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ABSTRACTS FROM SYMPOSIUM ON NEXT-GENERATION BIOCONTROL OF INVASIVE VERTEBRATE PESTS, ROYAL SOCIETY OF VICTORIA, SEPTEMBER 2022

*Note: Abstract not provided

NEXT-GENERATION BIOCONTROL OF INVASIVE VERTEBRATE PESTS

Symposium held at the Royal Society of Victoria
September 2022

PREAMBLE

The symposium was convened in partnership with the Invasive Species Council; the Victorian Department of Environment, Land, Water & Planning; Zoos Victoria; Rabbit-Free Australia; the Victorian National Parks Association; and the Centre for Invasive Species Solutions.

The idea at the core of this symposium was to explore developing technologies in the field of invasive species management in Australia. The technologies discussed in this symposium are often species-specific and may rely on the release of biological control agents (such as rabbit calicivirus) or interference with sex ratios and/or reproduction. Modelling the outcomes of such technologies on population size is valuable to determine the expected rate of decline in a target species and identify which strategy may be the most effective.

In addition to researchers who discussed new discoveries and technologies relating to reproduction in the major feral species (wild horses, cats, deer, etc) and the use of biological control agents, the symposium also included discussion of mathematical predictions of population decline, problems with delivery of treatments to a restricted range of species, and the ethical and community aspects of such approaches. Importantly, input from Indigenous, wildlife and agricultural experts was sought to provide a community-wide balance of opinion.

The symposium was open to the public through an online link. A reason for involving the public arose from a remit of the RSV to explain important scientific concepts to the public. So, rather than making this a conference of academic scientists talking to other experts, the public was given the opportunity to listen and participate in the discussions that followed each session.

As outlined in the *Proceedings* abstracts which follow, recent advances in understanding feral population sizes included:

- Contraception vs species-specific treatments that reduce the incidence of pregnancy
- Recent advances in DNA technology and the concept of 'gene drive'
- Mathematical modelling of population size after reduction of fecundity: species-specific effects and trajectory over time
- Challenge of delivery of biological treatments that interfere with reproductive efficiency
- Bioethical and public interest challenges in interfering with species-specific reproductive potential.

Rob Gell (President, RSV)
Mike Flattley (CEO, RSV)
David Walker (Council Member, RSV)
Bill Birch (Editor, *Proceedings* of RSV)

SESSION 1: THE PROBLEM

AUSTRALIA'S INVASIVE VERTEBRATES: THE EXTENT OF THE PROBLEMS AND THE NEED FOR INTEGRATED, COMMUNITY-BASED SOLUTIONS

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How big is the invasive species problem? Short answer: it's huge. Recent estimates into costs incurred since the 1960s indicate close to $400 billion dollars has been spent on invasive species management, with cats and rabbits in the top three most expensive species to control. Impacts of invasive species in Australia are diverse. For example, foxes and cats consume billions of animals each year, rabbits negatively impact on endangered plants and critical habitats for threatened species, while large herbivores such as feral horses impact delicate ecosystems including alpine areas. The scale of invasive species impacts is broad, affecting our environment, economy and health, and is felt throughout communities all over Australia. Invasive species' impacts between and within ecosystems in conjunction with other factors such as climate change, fires and habitat loss can exacerbate outcomes, resulting in biodiversity decline and species extinctions. Management of whole ecological communities and ecosystems is critical, as opposed to focusing on the control or removal of individual species only. To ensure management of invasive species and their impacts is effective, we must consider multiple management tools and approaches, restoration of ecological health and top predator reintroductions, maintaining habitat complexity and cover, and, ultimately, implement planning that explicitly considers and accounts for multiple possible outcomes of any management strategies and actions

THE ON-FARM COST OF INVASIVES

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Gerald outlined the personal impacts invasive species take on his farm in northern Victoria and the enormous costs and time required to keep invasive species at bay. Rabbits' impacts on the grazing land requires up to 190 hours per year to keep under control. Fox predation on lambs during lambing season requires careful planning regarding timing of lambing season and protection of newborn lambs up until one week old to increase their chances of surviving with foxes in the landscape. Mice present the most expensive and time-consuming invasive species for the farm. Losses in 2017 due to mouse activity were over $200K for the farm, which is a considerable loss of revenue. Projections surrounding mouse populations may point towards 'boom and bust' events becoming more frequent. Feral pigs and goats have established populations within the neighbouring national park and present considerable concerns regarding transmission of disease to livestock and zoonotic disease spread to both humans and animals. Feral cats in the area have also transmitted *Toxoplasmosis gondii* to ewes, resulting in late-term abortions. The financial and emotional impact of invasive species on the farming community must be taken seriously and more funding and research into strategies to support farmers are essential.

