs and 3) the adults (Table 1). There are nine artificial water bodies on the island and these are easily accessible. Only one of these water bodies is fed by a natural spring. The water bodies contain a few native fish and invertebrate species, dominated by diadromous species that should naturally recolonise these habitats from the sea, or by aerial dispersal of adult insects.

Table 1: Planned Eradication Methodology

Target Pest	**Cane Toad eggs, larvae and tadpoles**
Method	All water bodies has been identified and mapped. The fate of these water bodies has been discussed with the people of Viwa as some waterholes will be filled in, some drained, while others will be fenced off to prevent cane toads from entering or leaving the waterholes. Rotenone (or it may be possible to use a local Fijian powder from *Derris malacensis*), will be used in water bodies.
Details	Sprinkle a known quantity of rotenone (or *D. malacensis*) in water bodies where the eggs, larvae and tadpoles of cane toads are found. NB: the later stages of cane toad tadpoles may be able to gulp air from the surface so this method may not be as effective against the older tadpoles.
Other	The rotenone poisoning work will not affect Fiji ground frogs as they are direct developers and do not require water to lay their eggs or breed.
Target Pest	**Cane toad metamorphs**
Method	Use rotenone (or similar) and/or slaked lime around the water bodies.
Details	Sprinkle rotenone and place slaked lime around the edge of all water bodies where metamorphs are found.
Other	Metamorphs are generally found close to water once they leave it (within 5 metres). Further work on metamorph movement is being investigated by Dr. T. Markwell (USP).
Target Pest	**Cane toad adults**
Method	A variety of traps will be deployed along the grid network and in high density toad sites. The types of traps that will be deployed are: water, light, acoustic, invertebrate bait, drift-nets, and pitfall traps. All remaining water bodies will be fenced off. All toads will be collected by hand. Although the collection of cane toads will be continual, we predict the greatest chance of capturing the toads will be during the dry season when the toads are forced to rehydrate every 3-4 days. Therefore, the greatest trapping and collection effort will occur during the dry season which is June-September (see Figure 1).

Table 1: Planned Eradication Methodology (continued)	
Trap details	All traps will be made from lightweight portable materials so they can be used in a range of terrains. The traps will be fairly substantial to help contain a large number of toads, be inexpensive, and must be useable in the wet season. Each trap will be labelled the number and size of cane toads captured per trap will be recorded.
Fence details	The ponds too large to drain will be fenced. The drift-net fences will be 50 cm high with the top 10 cm of the barrier hanging to the outside to prevent cane toads climbing the barrier (as used by the Townsville Frog Society to stop toads getting into frog ponds). The fencing material will be buried into the ground (20 cm) to prevent the toads from burrowing in or out of the ponds
Collection	Eradication team workers will collect the adult cane toads daily by hand using gloves. We are also investigating the use of muzzled cane toad dogs to detect remaining adults at low densities.
Euthanasia	All live toads collected will be killed humanely. The recommended method by most animal ethics committees is by concussion of the brain by striking the cranium, followed by pithing, decapitation or maceration.

Figure 1: Suva total monthly rainfall average (mm) from 1961-1990 (from Fiji Meteorological Service).



Conservation outcomes

Many other organisms should benefit from this project and they include the banded iguana (*Brachylophus fasciatus*), Pacific boa (*Candoia bibroni*), oceanic gecko (*Gehyra oceanica*), Pacific slender-toed gecko (*Nactus pelagicus*), several skink species (*Emoia cyanura, E. impar*, *Lipinia noctua* and *E. concolor*) and birds including the golden dove (*Chrysoenas luteovirens*), many-coloured fruit dove (*Ptilinopus perousii*) and banded rail (*Gallirallus philippensis*). Some of the rarer endemic species that naturally occurred on Viwa in the past could be reintroduced once the eradication work is completed (e.g. *Emoia nigra*). No native species will be at risk at a population level from this operation.

In order to assess whether the removal of the mammals and cane toads is beneficial to the islands' flora and fauna and to assess what specific changes have occurred following the eradications, several baseline studies have been undertaken. These include studies on Fijian ground frogs and cane toad densities and habitat preferences using repeated measure techniques and mark-recapture data. The ground frogs are being marked with passive integrated transponder tags while the toads are being counted along permanent transect lines.

Skinks and geckoes will be monitored using artificial shelters, pitfall traps and visual encounter survey techniques in at least 5 different microhabitats on four occasions during each year. The ground invertebrates will be collected using a motorised G-vac and Winkler sacks. G-vac samples involve collecting 10 samples within each habitat type (each sample consists of 10 sub-samples (= G-vac diameter) run for 10 seconds). Two leaf litter sub-samples (1m x 1m) from each habitat will also be scooped by hand, sorted and placed into Winkler sacks. The later method collects the larger ground invertebrates whilst the G-vac collects the smaller ground invertebrates. Four permanent quadrats (8m x 8m) have been set aside in key habitats and ten 2m x 2m x 1m high rat and cane toad exclosures (with appropriate 2m x 2m control plots) have been built to monitor any differences in plant growth.

The primary objective on Viwa is to minimise (or remove) the number of standing water bodies available for cane toad breeding, thereby disrupting their lifecycle (cane toads require water for breeding and for their eggs and tadpoles to develop). A secondary benefit is the provision of a permanent source of clean water and to provide a wastewater disposal system for the village. To date, we have commissioned a hydrology report which includes locating any potential ground water sources and natural drainage systems. We are also investigating methods to improve the drainage to minimise the number of standing water bodies. Some solutions include:

1. Clearing and channelling remaining watercourses (includes villages water issues).
2. Putting in a bore-hole and solar pump to obtain water rather than using the current standing pools of water.
3. Improve water storage capacity and collection systems (e.g. roof top rainwater) – by deploying spouting, increasing the number of water tanks and pipes.
4. Improve waste disposal systems – by developing soakage pits for water, and by constructing composting toilets that do not require water.
5. Monitor water use over a defined period to ensure there will be an adequate water supply for the villagers.

Ethical concerns

Cane toad traps may potentially catch both cane toads and Fiji ground frogs. Therefore, we will be using live traps. The Code of Practice for the Humane Killing of Animals under Schedule 1 to the *Animals (Scientific Procedures) Act New Zealand 1986* recommends the most suitable method to kill amphibians (under 1kg) is a concussion of the brain by striking the cranium, followed by pithing, decapitation or maceration in apparatus approved under appropriate slaughter legislation. A final concern is the disposal of the toad carcasses. The toads will be buried and placed into a deep pit.

Biosecurity and quarantine

Viwa is only 900 m from the shores of Viti Levu at low tide, yet mongoose (*Herpestes javanicus*), ship rats (*R. rattus*) and Norway rats (*R. norvegicus*) have not arrived in the past 100 years. This is promising for an eradication project as it signals that invasion by cane toads and rats is remote. Cane toads were deliberately introduced to Viwa by the Department of Agriculture and because of the boat traffic between the mainland and Viwa there is always a possibility of a reinvasion. Cane toads would be less likely to reinvade (as they were deliberately introduced in the past) than rats.

Biosecurity measures and controls will be imposed before any eradication work takes place. This is to prevent any reintroductions or new introductions on the island after the eradication work. This will involve border controls between the mainland and Viwa and an educational campaign. Post-eradication protocols to detect and remove any new cane toads arriving on the island will also be implemented.

Summary

This demonstration project has the potential to impress upon the Fijian people (and others) that conservation in the South Pacific is beneficial and that IAS should not be tolerated, and can be managed. With careful planning, continued consultation and organisation we believe the eradication of these invasive alien species from Viwa Island is achievable. The mammalian eradications should be straightforward and provide immediate conservation and education benefits On the other hand, a cane toad eradication has never been attempted before, and is highly experimental (i.e. the development of new control and eradication methods and technologies). We have, therefore, structured the project so that there are clear milestones and targets as progress is achieved.

The removal of cane toads from Viwa would be a world-first and a great step forward in ensuring the survival of the Fijian ground frog in Fiji. It will also have positive conservation spin-offs internationally by providing tools and shifting mindsets with regards to management of this IAS. Site accessibility will also ensure that Viwa Island will provide a powerful teaching tool to illustrate the role of IAS, and pest management biology to Fijians and students at USP. This proposal emphasises capacity building in Fiji (USP students and people on Viwa) with external assistance in research training from associated agencies in the Pacific region. The ease of access also means Viwa has the potential to become a significant ecotourism destination where people can view Fijian ground frogs, iguanas and ground birds.

Importantly, this project will raise the profile of the Fijian ground frog and conservation in Fiji. Perceiving the Fijian ground frog as a conservation icon will empower the people to take a greater responsibility for its continued preservation. Conceivably, an integrated pest eradication initiative on Viwa Island may act as the genesis for the development of many other Pacific Island community conservation projects.

Acknowledgements

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SESSION 6: TOXINS, ATTRACTANTS AND REPELLANTS

Cane toad toxin – an Achilles' heel?