THE IMPACT OF INVASIVE SPECIES ON NATIVE SPECIES

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The state of the environment report lists 230 non-native species currently identified as key threats to our native threatened taxa. Of the top ten, seven are vertebrate pests. Rabbits specifically threaten 333 EPBC-Act listed species, including birds, mammals, reptiles, plants, fish, invertebrates and amphibians, with their impact felt nationwide. Australia's biosecurity system comprises three parts: offshore, at the border and within Australia. The goal of the national biosecurity system is to minimise adverse impacts of pests and diseases on Australia's economy, environment and the community while facilitating trade and the movement of plants, animals, people and products. The National Biosecurity Strategy outlines the key strategies for the next ten years that aim to protect people, the environment and our economy against biosecurity threats. There are 168 species (on the National Priority List of Exotic Environmental Pests, Weeds and Diseases) that have not yet arrived or become established in Australia but pose risks to our economy, environment and health. The role of the Australian Government's Environmental Biosecurity Office in established pest animal and weed management is national coordination, strategic investment with states and territories, and investment in research, development and extension. The Office of the Threatened Species Commissioner oversees development, implementation and reporting of programs that support threatened species.

SESSION 2: THE TECHNOLOGIES

EMERGING BIOCONTROL TECH: AN HISTORICAL OVERVIEW OF NATIONAL COLLABORATIVE RESEARCH AND DEVELOPMENT

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Australia has made major advances in both vertebrate pest genetic biocontrol and classical biocontrol through a continuous series of national scale Research and Development (R&D) collaborations initially supported by the Commonwealth Cooperative Research Centre (CRC) program and more recently through the Centre for Invasive Species Solutions. This is a 22-member and partner collaboration between the Commonwealth, all states and the ACT, two R&D Corporations, CSIRO, four national natural resource management and conservation organisations, one industry body, New Zealand Department of Conservation, and five universities. This presentation outlines the major areas of research since the early 1990s to present day.

In the early 1990s to 2005, two CRCs led by CSIRO progressed world-leading research on virally vectored immune contraction technologies for mice, rabbits and foxes. This resulted in major advances in knowledge, but no viable control product was developed. A subsequent CRC pursued research to develop ‘daughterless carp’ technology complemented by research to evaluate Australia’s first potential classical biocontrol agent — *Cyprinid herpes virus 3* (CyHV-3). While the research demonstrated that ‘daughterless carp’ technology was viable, governments decided to only progress CyHV-3, which was further evaluated before being taken forward through the National Carp Control Plan. In tandem, since 2010 our former CRC collaboration evaluated new strains of Rabbit Haemorrhagic Disease Virus to improve national effectiveness of this biocontrol agent, leading to the national release of the RHDV1 K5 strain in 2017 — the first new rabbit biocontrol agent in 20 years. Our current Centre collaboration evaluated a further potential rabbit biocontrol agent — RHDV2 as well as Australia’s first potential classical biocontrol agent for tilapia — Tilapia Lake Virus. This has been complemented by development of a national decision framework for genetic biocontrol, and investment in proof of concept research in a mammal model (mice) and a fish–amphibian model (zebra fish). Given potential impact of these strategic technologies in reducing vertebrate pest impacts at large-scale, classical and genetic biocontrol R&D needs to remain a priority for Australia.

IMMUNOCONTRACEPTION FOR FERAL CAT MANAGEMENT

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Since their introduction, the impact of feral cats on Australian native wildlife has been devastating. Feral cat numbers are estimated to be 6.3 million and a single cat is responsible for the killing of between 5 and 30 native animals per day. Their activity has been a driving force behind the decline, and even extinction, of many land-dwelling birds, reptiles and mammals. Virally vectored immunocontraception (VVIC) has been identified as a potential population control method for invasive vertebrate species. The technique relies on stimulation of the host immune system to suppress either the occurrence or continuation of a pregnancy. Properly designed, immunocontraceptives have the potential to be more humane and effective, while requiring less human input with regards to delivery than current population suppression methods.