Tony Robinson, CSIRO Entomology, Canberra

Abstract

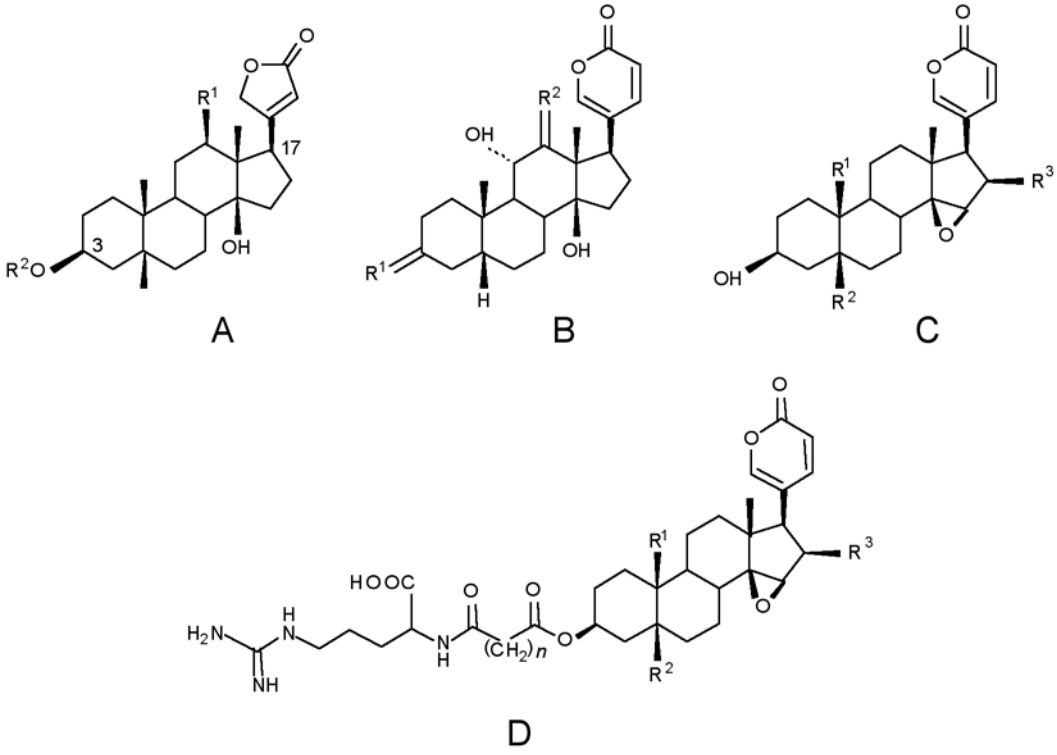
Cane toads (*Bufo marinus*) are amongst the most poisonous amphibians in the world. There has been considerable research on the nature of the toxins in *Bufo* venom. They resemble digitalis and act on the Na^+,K^+ATPase or sodium pump of the cell. Cane toads have modified their sodium pump so that they do not bind their own toxin. The molecular nature of those modifications are known and it is proposed that this knowledge could be used to design a cane toad-specific toxin.

Cane toad toxins

Cane toads (*Bufo marinus*) are amongst the most poisonous amphibians in the world (Lever 2001). They are a member of the family *Bufonidae* most members of which are toxic to some degree (Meyer & Linde 1971). Unlike the well known South American "poison dart" frogs which concentrate their toxin from their diet, the *Bufonidae* synthesise their toxins *de novo*. There are specialised glands in their skin which synthesise the toxins and in *Bufo sp* there is a concentration of these glands on the shoulders of the toad into a structure called the parotoid gland. An adult toad can contain up to 580 mg (dry weight) of "venom" in these glands which is more than sufficient to kill an average sized predator. The LD_{50} for cats for the various toxins in the secretion ranges from 0.08-4.0 mg/Kg (Chen and Kovarikova 1967).

There has been considerable research on the nature of the toxins in *Bufo* "venom" (reviewed in Meyer & Linde 1971, and Chen and Kovarikova 1967). It was recognised early on that the toxins resemble digitalis which is found in a number of plant species, notably the foxglove (Thomas *et al.* 1990). Digitalis is in fact a mixture of compounds collectively known as cardiotonic steroids or cardiac glycosides named for their well known effect on the heart. These compounds increase the strength of the heart beat (inotropic effect) and have been used, and are still used, to treat congestive heart failure. A problem with these compounds is that there is a very narrow therapeutic index and small overdoses can be fatal. The early interest in *Bufo* toxins was to see if any had value in human medicine and in particular if any had a more acceptable therapeutic index.

Figure 1: Basic chemical structures of cardiotonic steroids. A. Digitalis-like compounds, B and C. Bufadienolides, D. Bufotoxins. (From Steyn and van Heerden 1998). (These images were drawn using free shareware WINPLT v. 7.1.11.).



Bufo "venom" contains two groups of toxins, the bufadienolides or bufagins and the bufotoxins (Chen and Kovarikova 1967). These compounds are also grouped with the cardiotonic steroids and, in common with the digitalis compounds, have inotropic effects (Steyn and van Heerden 1998, Molero *et al.* 2000). They differ from the digitalis compounds in that they lack the sugar residues on C3 and also have a differently structured lactone ring on C17. The bufadienolides have an –OH group at C3 and the bufotoxins a suberylarginine chain (Figure 1). There are a range of other modifications to the steroid backbone to give the range of bufadienolides and bufotoxins that have been isolated from the various Bufo "venoms".

Na^+,K^+ATPase – the cane toad toxin target

The toxic action of these compounds is exerted by binding to a membrane bound molecule present in all cells called the sodium pump or Na^+,K^+ATPase. The Na^+,K^+ATPase is one of the main mechanisms for maintaining an appropriate Na^+ and K^+ level in cells which ensures an essential electrical potential across the membrane (Kaplan 2002, McDonough *et al.* 2002). In so doing it also influences the level of Ca^{++} in the cell which is the means by which it exerts its inotropic effect.

The Na^+,K^+ATPase is exquisitely sensitive to the cardiotonic steroids in plants and *Bufo* "venom". Sub-milligram amounts per kilogram of body weight can kill by stopping the heart and digitalis or foxglove infusions have been used as a poison for centuries. The cane toad though is insensitive to the level of toxin that is naturally circulating in its blood and is relatively resistant to much higher doses. How does it do this?

The Na^+,K^+ATPase consists of two polypeptide chains, an α- and a ß-chain. The α-chain is the larger of the two at 112kD with the ß-chain being 55kD. There are four isoforms of the α-chain recognised and two isoforms of the ß-chain (Blanco and Mercer 1998). α1 is generally distributed but the other three tend to have restricted tissue distributions. There are various combinations of the two ß-chains with the four α-chains. Resistance to cardiotonic steroids is mediated through the α-chain and in-vitro mutagenesis studies have pin-pointed the main region of resistance as being restricted to a region between amino-acids 111 to 122 (M1-M2 region) (Price and Lingrel 1998, Price *et al.* 1990, Croyle *et al.* 1997). This region is the first external loop of the molecule that winds ten times in and out through the cell membrane (Lingrel and Kuntzweiler 1994). Other parts of the molecule can influence cardiotonic steroid binding but the M1-M2 region is by far the most important.

Cane toads have modified their Na^+,K^+ATPase

Sequencing of the cane toad Na^+,K^+ATPase has shown that M1-M2 region is modified to a resistant form (Jaisser *et al.* 1992). Cane toads are not the only animals that show such a resistant phenotype. The rat α1 isoform also has a resistant phenotype (O'Brien *et al.* 1994) as do a number of insects that feed on plants that produce digitalis-like compounds (Labeyrie and Dobler 2004). Sequencing of these Na^+,K^+ATPases show that they have modified M1-M2 regions consistent with the in-vitro mutagenesis studies.

The question can be asked as to why these plant and animal toxins have evolved to bind to the Na^+,K^+ATPase M1-M2 region. It has been speculated that there are naturally occurring endogenous cardiotonic steroids that bind to and regulate the activity of the Na^+,K^+ATPase and that plants and the *Bufonidae* have evolved to exploit this regulatory mechanism. In fact such endogenous compounds have been found in humans and are thought to be produced by the adrenal gland (Mahon and McKenna 2000, Manunta and Ferrandi 2004). They are called ouabain-like compounds (ouabain is a well known plant cardiac glycoside) and one is almost indistinguishable from the cane toad toxin marinobufogenin.

A toxin for cane toads?

So how does this relate to cane toad control? One possibility could be to devise a compound that would bind to the cane toad Na^+,K^+ATPase and not to that of animals that have cardiotonic steroid sensitive pumps. There is a considerable literature on the structure/activity relationships of the cardiotonic steroids with the Na^+,K^+ATPase and computer based models of binding which could be the basis for designing a compound that would bind specifically or preferentially to the cane toad pump (Farr *et al.* 2002, Molero *et al.* 2000,Thomas *et al.* 1990). One problem with this approach could be that although the sequence of one of the main isoforms of the cane toad Na^+,K^+ATPase has been determined, there could be variation with the cane toad population which would influence the effectiveness of a synthetic toxin. This is a possibility that we are currently exploring by sampling animals from across the toad's range in Australia and Venezuela. We will also be looking at the different isoforms of the Na^+,K^+ATPase in cane toads and their tissue distribution.

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Cane toad pheromones

Michael J. Tyler, Environmental Biology, School of Earth and Environmental Sciences, University of Adelaide, S.A. 5005
michael.tyler@adelaide.edu.au

Abstract

Pheromones are either attractants or alarm signals. Widely distributed in the animal kingdom, both kinds have been reported in frogs: a sex attractant (splendiferin) in *Litoria splendida* and an alarm reaction in the tadpoles of two toads, one species of which is the cane toad. An understanding of the compounds involved may permit control to be explored.

Introduction

There is a tendency for humans to assess senses based upon their own. Thus sight and hearing are paramount. We tend to fail to recognise senses which are more refined in animals than ourselves; if anything, we denigrate them, treating them like dotty relatives who are not to be taken seriously.

The sense of smell is not well developed in humans. The flattening of the facial region due to paedomorphic arrest resulted in a reduction of the olfactory organs. We may be able to smell eggs and bacon at five metres, but many animals such as dogs are more sensitive by a factor of 1,000 to one million.

The word pheromone was coined by Karlson and Lucher to describe a chemical signal transmitted between members of the same species. The first pheromone to be described was bombykol from the female silkworm moth, *Bombyx mori*. 500,000 moths were needed to obtain sufficient material for analysis.

Amphibian pheromones

Pheromones are either attractants or repellents. The known attractants are all sex pheromones whilst the repellents are alarm pheromones. Knowledge of both in anurans is extremely limited. The first peptide sex pheromones in the Amphibia were reported in the aquatic newt *Cynops pyrrhogaster* by Kazutoshi *et al.* (1995) and were named sodefrin. Then Kikuyama *et al.* (2000) reported silefrin from C. *ensicauda*, and Rollman *et al.* (1999) described a protein sex pheromone in the male of the terrestrial salamander *Plethodon jardani.*

Newts and salamanders have elaborate courtship and mating behaviour in which it is probable that pheromones have a significant role. The nudging of areas of glandular skin against the partner suggests the transfer of secretions.

The first report of a pheromone in frogs was by Wabnitz *et al.* (1999) who identified a compound in the male of the Australian Hylid species, the Magnificent Tree Frog *Litoria splendida*. They named this polypeptide splendiferin. This compound attracts females at concentrations of 10^{-11} to 10^{-9} Molar. Previously the male advertisement call was assumed to be the only sex attractant in frogs. The existence of a pheromone is unlikely to be confined to one species. Wabnitz *et al.* (1999) demonstrated that splendiferin is species-specific; it has no effect upon the closely related species *L. caerulea*. Good candidates for pheromone communication are species lacking vocal sacs and having very soft calls.

Alarm pheromones are poorly understood and have not been identified. They were first discovered in a small European fish: the minnow. The slightest injury to a fish in a school results in evidence of mass fright in the remaining individuals. Specific cells in the skin of the minnow have been identified as the source.

Alarm reactions have been observed in tadpoles of both *Bufo bufo* and the cane toad. These species form dense shoals and the introduction into the shoal of an extract derived from a tadpole produces a fright reaction in which the shoal disperses, and the individual members rush around in a frenzy. It is highly likely that this reaction is caused by a peptide.

It has been demonstrated by Wabnitz *et al.* (1998) that host defence peptides have been found in tadpoles of *L. splendida* from early stages of their development. Distinct granular glands have been detected at Gosner (1960) tadpole developmental stage 39. They are in the form of basophilic cells which later differentiate into flattened cells surrounding a cavity filled with eosinophilic granules (Walters, Wabnitz, Tyler & Bowie, unpublished).

With the availability of greatly improved analytical resources, and the capacity to detect compounds at a much higher resolution than formerly, there is a good chance of identifying the alarm pheromone of cane toad tadpoles. If there is a sex pheromone emitted by adults it, too should be detectable. Thus the possibility of interfering with the normal development of the species exists. Pheromones have been harnessed to congregate lampreys to permit their destruction. A similar use for amphibian pheromones should be expected.

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Increasing the effectiveness of toad traps: olfactory and acoustic attractants

Lin Schwarzkopf, Ross A. Alford
School of Tropical Biology, James Cook University, Townsville, Queensland 4811

Abstract

Trapping may be a useful control technique for cane toads, especially if included as part of an integrated pest management scheme. However, trapping must be very effective to reduce toad populations. Therefore, techniques increasing the number of toads trapped are worth investigating. We conducted experiments to determine whether olfactory or acoustic attractants could be used to enhance trap effectiveness. In a Y-maze, toads were not attracted to toads of the same or the opposite sex when given a choice between toad scent and no scent. However, they were attracted to members of the same sex when choosing between toads of both sexes, and were repelled by dog food. Toads were not attracted to the scent of pond water. These results indicate that toads can use olfactory cues to orient themselves, but that substantial additional work is needed to determine whether any olfactory cue will act as an effective attractant. In an experimental arena, toads were attracted to quiet (40dB) toad calls, but not to loud (60dB) calls, or pink noise. The effectiveness of traps in the field was enhanced three-fold when traps were equipped with a speaker playing toad calls continuously. Acoustic attractants show promise as a method for enhancing toad trapping.

Introduction

Since its intentional introduction in Australia in the early 1930s as a biological control agent, the cane toad (*Bufo marinus*), has proven to be the most successful invasive amphibian in the world (Estoup *et al.* 2004). Over the past eighty years, cane toad populations have not only increased in density, but have expanded their intended range, and continue to spread (Alford *et al.*, this volume, Estoup *et al.* 2004, Sutherst 1995). *B. marinus* eat native species (Werren and Trenerry 1993), compete with them for resources (Crossland 1998) and are toxic to individuals that prey upon them (Phillips *et al.* 2003). Thus, cane toads are likely to threaten the health of Australian ecosystems where they occur. In spite of this threat, there is presently no coordinated control program for *B. marinus* in Australia (Baskin 2002, CSIRO 2004).

One method to reduce abundance in invasive species' populations is broad-scale trapping. Trapping programs can successfully control species in a variety of habitats (Baskin 2002). When deployed correctly, traps can reduce the number of individuals in a population, decreasing their impact on native populations and ecosystems.

Recently, The Northern Territory Conservation Commission sponsored a contest to design toad traps. They evaluated the finalists using field trapping tests. Wire cage traps with trap doors, lights attractive to insects (and possibly directly to toads), and trap doors preventing exit of trapped animals proved the most effective trap designs, catching toads and few, if any, non-target species. However, if trapping programs are to effectively control toads, they must trap a very high proportion of the population (25–40 % of the population, R. Thresher and H. McCallum, pers. comm.).

The purpose of our study was to evaluate possible methods to enhance trapping using olfactory and acoustic attractants. Adult amphibians are able to respond to olfactory cues from food (e.g., Shinn and Dole 1979), conspecifics (e.g., Waldman and Bishop 2004), and predators (Flowers and Graves 1997). Thus, if particular odours were attractive to adult cane toads, they could potentially be used to attract individuals to traps, enhancing their trappability. Surprisingly, cane toad responsiveness to the smells of conspecifics and food have not been studied, although there are reports that cane toads can find food using olfaction (e.g., Boland 2004).

Many anurans respond to the calls of conspecifics with phonotaxis (Gerhardt 1994), so cane toad calls may be attractive to toads, making them more likely to enter traps associated with calls. However, as with olfactory responses, cane toads have not been examined to determine whether they orient to or move in the direction of conspecific calls.

We experimentally examined olfactory attractants (conspecifics, food and water) as possible attractants for toads. In addition, we determined whether toad calls were attractive to toads under experimental conditions and whether they increased trapping rates in the field when used as lures.

Methods, results, and discussion

Y-maze experiments

We used Y-maze experiments to determine preferences of cane toads for scents. The Y-maze was completely enclosed, except for vents. Air was drawn through the maze, past the stimuli and towards the focal toad by means of a quiet fan. Stimuli were hidden from the focal toad by baffles that allowed air to pass but obstructed vision. The room was illuminated using an infrared light source, and toads' movements were recorded with a video camera. Stimuli were placed in their holding areas, the focal toad was placed into its holding area, and the trial began when the observer withdrew from the room and shut the door. Responses of the focal toad were recorded for 1 hr after the observer withdrew. Although some toads failed to move, most moved and made a choice in the maze within 1 hr. A choice was scored as having been made when the focal toad entered one arm of the maze. No choice was made if the focal toad failed to enter an arm, or entered both arms.

Individual toads were tested on the same night with all possible stimuli, to avoid choice biases caused by particular individuals (Table 1). Stimuli were presented in random order, and the arm of the maze in which the stimulus was presented was also randomised. The maze was cleaned thoroughly with alcohol, then rinsed with water, and dried completely between each trial.

A separate series of trials were conducted with water as the stimulus in the maze. A petri dish of fresh pond water was provided as the stimulus, placed in a random arm of the maze. Trials were otherwise conducted as described above.

Table 1. Stimuli to which toads (*Bufo marinus*) were exposed in a Y-maze.

One arm	Other arm
Empty	Empty*
Same sex	Empty
Opposite sex	Empty
Same sex	Opposite sex
Dog food	Empty
Pond Water**	Empty

NB. Arm and order of presentation of treatments were randomised
*Control to determine if individual has a side preference
**Pond water trials were conducted separately

In all treatments, males and females usually entered one arm of the maze, although males were slightly more likely to do so than were females. Neither males nor females showed any preference for the left or right arm of the maze. A significant number of males preferred to avoid males, if given the choice between a male and an empty arm, whereas a significant number of males chose to go towards a male if given the choice between a male and a female. Females also showed a tendency, approaching significance, to move toward females when given the choice between males and females, and females were significantly more likely to avoid the dog food than to move towards it. When the data for males and females were combined, both sexes preferred to move towards their own sex, and both sexes avoided dog food. Neither males, females, nor the combined data for both sexes showed any preference for pond water.