I presented work detailing the construction of feline immunocontraceptive candidates derived from feline herpesvirus (FHV-1) which contain critical reproductive genes essential for reproduction-related processes in both males and females. We predict that viral expression of these genes will induce an immune-directed disruption of the natural activity of both of these genes, rendering the cat partially or fully sterile.

This project is expected to provide new insights into the use of VVIC control in Australia for large-scale population control of invasive vertebrate species.

BIOLOGICAL CONTROL PIPELINE STRATEGIES FOR SUSTAINABLE CONTROL OF RABBITS IN AUSTRALIA

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European rabbits remain one of the most damaging environmental and agricultural pests in Australia. Self-disseminating viral biocontrol agents have proven to be the only effective means of continental-scale rabbit control. The two rabbit-specific pathogens myxoma virus (MYXV) and the calicivirus rabbit haemorrhagic disease virus (RHDV) were deployed as biological control tools in the 1950s and 1990s, respectively, resulting in savings exceeding $70 billion AUD to the agricultural industries over 70 years. In addition, the sustained landscape-scale reduction of rabbit numbers and impacts has allowed many fragile ecosystems to partially recover from the devastating impact of rabbits. Despite these successes, biological control is never a silver bullet, as building population immunity and ongoing host-pathogen co-evolution will eventually reduce their effectiveness, and a pipeline of tools and strategies is needed to be rolled out in intervals, to maintain the valuable gains made. A novel variant of RHDV (RHDV-K5) was nationwide released in 2017 to boost biocontrol effectiveness. Its release was overshadowed by the arrival of the emerging RHDV Type 2 (RHDV2), which rapidly replaced endemic strains and led to an overall reduction of wild rabbit populations by ~60%. Current recommendations for the rabbit biocontrol pipeline strategy going forward include developing strategies to better integrate existing biocontrol tools with conventional controls, accelerated natural selection of new RHDV variants, searches for novel rabbit pathogens and, in the longer term, investigating genetic biocontrol approaches for rabbits.

GENETIC BIOCONTROL OF INVASIVE FISH VIA SELF-STOCKING INCOMPATIBLE MALE SYSTEM

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European Carp are a widespread invasive species in Australian waterways. They comprise 90% of fish biomass in some regions and are the most abundant fish species in the Murray–Darling Basin. Carp have a significant impact on our freshwater ecosystems, leading to the destruction of aquatic vegetation and alteration of the composition of native invertebrate species. Current biocontrol efforts have been found to be inadequate, as although they can do a good job of knocking down the population, the fish bounce back very quickly.

I presented our work on the development of a genetic biocontrol approach to population control — Self-Stocking Incompatible Male System. This system provides a way to deliver a small number of genetically modified fish to a waterway, and which is both self-amplifying and self-limiting. Our modelling has shown it can lead to effective control of the carp population. I presented the initial, successful data we have on the different genetic components of the system that we have tested in our model zebrafish.

DEVELOPING GENE DRIVE TECHNOLOGY FOR INVASIVE RODENTS

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Invasive mammalian pests are among the greatest threats to global biodiversity and constitute an unprecedented form of global change. Commensal rodents, including house mice (*Mus musculus*), have spread throughout the globe, causing significant environmental damage and loss of agricultural productivity. Islands are biodiversity hotspots and are particularly susceptible to the impact of invasive rodents, where they contribute to widespread extinction and endangerment, particularly of migratory bird species, reptiles and plant stocks that have not evolved with rodents. Current control methods rely principally on the widespread distribution of anticoagulant rodenticides, an approach that is costly to apply at scale, carries ethical concerns regarding the mechanism of toxicity, and is not species-specific.