Our Y-maze data clearly indicated that adult toads could respond to scents, exhibiting clear preferences when presented with some dichotomous choices. Although other anurans are known to respond to scents (Waldman and Bishop 2004), this is the first demonstration that cane toads can use them to orient themselves.

Because cane toads do use scent to orient themselves, our experiment suggests that olfactory lures might be used to attract them into traps. However, our results also indicate that toads are not simply strongly attracted by conspecific odors under all circumstances. None of the stimuli we used repelled or attracted all the toads. The patterns of attraction or repulsion we found were context dependent. Toads moved towards their own sex when given the choice between two toads of different sexes, but avoided (males), or did not respond (females) to their own sex when given the choice between a toad of their own sex and an empty arm. This suggests that there may be a combination of unequal-strength repellents and attractants at work, and detailed studies of the chemical composition of scents associated with males and females are required to investigate this further.

When sexes were combined, toads avoided the dog food we offered. This was surprising, because people regularly note that cane toads eat pet food (e.g., Cameron 2002). Possibly, cane toads are attracted to insects that are attracted to pet food, or perhaps the particular brand of pet food we used was unacceptable to toads.

Pond water was not attractive to cane toads in the maze. While toads deprived of water do show increased activity and water-seeking behaviour (Jørgensen 1991), the toads in our experiments were not dehydrated. Since it is unlikely that most toads would be dehydrated in the field where trapping would take place, water may not be a good attractant to use in traps on its own, although it may enhance the attractiveness of food or other scents, and could be attractive to toads seeking a source of water for rehydration.

Acoustic experiments

Acoustic trials were conducted in a large (8-m diameter) circular arena with a mowed grass substrate. Dummy speakers the same size, shape and colour as real speakers, were placed at 30° intervals around the arena. When a trial commenced, one randomly chosen dummy speaker was removed and replaced with a real speaker. A cane toad call was played continuously throughout the trial. We conducted trials at two volume levels 40dB and 60dB, measured at 1 m from the speaker. Focal toads were placed in the centre of the arena in a small wire cage that was attached to a rope. The observer switched on the speaker, retreated behind the wall of the arena, and lifted the cage confining the toad using the rope. After 5 min, the observer stood and illuminated the arena with a spotlight, and recorded the location of the toad in the arena.

Toads were exposed to toad calls and pink noise at both volume levels. Pink noise is a random noise signal in which the spectrum density varies as the inverse of frequency.

Male and female toads moved distances and directions very similar to random expectations when exposed to pink noise at either volume level. Male toads moved towards calls at both the 40dB and 60dB volume levels, responding more strongly to the quieter calls. Female toads did not move significantly towards the louder (60dB) calls, but did move significantly towards the 40dB calls.

Toads responded strongly to the quiet calls in our experimental set-up. In nature, toads move towards the sound of distant choruses (Schwarzkopf and Alford 2002), and the quieter calls in our experiment may have sounded like a distant chorus. Toads may not orient towards calls when they perceive that they are very close to them in order to avoid behavioural interactions with the calling individual.

We carried out a field trapping experiment using three-door cane toad traps purchased from FrogWatch in the Northern Territory to determine whether playbacks of toad calls increases the effectiveness of traps. Traps were set in pairs, one with a playback and one without. All traps had an attached 8-watt fluorescent light, the standard means of attracting toads by attracting insect prey. Traps within a pair were separated by at least 50m to avoid attracting animals to control traps with sound from the experimental traps. Pairs of traps were separated by at least hundreds of metres to avoid any form of interference between pairs. Trapping locations were characterized by large toad populations, verified because we used these as toad collecting locations for other parts of the study.

Traps with playbacks were set-up with a small, portable speaker attached to an MP3 player positioned on top of the trap. An umbrella protected the speaker and MP3 player from rain. Traps without speakers were assembled in a similar fashion but without the speaker, MP3 player or umbrella.

Capture success in traps was relatively low, compared to our success at hand capture, but we captured 3 times as many toads in the traps equipped with toad calls than in traps without sound. This advantage of traps supplemented by playbacks was highly statistically significant. Thus, cane toad trapping could be enhanced by equipping traps with sound.

Conclusions

Cane toads responded to both olfactory and acoustic attractants. Responses to olfactory attractants were relatively weak and complex, and depended on the sex of the toad and the nature of both of the stimuli presented. Clearly, cane toads are able to respond to olfactory attractants, but more research is required to determine factors enhancing attraction. Water did not attract hydrated cane toads, and complex stimuli such as whole cane toads, and dog food, may contain combinations of attractants and repellants. Further research into sources of olfactory attraction and repulsion are required before olfactory attractants can be used in traps.

Acoustic attractants in the form of cane toad calls were clearly attractive to toads. Quiet calls (in the 40dB at 1 metre range) attracted toads in an experimental arena, and toad calls were attractive to toads in a field trial using toad traps, enhancing trapping success.

Our results have implications for control efforts. We recommend equipping traps with speakers playing toad calls to significantly enhance cane toad trapping. Enhanced toad trapping could effectively form part of an integrated control strategy for toads. Scents may also be used as attractants for toads, but more research is required to find a strongly attractive scent.

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Cane toad chemical ecology: getting to know your enemy

R. A. Hayes[1], G. C. Grigg[2] & R. J. Capon[1]
[1]Centre for Molecular Biodiversity, Institute for Molecular Bioscience, The University of Queensland, St Lucia Qld 4072
[2]School of Integrative Biology
The University of Queensland, St Lucia Qld 4072

Abstract

Surprisingly little is known about the way chemical secretions modulate behaviour in amphibians, and this is particularly so with respect to cane toads. For example, anecdotal observations and some preliminary experimental data suggest that cane toads may deploy sex, alarm or aggregation pheromones. If true, the existence of such ecologically significant cane toad chemicals would support our proposition that "…knowledge of cane toad chemical ecology will reveal potential control strategies…" To test this proposition we have recently assembled a multidisciplinary research team based at the University of Queensland, with a view to answering the following questions. (1) Can we establish methodologies capable of the qualitative and quantitative chemical analysis of ecologically significant cane toad chemicals? (2) Can we isolate, characterize and identify these chemicals? (3) Does cane toad chemistry vary between individuals, with life cycle, male vs. female, sexual maturity, season, geographic location etc…? (4) Can we establish cane toad behavioural assays, and do any cane toad chemicals display activity in these assays? (5) Can we use knowledge of cane toad chemicals to disrupt cane toad survival?

Introduction

Most animals (and indeed many plants) use chemicals (pheromones, toxins, venoms…) to improve their individual survival, and in doing so contribute to the success of their species. That is, those species that can produce or acquire key chemicals that significantly enhance survival will prosper and proliferate within an ecosystem, at the expense of competing species that are less chemically adept. The chemicals can provide advantage in the form of sex, alarm and trail pheromones, which facilitate intra-species communication for the purpose of enhancing reproduction, defence and feeding. They can also take the form of venoms or defensive secretions which can be used to improve inter-species interactions - for example - a venom that rapidly kills or immobilizes prey, or an unpleasant tasting secretion that deters (or even kills) would be predators – each in their own way facilitates the desire to feed well, and not be fed upon! The study of this diverse array of chemicals and their biological roles is encapsulated in the science of chemical ecology. Species that co-evolve within an ecosystem eventually establish a stable balance, an accord between competing chemical ecologies where prey and predators acquire, lose, and reacquire immunity to successive generations of venoms and defensive secretions – and as individual species trial and refine new pheromonal solutions. While this balance will ebb and flow as species and indeed ecosystems evolve, the peace can be shattered by the enforced relocation of an invasive species into an unfamiliar ecosystem – unaccustomed or prepared for the chemical onslaught launched by the invader. The appearance of the cane toad in Australian ecosystems may be characterised as just such an onslaught. An understanding of the chemical ecology of cane toads could offer a way to control them. As new participants in the quest to control cane toads in Australia, we bring a molecular skill set, quite different from but complementary to that which has been historically applied to this problem.

Our ability to explore and challenge the cane toad at the molecular level allows us to address our primary proposition that:

Knowledge of cane toad chemical ecology will reveal potential control strategies.

As Anuran amphibians (frogs and toads) have a pronounced calling behaviour, it has long been assumed that chemicals played little or no role in their behaviour and ecology (*e.g*. Houck and Sever 1993). Recent results have changed this view (Brizzi, *et al.* 2002, Lee and Waldman 2002, Pearl, *et al.* 2000, Wabnitz, *et al.* 1999, Waldman and Bishop 2004), and it is now clear that many, if not all, Anurans have a robust chemical ecology and use chemical signals to modulate their behaviour.

In reviewing the scientific literature on cane toad chemistry we were surprised to note that it was largely decades old, was superficial and incomplete by modern standards, and was bereft of ecological context. Furthermore, these studies were limited to narrowly defined investigations into the organic soluble defensive secretions from cane toad parotoid glands, and the appearance of related chemistry in toad egg masses – and were overwhelmingly focused on the bufadienolide class of metabolites – which are broadly represented across many animal and plant species and are known to be cardioactive toxins. For example, marinobufagin is representative of the ten bufadienolides that have been reported from adult cane toads and egg masses (Chen and Osuch 1969, Matsukawa, *et al.* 1997). The skin secretions of the toad also contain alkaloids and bioactive peptides and proteins (Heatwole 1994). The incomplete nature of this chemical knowledge begs more questions than it answers, and prompted us to formulate and seek to address a series of questions.

Question 1

Can we establish methodologies capable of the qualitative and quantitative chemical analysis of ecologically significant cane toad chemicals?

We make no assumptions on the molecular nature of cane toad chemical ecology, and require that methodologies we develop are capable of analysing small (ie bufadienolides) and large (i.e. proteins and peptides) molecules, across all biosynthetic structure classes, whether they are water or organic soluble, volatile or non-volatile, stable or non-stable, and whether they are major or very minor components. Our analytical technologies of choice are automated HPLC/DAD/ELSD/ESIMS and GC/MS, and build on our extensive experience in the analysis of molecules across all biosynthetic structure classes. Preliminary studies on parotoid gland secretions have already revealed a far more complex molecular picture than previously recognized – and hint at issues of chemical stability and interconversion post-secretion.

Question 2

Can we isolate, characterise and identify these chemicals?

Knowledge of molecular structures is a key step in understanding origin, relationship and function. Pure standards can be used to calibrate analytical procedures, to more accurately monitor variations (see question 3), and to screen for ecologically relevant properties (see question 4). Our technologies of choice are HPLC/DAD/NMR, and advanced chromatographic and spectroscopic methods.

Question 3

Does cane toad chemistry vary between individuals, with life cycle, male vs. female, sexual maturity, season, geographic location etc…?

Armed with effective chemical analysis methodologies we can address the issue of cane toad chemical plasticity. Chemicals that cycle in abundance are obvious candidates for consideration as pheromones, which need to be regulated on vs. off in order to control ecological outcomes. Similarly, we can assess whether the character of the defensive chemicals in adults vs. eggs vs. tadpoles vs metamorphs reveal a common biosynthetic origin (i.e. deposited by the adult vs. de novo biosynthesis), and whether this knowledge highlights vulnerability in the life cycle that would allow us to disrupt critical intra-species communication, and/or enhance susceptibility to predators and/or infection. Our technology of choice will be HPLC/DAD/ELSD and HPLC/MS supported by databases keyed to the chromatographic (HPLC) and spectroscopic profiles of individual samples and pure standards.

Question 4

Can we establish cane toad behavioural assays, and do any cane toad chemicals display activity in these assays?

Discussions with colleagues suggest that cane toads may deploy sex (as in Brizzi, *et al.* 2002, Pearl, *et al.* 2000, Wabnitz, *et al.* 1999), alarm (Alford 1994, Hearnden 1991) and aggregation pheromones. Also, there is anecdotal evidence that female toads select locations for egg laying that are devoid of other egg masses/tadpoles. Our preferred approach to addressing these questions is collaboration, although we are establishing a toad colony that may be suitable to support selected ecological and behavioural studies.

How exactly does cane toad venom (ie defensive secretion – see below) exert a toxic effect, and are individual chemicals more or less toxic? Can knowledge of the mode-of-action of these toxins suggest likely approaches to developing cane toad specific toxins? Are egg and tadpole chemicals more toxic than adult chemicals (Cohen and Alford 1993, Hearnden 1991)? Can we identify a sex pheromone (Brizzi, *et al.* 2002, Pearl, *et al.* 2000, Wabnitz, *et al.* 1999)? Can we identify an aggregation pheromone? Can we identify an alarm pheromone (Kraft, *et al.* 2005, Summey and Mathis 1998, Wilson, *et al.* 2005)? Can female cane toads detect chemical cues that influence choice of location for the laying of eggs, and if so can we identify the chemical(s) responsible for this effect?

Question 5

Can we use knowledge of cane toad chemicals to disrupt cane toad survival?

Clearly we will be better positioned to answer this question once we know about cane toad chemical ecology. However, as a basic principle we take the view that such knowledge will reveal potential solutions for cane toad control. Some possible outcomes include, but are not limited to:

- a synthetic sex pheromone that can be used to either disrupt mating over a wide area, and/or as a species/sex selective lure to assist trapping programs.
- a synthetic alarm pheromone that may disrupt female cane toad egg laying behaviour by chemically tagging suitable locations as "used", or that may disrupt tadpole/metamorph behaviour, leading to reduced survival.
- a synthetic aggregation pheromone that may disrupt the natural aggregation of cane toad tadpoles, leading to reduced survival.

- a synthetic chemical that may disrupt the production and/or distribution of cane toad toxins, rendering the cane toad vulnerable to predators and/or infection, leading to reduced survival.
- a synthetic chemical that is a toad selective toxin that might be used to enhance trapping and baiting programs.

Research team and progress

Our approach to studying cane toad chemical ecology has been to assemble a specialist team with a broad chemical skill base, supported by experts in ecology and biology. The team is built around four University of Queensland research groups, these being Prof Rob Capon (Project Leader, chemistry – small molecules), Prof Paul Alewood (chemistry - peptides), Prof Richard Lewis (biology – bioassays) and Prof Gordon Grigg (zoology – toad colony). Dedicated project appointments include Dr Andrew Hayes (chemical ecology), Dr Jie Zhang (peptide chemistry) and Alexis Barrett (toad ecology). The project received 2 yrs funding from the Qld Government, via a contract research agreement with the Invasive Animals CRC, and commenced operations on Feb 1st 2006. While very early days, the players are in place, and the dynamics of the research program are taking shape. The next 12 months promise to be a significant period, as we come to terms with cane toad chemistry.

As a postscript … to know your enemy is to respect your enemy

As described above, the existing knowledge base on cane toad chemistry is modest at best, and is inadequate to provide a scientific basis to support many of the truisms that seem to pervade the public psyche when it comes to cane toads. With a certainty verging almost on hysteria, some attribute cane toads with almost legendary powers to poison all in their path, including waterways. That such hyperbole infuses the public and legislative debate is unhelpful. For example, even the language used when discussing cane toads can be unnecessarily emotive. Use of the term cane toad venom conjures an image of a belligerent aggressor, poised and willing to attack, to inject, whereas in reality cane toad poisonings are merely the unfortunate demise of a would be predator encountering cane toad parotoid secretions. Even the ecological role of these secretions is unclear. While they undoubtedly contain toxic components, such as the bufadienolides, it is not certain that this is the primary role of these secretions. It may be that the toxicity of these chemicals is a by-product of secretions developed for a different purpose, such as communication, or (more likely) that these components serve a dual role. Until the molecular knowledge is gained, and combined with accurate ecological information, the most sensible terminology to use is skin secretions. We do not then presuppose a function, but allow a more measured assessment.

That we identify more closely with the demise of the native predator (quoll, lizard, snake etc…) is not surprising, but under different circumstances one might reflect on the cane toad as victim not villain – not simply because it kills native animals only in defence, and can die in the process, but because it finds itself an unwilling transportee to Australia, an invasive pest, the enemy, through no fault of its own. We look forward to contributing to the debate on, and to developing technologies that may contribute to, the control of cane toads in Australia – and to do so in a humane and respectful manner.

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SESSION 7: ADAPTATION

Cane toads and northern quolls: can quolls persist in the face of an invasive toxic onslaught?

Karen Firestone[1],[2] and Tony Robinson[3]
[1]Australasian Conservation Genetics Centre, Zoological Parks Board of New South Wales, PO Box 20, Mosman, NSW 2088 Australia
[2]School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052 Australia
[3]Invasive Vertebrate Species Group, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601 Australia

All Correspondence to: Dr Karen Firestone, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052
email: kfirestone@unsw.edu.au, ph: 02 9385 3446

Abstract

Cane toads are a large amphibian species native to Central and South America that were introduced to northern Queensland in 1935 in an attempt to control two species of native cane beetles that damage sugar cane crops. Cane toads are poisonous at all stages of their life cycle including eggs, metamorphs and adults. While cane toads were singularly unsuccessful at biocontrol of their intended target species, they nevertheless were able to establish populations, breed successfully, and colonize new areas. Northern quolls are top-order native marsupial predators with a distribution across the northern third of the Australian continent. This species has declined substantially over recent times and is now restricted to six highly disjunct and isolated populations across northern Australia and a number of offshore islands. A number of factors have been implicated in the decline of northern quolls including, increasingly, the fatal toxic ingestion of cane toads. The spread of cane toads into core northern quoll habitat in Arnhem Land and Kakadu National Park has lead to dramatic localised extinctions of northern quolls in one of the few remaining hot spots for the species. Due to the rapid decline of northern quolls in toad infested areas, the conservation status of northern quolls was reviewed and this species is now considered to be Endangered under EPBC Act 1999 and by the IUCN in the 2007 draft Red List of Threatened Species. While Northern Territory populations of northern quolls are facing severe localised extinctions, some Queensland populations appear to persist despite the long-term presence of cane toads in their environment. The mechanism for quoll persistence in the face of toad presence is unclear and may be due to one or more factors including behavioural (e.g. learned avoidance), environmental (e.g. micro-niche partitioning), biochemical (e.g. decreased toxicity in smaller toads), molecular (e.g. genetic resistance to toad toxins) or a combination of these factors. We present here a project proposal to examine the genetic factors of toad resistance in northern quolls by examining loci that are directly affected by cane toad toxins. Results of our research will have implications for the conservation management of northern quolls.