Genetic biocontrol technologies including gene drives offer the potential for landscape-scale modification or suppression of invasive populations. Gene drives are natural or synthetic genetic elements that spread through a population via super-Mendelian transmission. Synthetic 'homing' gene drives have recently been developed in several insect species, including *Anopheles* malaria vectors. However, in mice, homing gene drives have been challenging to develop, prompting us to consider alternative strategies. The *t* haplotype is a naturally occurring gene drive element commonly found in wild mouse populations that functions as a male meiotic drive, biasing transmission from carrier males by up to ~95%, depending on the variant. In non-lethal *t* haplotype variants such as t^{w2}, homozygous males are sterile and homozygous females are viable and fertile. Given the significant bias in *t* transmission, it is possible that this natural male meiotic drive could be leveraged for mouse population suppression or even eradication on islands.

I presented work describing a novel gene drive strategy termed t_{CRISPR}, in which fertile females are progressively depleted due to a CRISPR transgene embedded in the *t* haplotype that targets a haplosufficient female fertility gene. Using spatially explicit individual-based *in silico* modelling on a hypothetical island, we demonstrate that t_{CRISPR} has eradication potential across a range of realistic scenarios. We further demonstrate the feasibility of this strategy by engineering and testing t_{CRISPR} in a genetically-contained 'split drive' format, where the Cas9 and gRNA expression cassettes are integrated into different chromosomes. We hope that these data will promote meaningful discussion and debate on the feasibility, benefits and risks of using gene drives to mitigate the devastating impact of invasive rodents on global biodiversity and the environment.

FISH VIRUSES: FRIEND OR FOE?

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Invasive fish have been documented to have severe impacts on freshwater ecosystems primarily through displacement of native species and habitat alteration. For example, invasive carp comprise up to 90% of the fish biomass in parts of the Murray–Darling Basin (MDB) and Mozambique tilapia is listed in the top 100 of the world's worst invasive alien species. Eradication attempts using chemical and physical removal are rarely successful in open waterways. Biological control, where it is feasible, can be a cost-effective, safe (species-specific) and practical solution to managing invasive species because it does not require reapplication of chemicals or poisons, and once established should be self-sustaining. This presentation provides an overview of viral biocontrol for carp and tilapia using *Cyprinid herpesvirus 3* (CyHV-3) and Tilapia lake virus, respectively. Safety and efficacy, two major concerns for a successful biocontrol virus, need to be taken into consideration before the use of any exotic biocontrol virus is considered. The virulence (efficacy) and apparent species-specificity (safety) of both viruses may offer an important means of biocontrol. Modelling suggests that combined viral biocontrol and genetic technologies would be a better approach for effective carp, and possibly tilapia, control.

For more information on viral biocontrol of invasive carp, visit National Carp Control Plan and for tilapia, visit Tilapia biocontrol and Bioprospecting.

DEVELOPING PIPELINES FOR GENETIC BIOCONTROL OF VERTEBRATES

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Engineered suppression gene drives are among the most promising genetic biocontrol strategies for suppressing or even eradicating invasive vertebrate pest populations. Since they can propagate exponentially through a population simply via natural breeding, gene drives targeting female fertility are entirely humane, unlike most traditional methods of pest control. Since they require many generations of breeding to achieve suppression, ecosystems can adjust gradually to the removal of a non-native predator or prey species.

While functional gene drives have been successfully engineered in insects, further research is required in vertebrates. My laboratory is using the zebrafish as a vertebrate model to optimise gene drive design for vertebrates by testing the capacity of candidate gene promoters to drive expression of Cas9 at the prophase stage of meiosis in sperm-producing germ cells. This is expected to provide the crucial data needed to demonstrate that suppression gene drives are a viable strategy for vertebrate pest control.

In parallel, my laboratory is also developing pipelines to address an additional challenge: producing animals of non-model species (e.g. carp, cane toad, fox, rabbit) with targeted insertions of large DNA fragments. We are largely focusing on a nuclear transfer-based approach, which allows CRISPR editing to be first (and more easily) performed in cultured cells, which are then used to create embryos via nuclear transfer to enucleated oocytes.

SESSION 3: THE CAVEATS

A PERSPECTIVE ON NEXT-GENERATION TECHNOLOGY DEVELOPMENT FROM A CONSERVATION STAKEHOLDER

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Despite conservationists' best efforts, global biodiversity remains in crisis with ongoing species loss. Yet in many systems, while research leverages such issues for resourcing, the work conducted frequently fails to deliver to priority stakeholder needs. On the whole, research for conservation is not having the impact that it could have and is needed.