Introduction

Cane toads (*Bufo marinus*) are a large amphibian species native to Central and South America, with an average adult size of 10-15cm and weight between 500 to 800g (Lever 2001). They were introduced to northern Queensland in 1935 in an attempt to control two species of native cane beetles that damage sugar cane crops (McLeod 2004).

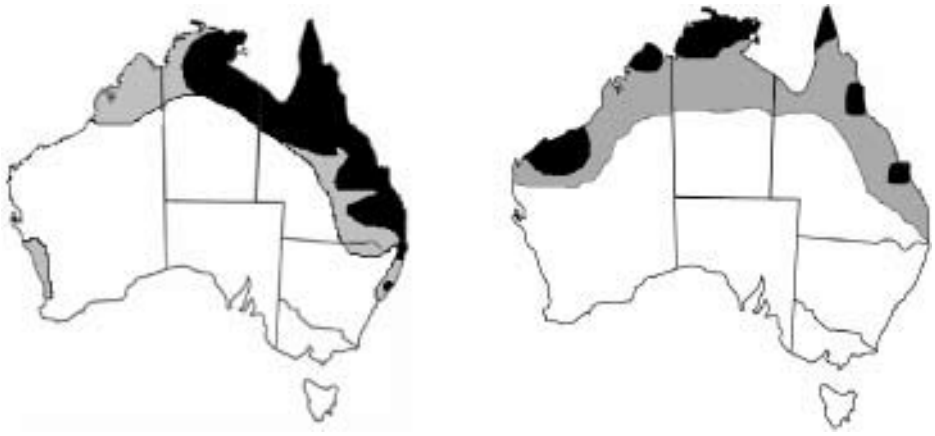
Cane toads are poisonous at all stages of their life cycle including eggs, metamorphs and adults (Lever 2001). Adult cane toads possess prominent parotoid glands at the base of the skull behind the ears. These glands exude a toxic milky substance containing a cocktail of 27 bufadienolides and 9 bufotoxins (Chen and Kovarikova 1967). Bufotoxins and bufadienolides are small, fat soluble, easily absorbed, and very fast-acting compounds which diffuse rapidly into the body. They act as animal analogues of the plant compounds digoxin (one of the compounds of digitalis) and ouabain, and affect heart function as cardiotonic glycosides. The transmembrane Na^+/K^+-ATPase enzyme is the primary binding site for these compounds (Hansen 1984, Thomas *et al.* 1990). When ingested, toad toxins block cellular functioning of the Na^+/K^+-ATPase pump (which regulates the electrical potential across cell membranes) and cause cellular- and ultimately whole-animal death, often within minutes (Covacevich and Archer 1975).

While cane toads were singularly unsuccessful at biocontrol of their intended target species, they nevertheless were able to establish populations, breed successfully, and colonize new areas. By 1983 cane toads had crossed the border into the Northern Territory and by January 2001 they had reached Kakadu National Park (van Dam *et al.* 2002). The species continues to spread west at a rate of up to 30 km per year, and though their spread southward is slower, it is predicted to eventually occupy approximately 2 million km^2 throughout northern and eastern Australia (Sutherst *et al.* 1995, Figure 1a).

Cane toads are currently 270 km east from the Victoria River in the Northern Territory (G. Graham, CALM, pers. com.) and it is predicted that they will cross the border into Western Australia in perhaps as little as two to three years. In 2005, cane toads were listed as a Key Threatening Process under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act 1999) (TSSC 2005a).

Figure 1:

A) Current (black) and predicted (grey) range of cane toads (*Bufo marinus*) (modified from WA Department of Agriculture website).
B) current (black) and past (grey) distribution of northern quolls (*Dasyurus hallucatus*) (modified from Strahan 1998).



A wide range of native species including reptiles, birds and mammals are adversely affected by the presence of cane toads in their environment (Covacevich and Archer 1975, Burnett 1997, Watson and Woinarski 2002). We intend to use a carnivorous marsupial, the northern quoll (*Dasyurus hallucatus*) as our model species to examine the genetic effects of toads on carnivores. Northern quolls are top-order native marsupial predators with a distribution across the northern third of the Australian continent (Figure 1b). This species has declined substantially over recent times (Braithwaite and Griffiths 1994) and is now restricted to six highly disjunct and isolated populations centred on the Pilbara and Kimberley regions in Western Australia, the Top End in the Northern Territory, and Cape York Peninsula, the Atherton Tableland, and Carnarvon Range in Queensland (Strahan 1998). Northern quolls are also found on a number of small offshore islands.

A number of factors have been implicated in the decline of northern quolls including predation and competition by introduced carnivores, altered fire regimes, loss of habitat, and, increasingly, the fatal toxic ingestion of poisonous cane toads. Ingestion of toads by quolls leads to direct poisoning of individuals as well as extreme localised population extinctions. The spread of cane toads into core northern quoll habitat in Arnhem Land and Kakadu National Park has lead to dramatic extinctions of northern quolls in one of the few remaining hot spots for the species (Watson and Woinarski 2002, Oakwood 2004), and northern quolls are considered to be the species most severely affected by cane toads.

Due to the observed rapid decline of northern quolls in toad infested areas, the conservation status of northern quolls was recently reviewed and this species is now considered to be Endangered under both the EPBC Act 1999 (TSSC 2005*b*) and by the IUCN 2007 draft Red List of Threatened Species, and Vulnerable by the Northern Territory state government. In 2003, populations of northern quolls were established as a safeguard measure on two offshore islands (Pobassoo and Astell Islands in the English Company Island group) through translocation programs coordinated by the Northern Territory's Department of Natural Resources, Environment and the Arts (NRETA; formerly the Department of Infrastructure, Planning and Environment) and local traditional owners of the islands.

While Northern Territory populations of northern quolls are facing severe localised extinctions, some Queensland populations appear to persist despite the long-term presence of cane toads in their environment. Notably, populations in the Cape York Peninsula, the Atherton Tableland area, and the Carnarvon Range appear to have declined, but not succumbed, though populations also do not appear to have recovered. Indeed, in some areas (inland from Mackay, Queensland), northern quolls and cane toads are readily trapped within a few meters of one another along the same trap lines (A. Dinwoodie, QPWS, pers. com.). Cane toads have not yet reached core northern quoll areas in Western Australia, but it is predicted that the range of toads will encompass part of this area.

The functional mechanism for quoll persistence in the face of toad presence is unclear at present and may be due to one or more factors including behavioural (e.g. learned avoidance), environmental (e.g. micro-niche partitioning), biochemical (e.g. decreased toxicity in smaller toads), molecular (e.g. genetic resistance to toad toxins) or a combination of these factors.

We intend to examine the genetic factors of toad resistance in northern quolls and will focus our efforts on the genes that have been shown to be related to ouabain-like susceptibility or resistance, i.e. the Na^+/K^+-ATPase. The Na^+/K^+-ATPase, also known as the sodium pump, is a transmembrane protein found in the cells of all higher eukaryotes and is the main mechanism for maintaining appropriate Na^+ and K^+ ions levels within the cell. The Na^+/K^+-ATPase molecule is composed of two subunits, α and β, with a third, tissue-specific γ subunit being associated with the α-chain. It is the α subunit that acts as the receptor for the cardiotonic steroids such as digoxin, ouabain, and the bufadienolides and bufotoxins found in cane toads. In rats and humans there are four genes for the α-chain and four for the β-chain and, although they have different tissue distributions, the functional significance of these isoforms is generally

not known. Some of the genes also produce alternative spliced products to produce further isoforms of the α subunit. There is also evidence in humans that there is an endogenous cardiotonic steroid that could act as a natural regulator of the sodium pump (Bagrov *et al.* 1998, Kawamura *et al.* 1999, Mahon and McKenna 2000, Cusi 2002, Manunta and Ferrandi 2004) and there is speculation that the plant and toad toxins have evolved to exploit this regulatory system as a defense mechanism. Some insects have been shown to express a modified Na^+/K^+-ATPase that resists the effects of plant cardiotonic steroids (Labeyrie and Dobler 2004) and similarly cane toads have a Na^+/K^+-ATPase which confers resistance to its own toxin (Jaisser *et al.* 1992). There are precedents, therefore, for genetic adaptation to deal with the adverse effects of cardiotonic steroids.

The genetic basis for this adaptation has been established in experimental models and the genetic structure of the ouabain resistant forms of Na^+/K^+-ATPase in insects (Labeyrie and Dobler 2004), cane toads (Jaisser *et al.* 1992), and rats (Price and Lingrel 1988) is consistent with these models. The modifications that convert a sensitive phenotype to a resistant phenotype have been investigated using *in vitro* mutagenesis of the sheep (Price *et al.* 1990, Labeyrie and Dobler 2004), dog (Jaisser *et al.* 1992), and rat (Coppi *et al.* 1999) Na^+/K^+-ATPase. The changes to the primary amino acid sequence of the α-chain that confer the greatest resistance to ouabain are found in the first extracellular loop called the H1-H2 region (or the M1-M2 region for the region between the first and second transmembrane domains) which consists of 12 amino acids consisting of residues 111 to 122 in sheep. In the sheep model, the combined substitutions of glutamine with arginine and asparagine with aspartic acid at residue 111 and 122 respectively (i.e. Q111R and N122D) confers over a 1000-fold resistance to ouabain; substituting glutamine with aspartic acid and asparagine with arginine (Q111D and N122R, respectively) confers a 50,000-fold resistance. A variety of other substitutions in the H1-H2 region confer increased resistance in the range of 2- to 65-fold. Individual mutations in other regions in either sheep, rat, or dog Na^+/K^+-ATPase (i.e. H1 transmembrane, H3/H4 extracellular, H4/H5 cytoplasmic, H5 transmembrane, H5/H6 extracellular, H7 transmembrane, H7/H8 extracellular and H10 transmembrane regions) can confer between 1- and 80-fold increase in ouabain resistance.

Given that amino acid substitutions in the α-chain of the sodium pump can confer resistance to cardiotonic steroids and that this is the most likely mechanism whereby resistance will develop, this provides a means of screening individuals for potential resistance. In this project we will sequence across key regions of the Na^+/K^+-ATPase in quolls for evidence of cane toad toxin-resistant genotypes in the population. This information may lead to strategies for managing and conserving quoll populations in an environment where cane toads are likely to persist for many years.

Our hypothesis is that there may be a degree of natural genetic diversity present in northern quoll populations at the locus that codes for the sodium pump and that some of these alleles may confer a natural resistance to toxins affecting the pump. It is further hypothesized that the animals that possess the resistant alleles will be able to survive cane toad influxes and ingestion of toads; animals that do not have a resistant allele will succumb upon predating toads. Populations of northern quolls that manage to survive cane toad invasions may show a greatly increased frequency of variants in this H1-H2 critical region and show genetic features of reduced population numbers which may be manifest through characteristic bottleneck signatures found using microsatellite loci.

Our aims are to develop and characterise loci that code for the α1-chain locus of the Na^+/K^+-ATPase pump, identify and characterise the genetic diversity within the Na^+/K^+-ATPase in populations that have had differing histories of exposure to toads, measure population parameters of concern for conservation management, gain an understanding of the evolutionary forces acting on populations affected by toads, and develop a predictive model of population response to toad invasions based on prior history of exposure.

Methods

Characterising the α1-chain of the Na^+/K^+-ATPase pump

What is the genetic sequence of the α1-chain locus of the Na^+/K^+-ATPase pump in quolls? Is there any allelic diversity at this locus? Can we correlate this diversity (or lack of diversity) to different histories of exposure to cane toads? These are the central questions in our hypothesis. Preliminary data (Firestone *et al.*, unpublished data) indicates that populations that have had long-term exposure to cane toads show decreased allelic diversity at selectively neutral loci. We also know that some populations manage to survive in toad infested areas despite the likely occurrence of meeting toads in their environment. Furthermore we know that the Na^+/K^+-ATPases are the most likely targets of the bufotoxins and bufadienolides (Flier 1978; Flier *et al.* 1980). Is there a genetic component to population survival? Is it related to the sodium pump loci?

In order to characterise the α1-chain locus, we will need to develop cDNA libraries from total mRNA extracted from quolls and screen the libraries for the α1-chain locus to generate an exact sequence. We focus on the α1-chain, as it is ubiquitously expressed in all tissues (unlike the α2 which has high concentrations in skeletal and heart muscle or the α3 which is expressed in brain tissue). We currently have available a number of different tissue samples from two northern quoll specimens (obtained from the collection of the Zoological Parks Board of New South Wales) that have been appropriately stored in liquid nitrogen for RNA extraction. These samples are a critical first step for extracting mRNA and making cDNA libraries.

Once a full sequence for this locus is available the genomic intron/exon structure in our target species can be determined and the information used to design probes to examine genomic DNA samples from our population samples. This approach will give us a clear picture of the locus coding for the α1-chain and avoids the problem of amplifying intronic material of unknown size by PCR. For example, one of the sites of interest is the last codon of exon 4 of the α1-chain. Exon 4 is 500-800bp in most eutherian mammals, but 1200bp in the marsupial *Monodelphis*. It is therefore necessary to obtain the correct quoll sequences via reverse transcriptase-polymerase chain reactions (RT-PCR) before attempting to amplify the long PCR product stretching across exon 4, intron 4, and exon 5.

The ideal starting point to establish a base-line sequence for the quoll Na^+/K^+-ATPase α1-chain would be to sequence the genes or key regions of these genes from an individual that has been shown to be sensitive to cane toad toxins. This, however, would be difficult to achieve, so we will begin with an ATPase from an animal that has been derived from an area where there were no cane toads, to establish normal sequences and later test animals from contact areas.

Identify and characterise the genetic diversity within the Na^+/K^+-ATPase pump

Is there genetic diversity within the Na^+/K^+-ATPase α1-chain? Is there a different pattern of diversity within populations of quolls based on prior history of exposure to cane toads? Do populations of quolls that have had extended exposure to cane toads show evidence of genetic bottlenecks at this locus or at microsatellite loci?

Our hypothesis is that there may be a degree of natural genetic diversity present in northern quoll populations at the α1 locus and that some of these alleles may confer a natural resistance to toxins affecting the pump. It is further hypothesized that any animals that possess a resistant allele will be able to survive cane toad influxes and ingestion of toads; animals that do not have a resistant allele will succumb upon predation of toads. Populations of northern quolls that survive cane toad invasions may show genetic features of reduced population numbers which may be manifest through characteristic bottleneck signatures found using microsatellite loci.

The strategy employed here will be to sequence or otherwise screen PCR products from individuals at the α1-chain locus to determine the degree of genetic diversity within populations at the Na^+/K^+-ATPase. One region of interest is located at amino acid residues 112-121 in humans (or in sheep at amino acid residues 111-122). Mutations in this region can confer up to a 50,000-fold increase in resistance to ouabain-like compounds.

Population genetics

In addition to our focus on the sodium pump, we intend to examine the broader population genetics questions in top-order carnivorous marsupials using non-coding microsatellite loci and mtDNA sequencing currently available. This is an area of concern for on-ground managers who require this information in order to be armed for effective conservation management. The questions that we will address are: how diverse are these populations? Are they genetically healthy? Do they suffer from bottlenecks or show signs of inbreeding? How have populations fared in terms of exposure to toads? How different are populations from each other and specifically how different are mainland and island populations? We know, already, that northern quolls in Queensland show lower levels of diversity in comparison to populations from the Northern Territory (prior to toad invasion) or Western Australia (Firestone *et al.*, unpublished data). However, this work was based on a limited number of populations and samples available at the time and can not be extrapolated to very meaningful results across the broad range of the species. We are using microsatellite loci that have been previously developed (Firestone 1999) as well as sequencing from the mtDNA control region to examine population parameters. Populations have been and will continue to be sampled from throughout their range, filling gaps in our current database (Table 1). We intend to measure a number of parameters of concern (e.g. genetic diversity, and differentiation, effective population size, relatedness). This data will provide us with the knowledge of which populations are of high conservation value and will also give us a baseline for future population monitoring. In addition, using non-coding loci in combination with functional, protein-coding loci will provide a counterpoint to test whether neutral loci are actually a good tool for these types of population measures.

Table 1. Samples currently available.

State	Sample site	Year	Treatment	N	Source
NT	Kakadu NP	1994/1995	novice	26	M. Oakwood
NT	Darwin area	2000/2001	novice	18	D. Spielman
NT	Groote Eylandt	2002/2003	novice	5	T. Naughton
NT	SW Darwin (T)	2003	novice (?)	27	J. Woinarski
NT	Darwin River (T)	2003	novice (?)	13	J. Woinarski
NT	Litchfield (T)	2003	novice (?)	4	J. Woinarski
NT	East Alligator (T)	2003	novice (?)	14	J. Woinarski
NT	Hayes Creek (T)	2003	novice (?)	5	J. Woinarski
NT	Astell Island F1	2004	novice	6	R. Taylor
NT	Pobassoo Island F1	2004	novice	6	R. Taylor
NT	East Alligator	2003/2004	novice (?)	7	M. Oakwood
NT	Vanderlin Island	2003	novice	1	J. Woinarski
NT	Marchinbar Island	2004	novice	10	R. Taylor
NT	Astell Island F2	2005	novice	71	B. Rankmore
NT	Pobassoo Island F2	2005	novice	24	B. Rankmore
QLD	Cape York	1994	veteran	1	L. Leung
QLD	Atherton Tableland	1995/1996	veteran	5	L. Pope

QLD	Carnarvon Gorge	2000	veteran (?)	3	M. Oakwood
QLD	Townsville AIMS	2000	veteran	19	S. Burnett
QLD	Crediton SF	2003-2005	veteran	3	A. Dinwoodie
QLD	Gamma	2003	veteran	5	A. Dinwoodie
QLD	Calen	2004/2005	veteran	13	A. Dinwoodie
WA	Boongaree Island	2003	novice	2	N. McKenzie
WA	Bigge Island	2003	novice	2	T. Start
WA	Faraway Bay	2002	novice	1	T. Partridge
WA	Mitchell River	2001	novice	4	A. Thomson
WA	Mitchell Plateau	2003	novice	6	T. Start/N. McKenzie
WA	Prince Regent Riv NR	2003	novice	5	T. Start/N. McKenzie
WA	Silent Grove	2003/2004	novice	5	T. Start
WA	West Pilbara	nd	novice	8	P. Kendrick
WA	Yampi Reserve	2002	novice	10	C. Palmer
WA	Bachsten Creek	2002	novice	3	C. Palmer
WA	Koolan Island	2006	novice	31	M. O'Connell

Evolutionary forces

Perhaps carnivorous marsupials can survive the presence of cane toads in their environment through adaptation and selection acting on natural genetic variation present in the gene pool? Results of our work will give an insight into how evolutionary forces are acting on populations of northern quolls as well as provide clues as to how this species can adapt and cope with changing environmental conditions. We predict that there may be some populations of northern quolls that have evolved to resist cane toads, despite the relatively short time span of their coexistence in Australia. Cane toads have been present in Australia for approximately seventy years, which equates to approximately sixty generations in quolls since these species breed annually beginning when they are a year old, and some individuals will breed in a second year. Though this is short in evolutionary terms, it has already been demonstrated in at least two instances that evolution is affecting both cane toads (Phillips *et al.* 2006) and species that predate on cane toads (Phillips and Shine 2004). This will provide a unique and exciting opportunity to examine these types of evolutionary questions at close range within carnivorous marsupials.