Better coordinated systems approaches would help address this issue. In the first instance, conservation research needs are best identified by conservation stakeholders rather than the research community. Priority needs for conservation and environmental impact can effectively be guided by the 'critical hurdles' to needed impact that stakeholders face.

While next-generation technology development could indeed help address such hurdles, far-horizon science by definition has greater risk and uncertainty, and longer time-frames and greater resourcing requirements for potential impact realisation. For making real-world difference for conservation, where expediency is often critical, such approaches should only be prioritised when closer-horizon applied and translational research likely cannot deliver.

And where next-generation technology development is prioritised, avenues pursued should be critically evaluated through both biological and socio-political lenses. For example, gene-drive development for long-lived species is not supported by population modelling of impact, while new virally-vectored biocontrol solutions are under increasing social scrutiny.

However, when closer-horizon research likely cannot deliver the needed impacts, not prioritising far-horizon research due to associated unknowns (technical, socio-political or cultural) is a fallacy. Rather, they are the reasons to prioritise the research, to find out what works, and to best fill knowledge gaps.

THE ECOLOGY AND EVOLUTION OF SUPPRESSION GENE DRIVES

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Gene drives are a self-disseminating biotechnology that holds considerable promise for controlling many pest species. The basic idea is to introduce a genetic modification that will invade a target population and cause drive carriers to be spread throughout the population. The end result is a population consisting entirely of male drive carriers, at which point the population will go extinct. It is now technically possible to do this, at least in the lab. In this talk I touch briefly on potential concerns with this technology, but also explain why it might be more challenging to achieve population suppression in a field setting than in the lab. I make the case that careful modelling of ecological and evolutionary dynamics will be required to understand the potential impact and potential efficacy of this technology.

WHAT CAN WE LEARN FROM THEORETICAL MODELS ON GENE DRIVES?

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I started by introducing the two main modelling approaches, viz. analytical models and computer simulations. The former are rather simple mathematical representations of a system, and usually are very general. The latter approach could allow for more complex systems to be modelled and therefore can be very specific. To obtain some generality with computer simulations, parameter space should be explored extensively with a large number of simulations. Computer simulations also come in various levels of complexity.

Current gene drive models using computer simulations often have simplifying assumptions, some of which are: non-spatial (panmictic) populations; not species-specific; non-overlapping generations; no fitness consequences for drive-carrying individuals; no multiple mating; and often very small population sizes. I presented our modelling framework where we overcome these assumptions, and therefore represent vertebrate pest populations more realistically.

I summarised some of the important results that emerge from our simulations. Briefly, spatial dimension is important, 'efficient' gene drives that are demonstrated to work well in non-spatial models may go extinct locally too quickly before having the opportunity to spread to other target populations/areas. Therefore, understanding movement and dispersal is crucial for effective eradication with gene drives. And also, gene drives are not a one-size-fit-all solution for all vertebrate pests. Expected median times to eradication in larger mammals with higher survival probabilities (and longer life spans) span over 140 years.

Lastly, I stressed that even with more complex and more realistic computer simulations, the results are only as reliable as the data that go into these models. It is crucial that more data from the field are collected for any species that is targeted for control with gene drives.

ANIMAL WELFARE —— POSING THE HARD QUESTIONS

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There is an urgent and ongoing need to protect and conserve our vulnerable and most threatened wildlife. However, it is essential that ethical principles for wild animal control are understood and adopted to ensure humane methods are used as well as to maintain social licence. Other principles include justification, clear and achievable outcome-based objectives, modifying human practices, systematic planning and basing decisions on specifics rather than labels. The most humane methods should be used and where this is not possible, clear justification for relatively less humane methods must be given. The Relative Humaneness Matrix, which is based on the Five Domains Model (recognises impacts on mental state including fear and stress) and developed by the NSW Department of Primary Industries is an excellent tool to assist in identifying the most humane methods currently available. All management programs should be reviewed on a regular basis to achieve continuous improvement in minimises pain and suffering associated with control methods. In addition, animal welfare assessments must be integral to the development of new methods.