A predictive model

Can native carnivorous marsupials persist in the face of a toxic onslaught? The short answer is yes. They are surviving (albeit in very decreased numbers) in Queensland despite long-term exposure to cane toads. The real question is how is this happening, what mechanism is allowing quolls to survive? Depending on the results of screening populations for Na+/K+-ATPase variants in populations with different toad histories (e.g. novice, veteran, or newly exposed populations) we may be able to predict how populations will respond if and when toads invade further quoll habitats. If toad-resistant alleles are found at the Na+/K+-ATPase loci within populations of northern quolls that have had long-term exposure to cane toads, this will give us evidence of how other northern quoll populations may respond to toad invasions in areas where toads do not currently exist. If we find no variant that indicates increased resistance to bufadienolides / bufotoxins, we may predict that these populations will crash if cane toads establish in those areas and different management actions and contingency plans will need to be formulated.

Management implications

The results of this work will give us additional information to help conserve and manage northern quolls in an appropriate manner. A number of potential scenarios exist. If, for instance, we find no variability in the α1-chain of the sodium pump in surviving Queensland populations that have had long term contact with cane toads, but some variability in those populations that have not yet been exposed to toads, then we might infer that toads have had an impact on the 'veteran' populations. We might also surmise that these veteran populations may have been subjected to a genetic sweep or a bottleneck and that what we observe now are the resistant remnants of prior diversity. If we find resistant alleles present in populations in the Northern Territory and Western Australia, then we might further infer that some individuals in those populations will be able to survive an influx of toads, and that these populations may be able to recover after an initial crash. If, on the other hand, we find no variability or only non-resistant alleles in the 'novice' populations, one management aim might be to translocate resistant northern quolls from long-term contact areas to help support populations as toads spread further west. An alternative management scenario is that we find no correlation between cane toad resistance and allelic diversity at the α1 locus. In this case, as cane toads continue to invade core habitat, our only options are to stock further offshore islands as a safekeeping measure until toads can be eradicated, maintain captive stock in breeding facilities, or design large-scale toad-proof exclosures.

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Inter- and intra-specific variation in squamate toad toxin resistance

Beata Ujvari and Thomas Madsen, School of Biological Sciences, University of Wollongong

Introduction

Human introduction of numerous exotic animal and plant species into the Australian continent has often resulted in catastrophic effects on the indigenous fauna and flora (Flannery 1995, Low 2001). A fairly recent introduction was the release of the South American cane toad (*Bufo marinus*) into the sugar cane fields of Queensland in 1935. A year later, Reginald Mungomery, the person in charge of the introduction of cane toads into Australia, made the following statement: "*This introduction into Queensland was made only after a careful analysis of the pros and cons, and, according to the behaviour of the toad up to present, there appears to be no reason for the assumption that we have made an error in our judgement*." Seldom has a statement been based on such a massive error of judgement. The devastating biological effects of the cane toad on the Australian native fauna such as the northern quoll (*Dasyurus hallucatus*) (Oakwood 2004) has resulted in this amphibian being listed as a key threatening species under the *Commonwealth Environment Protection Biodiversity Act in 1999*.

However, a few individuals of vulnerable taxa, such as northern sand goannas (*Varanus panoptes*), appear to be able to survive in cane toad infested areas (Madsen and Ujavri pers, obs). We suggest that the reason for their survival may be traced to their evolutionary history. Members of the family Bufonidae (toads) are found in most temperate and tropical parts of the world except Madagascar and Australasia (Cochran 1961). Thus, no toad species are native to Australia (Tyler 1998). Marsupial carnivores, such as the northern quoll, evolved on the Australian continent (Wroe 2003), and until the introduction of the cane toad to Australia, these predators had never been exposed to toad toxins, which has a unique molecular structure not found in any native Australian amphibians (Tyler 1998). Consequently, selection favouring evolution of toad toxin resistance in Australian marsupial carnivores is highly unlikely. Goannas, elapid and colubrid snakes, however, did not originate in Australia, and consequently have been exposed to toads throughout their evolutionary history. Indeed, the diet of all non-Australian goannas, many elapids, and colubrids frequently include toads (Pitman 1974, Fitzsimmons 1976, Auffenberg 1994, Wall 1921). The South American colubrids *Xenodon* spp. regularly feed on cane toads (Marques *et al.* 2000), strongly suggesting that all of these "toad-feeding" taxa are highly resistant to toad toxins.

The arrival of goannas and elapid snakes to the Australian continent is estimated to have occurred in the Miocene, approximately 15 million years ago (Keogh *et al.* 1998, Greer 2003), whereas colubrids arrived in the Mid-Miocene but probably as late as Pleistocene (approximately 100 000 years ago; Shine 1991). Thus, when arriving, the immigrant "toad-feeding" taxa most likely exhibited high levels of toad toxin resistance. However, the lack of toads on the Australian continent presumably resulted in a relaxed selection to maintain such a resistance. If evolutionary history is a major factor in explaining the profound inter-specific differences in cane toad toxin resistance among Australian predators, we predict that:

1. Marsupial carnivores that evolved in Australia, like the northern quoll, should not exhibit any resistance to toad toxin.
2. Ancient potential "toad-feeding" squamate immigrants should still exhibit intra-specific variation in toxin resistance.
3. Recent "toad-feeding" squamate immigrants should exhibit high levels of resistance to toad toxin.

Indeed, these predictions are strongly supported by the following observations:

1. Northern quolls have become extinct in all of the cane toad infested areas of Kakadu National Park (Oakwood 2004), strongly suggesting that all individuals lack resistance to cane toad toxin.
2. Ancient potential "toad-feeding" immigrants Australian elapid snakes, exhibit among-individual variation in toxin resistance (albeit only a few individuals are likely to harbour sufficient resistance to survive an attempt to feed on toad toxin (Phillips *et al.* 2003).
3. A recent "toad-feeding" immigrant, the keelback (*Tropidonophis mairii*), shows high levels of resistance to cane toad toxin (Phillips *et al.* 2003).

In cane toads, the toxin is not restricted to the paratoid glands and skin, where it is found in very high concentrations (1mM), but is also present in toad plasma where the concentration is estimated to 1µM, thousands of times higher than the level required to produce toxicity in humans (Flier *et al.* 1979). The high level of toxin in toad plasma demonstrates that the cane toad is immune to its own toxin.

Results and discussion

In 2005 we constructed primers that allowed us to amplify a gene that confers toxin resistance in cane toads. In a pilot study we have amplified the same gene in four snakes that show high levels of toad toxin resistance; keelback (*Tropidonophis mairii*), slaty-grey snake (*Stegonotus cucullatus*), grass snake (*Natrix natrix*) and Indian cobra (*Naja naja*). We also amplified the gene in four taxa susceptible to the toxin, death adder (*Acanthophis praelongus*), adder (*Vipera berus*), black whip snake (*Demansia atra*) and northern sand goanna (*Varanus panoptes*; Phillips *et al.* 2003, Griffiths & Holland 2004, pers.obs). Aligning the DNA sequences of the 8 taxa revealed that the gene in the toad-resistant predators was almost identical to that of the cane toad, whereas non-resistant taxa displayed large differences. Thus, our preliminary results strongly suggest that this gene is a major component in conferring resistance to cane toad toxin. Furthermore, our preliminary analyses of this gene in 10 highly vulnerable "toad-feeding" northern sand goannas (*Varanus panoptes*) revealed a significant intra-specific variation. Intra-specific variation in toxin resistance has been documented in several susceptible Australian squamate predators (Phillips *et al.* 2003). The intra-specific polymorphism in the resistance gene among the 10 northern sand goannas could therefore reflect a concomitant intra-specific variation in toxin resistance.

Our aim is to expand our analyses to cover more toad resistant taxa from Asia, Africa, Europe, North and South America, and compare the resistance gene with taxa known to be highly vulnerable to cane toad toxin such as Australian goannas, Australian pythons, Australian elapid and colubrid snakes. We will also expand our intra-specific analyses, and we aim to sequence all of our >200 *Varanus panoptes* and >100 death adder DNA samples. The latter part of the study will reveal whether the gene of some individuals exhibits sufficient similarities with the toad toxin resistant taxa, which, hence, may explain why a few individual squamates survive in cane toad infested areas.

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